EVALUATION OF THE ANTIPSYCHOTIC PROPERTY OF MORIN AND ITS MECHANISMS OF ACTION IN EXPERIMENTAL MICE

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ABSTRACT

Psychosis is a chronic neuropsychiatric disease characterised by severe behavioural perturbations. Current drugs used in the management of the disease are associated with serious side effects. Therefore, compounds with psychotropic-antioxidant effects are currently being sought as alternatives. Morin, a naturally-occurring neuroactive flavonoid isolated from *Morusalba* possesses strong psychotropic and antioxidant properties, however the mechanism of the antipsychotic property has not been fully elucidated. This study was designed to investigate the antipsychotic-like activity of morin and its mechanisms of action in mice.

Morin was administered intraperitoneally to male Swiss mice. Ninety mice randomised into 6 groups of each experiment (n=5): vehicle (normal saline, 10mL/kg), morin (25, 50, 100mg/kg), haloperidol (1mg/kg) and risperidone (0.5mg/kg); were pre-treated to assess the acute antipsychotic effects of morin on apomorphine-(2mg/kg), ketamine-(10mg/kg) induced stereotypes and woodblock-catalepsy test. For the chronic studies, fifty mice were given preventive treatments (n=5) with morin (100mg/kg/day), haloperidol (1mg/kg/day), risperidone (0.5mg/kg/day), or vehicle for 14 days prior to injection of ketamine (20mg/kg/day) from the 8th-14th day. For the reversal treatment, animals received ketamine for 14 days prior to the treatments. The antipsychotic and neuroinflammatory effects were also assessed in 25 mice following pretreatments with vehicle, morin, haloperidol and risperidone, in combination with lipopolysaccharide (0.1mg/kg/day) induced neuroinflammation for 14 days prior to ketamine (20mg/kg/day) treatment from the 8th-14th day. Schizophrenia-like behaviours in all chronic studies were evaluated using open-field, social-interaction and Y-maze tests. Thereafter, brain biomarkers of oxidative/nitrergic stress were determined, spectrophotometrically in specific-brain regions (striatum, prefrontal cortex and hippocampus) in preventive-reversal and neuroinflammatory studies. Specific-brain regions of dopamine, glutamate and serotonin concentrations, Glutamic Acid Decarboxylase-67 (GAD67), Brain-Derived Neurotrophic Factor (BDNF) and gp91-phox expressions were measured in the preventive-reversal study using ELISA or immunohistochemistry. Brain Tumor Necrosis Factor-alpha (TNF-α),interleukin-6 levels, cyclooxygenase-2 (COX-2) and Nuclear Factor-κB (NFκB) expressions were determined in the neuroinflammatory study using ELISA or immunohistochemistry. Dendritic arborization of the cortical pyramidal neurons of lipopolysaccharide-ketamine treated mice was assessed using silver-impregnation stain. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05.}$

Morin (25, 50, 100mg/kg) significantly suppressed stereotypy induced by apomorphine (23.4, 34.5 and 60.1%) and ketamine (33.7, 73.4 and 83.4%) relative to controls,and was devoid of extrapyramidal side effects in catalepsy test.Morin (100mg/kg) prevented and reversed ketamine-induced social and cognitive deficits relative to controls and ketamine-induced hyperlocomotion (61.6±5.2 vs109.8±5.3; 47.0±6.1 vs103.2±4.5), respectively. Morin prevented and reversed altered dopaminergic, glutamatergic, GABAergic and serotonergic neurotransmissions in the striatum, prefrontal cortex and hippocampus, respectively. Morin increased BDNF, glutathione, and decreased malondialdehyde, nitrite levels and pg91-phox expressions in the three brain regions. Morin reduced TNF-α (124.7±8.6 vs212.7±9.4; 117.3±9.7 vs278.5±13.9 pg/g tissue) in the striatum and prefrontal cortex, and morin also reduced interleukin-6 (321.3±24.2 vs704.7±26.3, 295.1±19.7 vs581.3±47.4 pg/g tissue) in the prefrontal cortex and hippocampus. It also reduced COX-2 and NFκB expressions in the three brain-regions, and increased dendritic arborization of the cortical-pyramidal neurons.

Morin demonstrated antipsychotic-like activity *via* mechanisms related to modulation of neurotransmitters, enhancement of neurotrophin, inhibition of oxidative/nitrergic stress and neuroinflammation.

Keywords: Morin, Psychosis, Oxidative stress, Neuroinflammation

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DEDICATION

To God Almighty, The Ultimate Planner and Father of all sciences.

To the memory of my late Father, Ben-AzuDiagbonya; the one who prepared me to be whom God want me to be.

To all sufferers of schizophrenia and mentally-related disorders in the world, your healing is on its way!

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CERTIFICATION

I hereby certify that this work is an original research carried out by Benneth **BEN-AZU** in the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, under my supervision.

……

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GLOSSARY OF ABBREVIATIONS

ACh Acetylcholine

AChE Acetylcholinesterase

α-7nAChR Alpha-7 nicotinic acetylcholine receptor

AMPA Alpha-amino-3-hydroxyl-5-methylisoxazole-4-proprionic acid

ANOVA One-way analysis of variance

APA American Psychiatric Association

APO Apomorphine

ATP Atropine

BBB Blood brain barrier

BDNF Brain derived neurotrophic factor

Ca^{2+} Calcium ion

CA CornusAmmonis

CaMKII Ca^{2+}/Calmodulin-dependent protein kinase II

CAT Catalase

COMT Catechol-o-methyltransferase

CNS Central nervous system

COX-2 Cyclooxygenase-2

CYP Cyproheptadine

DA Dopamine

DAT Dopamine transporter

DALYs Disability adjusted life years

DI Discrimination index

DISC1 Disrupted-in-schizophrenia 1

DMS Diagnostic and Statistical Manual of Mental Disorders

DTNB $5'$, $5'$–dithiobis-(2-nitrobenzoic acid)

DTNBP1 Dystrobrevin binding protein 1 or dysbindin

EAAC1 Excitatory amino acid carrier-1

EAATs Excitatory amino acid transporters

ELISA Enzyme-linked immunosorbent assay

EPSs Extra-pyramidal symptoms

FMZ Flumazenil

FST Forced Swim Test

GABA Gamma-amino butyric acid

GAD 67 Glutamic acid decarboxylase 67

GAT1 GABA transporter-1

GLU Glutamate

GLU Glutamate transporter-1

GIRK G-protein activated inward rectifying K

GSHPx Glutathione peroxidase

GSH Glutathione

GSK3β Glycogen synthase kinase-3β

GST Glutathione-s-transperase

HC Hippocampus

HLP Haloperidol

H^+ Hydrogen ion

H_2O_2 Hydrogen peroxide

5-HT_2 5-hydroxytryptamine-2(Serotonin)

5-HTIAA 5-Hydroxyindoleacetic acid

IHC Immunohistochemistry

iNOS Inducible Nitric oxide synthase

IL Interleukin

IL-6 Interleukin-6

IPSP Inhibitory post synaptic potential

KET Ketamine

KG Kilogram

KYNA Kynurenic acid

L-ARG L-arginine

L-NAME L-Nitro arginine methyl ester

LPS Lipopolysaccharide

LTP Long-term potentiation

LSD Lysergic acid diethylamide

MAO Monoamine oxidase

MDA Malonaldehyde

MET Methionine

MG Miligram

MTB Methylene blue

NF-κB Nuclear factor kappa B

MPO Myeloperoxidase

MOR Morin

MTG Metergoline

NADPH Nicotinamide adenine dinucleotide phosphate

NFs Neurotrophic factors

Nox2 NADPH oxidase-2

NO Nitric oxide

NORT Novel object recognition test

NMDA *N*-methyl-D- aspartate

NR *N*-methyl-D- aspartate receptor

NRG1 Neuregulin 1

OFT Open field test

$\cdot O_2^-$ Superoxide anion

PKB Protein kinase B

PKC Protein kinase C

PFC Prefrontal cortex

PRA Prazosine

PRO Propranolol

PV Parvalbumin

PCP Phencyclidine(1-phenylcyclohexylpiperidine)

PCPA Para-chlorophenylalanine

PET Positron emission tomography

PUFAs Polyunsaturated fatty acids

RACK Receptor for activated C kinase

RIS Risperidone

RNS Reactive nitrogen species

ROS Reactive oxygen species

RRT Rota rod test

SERT Serotonine transporter

SIT Social interaction test

SMA Spontaneous motor activity

SRM Social recognition memory

SO Stratum oriens

SOD Superoxide dismutase

SP Stratum pyramidalis

SR Stratum radiatum

ST Striatum

SUL Sulpiride

TBA Thiobarbituric acid

TBARs Thiobarbituric acid reactive substances

TD Tardive dyskinesia

VEH Vehicle

VTA Ventral tegmental area

WHO World Health Organization

YHB Yohimbine

YMT Y-maze test

CHAPTER ONE

1.0. INTRODUCTION

1.1. PSYCHOSIS

Psychosis (e.g., Schizophrenia) is a ubiquitous chronic neuropsychiatric disease characterized by severe behavioural perturbations, with distorted or non-existent sense of reality (Kreyenbuhl *et al.,* 2009). Schizophrenia is one of the most expensive mental illnesses in relation to human suffering because of the complex pattern of behavioral disturbances (McGrath *et al.,* 2015). It affects approximately 1% of the World population making it the seventh most costly debilitating medical illness (van Os and Reininghaus, 2016). Schizophrenia commonly begins in late adolescence and often associated with polygenetic, environmental and neuro-developmental vulnerability factors (Fanous and Kendler, 2008). Although many patients with schizophrenia also exhibits catatonic syndrome such as catalepsy, stereotypy, unusual posturing and mannerisms (Andreasen, 1995; Chatterjee *et al.,* 2015), it is presented by a group of heterogeneous perceptual and behavioural phenotypes characterized by positive (e.g., delusion, hyperactivity), negative (e.g., flat expressions, social impairment) and cognitive (e.g., deficits in learning and memory) symptoms (Monte *et al.,* 2013).

Schizophrenia is a complex disease of multiple pathologies including dysfunction in central dopaminergic, glutaminergic, gamma amino butyric acid (GABA), serotonergic and cholinergic pathways (Chatterjee *et al.,* 2012a; Monji *et al.,* 2013; Yuchio *et al.,* 2015). Increasing body of evidence also suggest the role of altered neuroimmune activation in the disease (Monji *et al.,* 2013). The hypothesis that neuroinflammation may play a prominent role in schizophrenia has been a subject of long history (Monji *et el.,* 2009, 2013; da Silva *et al.,* 2017). However, the dopamine dysregulation with hyperfunction of the mesolimbic dopamine system was the original tenet theory underlying the basis of schizophrenia (Pogarell *et al.,* 2012). The pharmacological dopamine agonist-induced schizophrenia was developed to simulate this condition which responds to drugs that affect majorly the dopaminergic transmission (Jone *et al.,* 2008). However, the model lacks the features to closely mimic the negative or cognitive symptoms seen in schizophrenia (Featherstone *et al.,* 2008). A widely used animal model of schizophrenia involves the acute or repeated administration of sub-anaesthetic doses of ketamine, an *N*-methyl-D-aspartic acid (NMDA) receptor antagonist (Chatterjee *et al.,* 2012a). The NMDA receptor antagonist treatment models have excellent face validity for all groups of (positive, negative and

cognitive) symptoms of schizophrenia (Chatterjee *et al.,* 2015; da Silva *et al.,* 2017). It induces hyperactivity, stereotypy behaviour, deficits in prepulse inhibition, social interaction, social cognition and memory in rodent, which models the positive, negative and cognitive symptoms of the disease, respectively (Chatterjee *et al.,* 2012a; Vasconcelos *et al.,* 2015). However, the influence of inefficient glutamatergic neurotransmission through interplay between the receptors of NMDA glutamate and GABAergic systems respectively in the pathology of schizophrenia has been consistently observed (Chatterjee *et al.,* 2012a; Koh *et al.,* 2016). Supportive evidence shows that hyperdopaminergic transmission relative to decreased GABAergic-mediated inhibitions of mesolimbic system is partially responsible for the cause of positive symptoms (Pogarell *et al.,* 2012). However, insufficient dopaminergic and glutaminergic transmissions in the mesocortical system may account for the negative and cognitive symptoms of the disease (Chatterjee *et al.,* 2012a). Notably, ketamine produces its psychotomimetic effects via the blocking of NMDA glutamate receptors distributed on GABAergic system, thereby causing decreased inhibitory control on NMDA-mediated mesolimbic dopamine release; resulting to behavioural disinhibition (Monte *et al.,* 2013). Moreover, other pleiotropic mechanisms of ketamine in the pathology of schizophrenia have been reported in different studies; including the involvement of oxidative stress (Vasconcelos *et al.,* 2015), nitrergic, cholinergic (Chatterjee *et al.,* 2012a) and neurotrophic (Janardhanan *et al.,* 2016) alterations, as well as upregulation of inflammatory cytokine (da Silva *et al.,* 2017).

Drugs prescribed for the treatment of schizophrenia are classified into typical and atypical antipsychotic agents (Meltzer, 2010). A large number of these antipsychotic agents inhibits or otherwise modulates neurotransmitter systems through multiplicity of mechanisms such as anti-oxidant, anti-nitrergic, neurotrophic, anti-inflammation, immunomodulation, among others, to ameliorate these symptoms (Meltzer, 2010; Vasconcelos *et al.,* 2015; Janardhanan *et al.,* 2016). However, evidences from current psychiatric therapy on the efficacy of antipsychotic drugs, particularly of typical antipsychotics, on these symptoms of the disease remain equivocal, further complicated by low adherence to treatment and extrapyramidal side effects (Meltzer, 2010). Although the atypical antipsychotic agents are capable of ameliorating all groups of symptoms, their clinical use have been largely limited by the serious metabolic adverse effects including diabetes, agranuloctosis, cardiovascular disease (Ames *et al.,* 2016). Moreover, these agents have failed to repair the underlying derangements of the disease such as oxidative/nitrergic,

neurotrophic and immune alterations, but only provide symptomatic relief that is associated with serious adverse effects (Wu *et al.,* 2015). Thus, evidence from clinical data (Ames *et al.,* 2016) have showed that significant improvement in the treatment of schizophrenic symptoms is most likely to emanate from agents with neuroprotective functions (Chen *et al.*, 2017), if possible, a flavonoid-based antipsychotic agents capable of targeting the underlying pathological culprits and could be used to improve the quality of life of schizophrenic patients (Zhang *et al.*, 2004).

1.2 MORIN

Morin or morin hydrate (C_{15} H_{10} 0_7; 2',4,3',5,7-pentahydroxyflavone) is one of the naturally occurring yellow crystalline bioactive phytochemical metabolites (flavonol), originally isolated from members of the Moraceae family (Kapoor and Kakkar, 2012); abundantly distributed in many fruits and vegetables including onion, seed weeds, mill, almond, red wine, osage orange and other Chinese herbs (Sreedharan *et al.,* 2009; Nandhakumar *et al.,* 2012). Morin has been reported to possess various pharmacological properties including antioxidant (Sreedharan *et al.,* 2009), antiinflammatory (Galvez *et al.,* 2001), chemoprotective (Li *et al.,* 2016), and anticancer (Hyun *et al.,* 2015). Supportive evidence showed that morin prevented oxidative damages in different human cell lines including erythrocytes, myocytes and hypatocytes due to free radical-mediated oxidative stress, and safely modulates cancer cell biology (Zhang *et al.,* 2018). Notably, studies have shown that morin demonstrate chemopreventive and immunomodulatory activities on chemically induced cancer of the tongue in rats (Li *et al.,* 2016), decreases the proliferation of human leukemia cells through inhibition of cell cycle via a mitochondria-dependent apoptotic mechanism (Zhang *et al.,* 2009; Li *et al.*, 2016); suggesting the possible in vitro and in vivo anti-cancer property of morin against neoplastic diseases (Zhang *et al.,* 2018). However, the pharmacological investigation of morin as novel therapeutic molecule for the treatment of neuropsychiatric disease such as schizophrenia stems from the finding of previous studies showing that morin demonstrated neuroprotective property in some neurological diseases associated with oxidative and immune imbalance (Gottlieb *et al.,* 2006; Chen *et al.,* 2017). For example, morin has been shown to attenuate behavioural hyperactivity, lowered [Ca2+]i induced excitotoxic insults and decreased neuronal death via mechanisms related to inhibition of production of reactive oxygen species (ROS) partially due to continuous glutamate receptor stimulation (Gottlieb *et al.,* 2006; Chen *et al.,* 2017). This interest raises the exciting possibility that morin could be a beneficial compound as an effective and safer neuroleptic agent against

schizophrenic disease. Besides, literature search revealed that the severities of schizophrenia-like behaviours are relatively based on the levels of the antioxidant defense and immune systems (Tsai *et al.,* 2013; Monji *et al.,* 2013).

1.3 JUSTIFICATION OF STUDY

Increasing lines of evidences have reportedly shown that neuroactive flavonols are positive modulators of central GABAergic neurotransmissions (Marder and Paladini, 2002; Wang *et al.,* 2005), one of the pathways implicated in the pathophysiology of schizophrenia thus, these neuroactive flavonol may induce sedative effects against behavioural disinhibition associated with schizophrenia (Gonzalez-Burgos and Lewis, 2008). Moreover, drugs enhancing the function of alpha-2 subunit containing $GABA_A$ receptors such as benzodiazepine-like drugs (Wolkowitz and Pickar, 1991; Nunes *et al.,* 2012), are currently being sought to reduce downstream glutamate release (Lewis *et al.,* 2008). Specifically, benzodiazepines have been reported to potentiate the action of antipsychotic drugs in resistant schizophrenia via decreased presynaptic dopamine release at the sub-cortical level and delayed postsynaptic adaptation of dopaminergic neurons to depolarization block by antipsychotics (Włodarczyk *et al.,* 2017). Also, benzodiazepines act on cortical regions where antipsychotics are less effective (Włodarczyk *et al.,* 2017). Accordingly, both naturally occurring and synthetic flavonols have been shown to bind to $GABA_A$ receptor with high affinity and to exert anxiolytic-like, sedative, and tranquilizing effects in rodents (Marder and Paladini 2002; Wang *et al.,* 2005). However, because of the increasing evidence on the diversity of $GABA_A$ receptor subtypes and its implication in the pathophysiology of behavioural hyperactive disorder such as schizophrenia (GABAergic hypothesis), increasing body of evidence showed that the number of cloned receptors of specific subunits has increased significantly (Wang *et al.,* 2005). Moreover, investigations of some naturally occurring and synthetic bioactive flavonols suggest that they enhance GABAergic neurotransmissions to alleviate certain behavioural hyperactivity (Wolfman *et al.,* 1994; Campbell *et al.,* 2004; Hall *et al.,* 2005). However, no data exist on the neuroactive action of morin on neuropsychiatric disorder such as schizophrenia. To this end, this study was designed to evaluate the antipsychotic-like effect of morin and to elucidate the possible mechanisms of action of the antipsychotic property in animal models predictive of human psychosis.

1.4 GENERAL AIM OF THE STUDY

The aim of the study was to evaluate the antipsychotic property of morin and its possible underlying mechanisms of action in experimental animal models.

1.5. SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of the study are to:

1. Evaluate the antipsychotic-like activity of morin on schizophrenia-like behavioural phenotypes induced by apomorphine and ketamine models of psychosis through:
    - Apomorphine-induced stereotypy (Positive symptom)
    - Ketamine-induced hyperactivity and stereotypy (Positive symptom)
    - Ketamine-induced social withdrawal (Negative symptom)
    - Ketamine-induced behavioural despair in forced swim test (FST) (Negative symptom)
    - Ketamine-induced memory impairments (Cognitive symptom)
    - Ketamine-induced deficit in social recognition memory (Negative-cognitive symptom)
2. Evaluate the acute and chronic effects of morin on catatonic and cataleptogenic through:
    - Wood block catalepsy test
    - Rota-rod test
3. Investigate the modulatory effect of morin on ketamine-induced oxidative and nitrergic alterations in the whole and specific brain regions including striatum (ST), prefrontal cortex (PFC) and hippocampus (HC) through the following spectrophotometric assays:
    - Superoxide dismutase and Catalase activity
    - Glutathione concentration
    - Nitric oxide estimation
    - Lipid peroxidation (malondialdehyde) level
    - Nicotinamide adenine denucluetide phosphate (NADPH) oxidase-2 (Nox-2) expression using immunohistochemistry
4. Investigate the effect of morin on monoaminergic modulations in relation to its effects on positive, negative and cognitive symptoms of schizophrenia through:

- Neurotransmitter receptor interaction studies with dopaminergic, GABAergic, adrenergic, serotonergic, cholinergic and nitrergic antagonists in open field, Y-maze and forced swim test tests
- Estimation of brain neurotransmitters [dopamine, glutamate and 5-hydroxytryptamine concentrations and glutamic acid decarboxylase-67 (GAD_{67}) expression (the enzyme for GABA synthesis)] in the ST, PFC and HC on ketamine-induced neurochemical alterations using enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry respectively

5. Determine the anti-neuroinflammatory effects of morin on LPS-ketamine-induced neuroimmune activation in the ST, PFC and HC through the following assays:

- Antioxidant and pro-oxidant (SOD, CAT, GSH, MDA, NO) spectrophotometric assays
- Myeloperoxidase (MPO) using spectrophotometric assay
- Interleukin-6 (IL-6) using ELISA
- Tumor necrosis-alpha (TNF-α) using ELISA
- Cyclooxygenase-1 (COX-2) using immunohistochemistry
- Inducible nitric oxide synthase (iNOS) using immunohistochemistry
- Nuclear factor kappa-B (NF-kB) using immunohistochemistry

6. Estimation of the effects of morin on ketamine- and LPS-induced neuronal/cellular alterations through:

- Histological and histomorphological evaluation of brain regions namely ST, PFC and HC and ST
- Assessment of Brain Derived Neurotrophic Factor (BDNF) in the ST, PFC and HC using immunohistochemistry
- Microanatomical study of the soma size and dendritic spine density of the pyramidal neurons of prefrontal cortex using Golgi silver impregnation stain.

1.6 SIGNIFICANCE OF THE STUDY

The significance of this study is the need to develop a new antipsychotic agent with higher potency particularly against negative and cognitive symptoms acting through a neuroprotective / neurorestorative compensatory mechanisms, with little or no cataleptogenic effects.

CHAPTER TWO
2.0. LITERATURE REVIEW

2.1.0. BASIC FEATURES OF PSYCHOSIS

Psychosis (e.g., schizophrenia) is a mental disorder often characterized by abnormal social behaviour and distorted or non-existent sense of reality (Thomas and Insel, 2010). The manifestation of a robust of schizophrenia disease generally begins during late adolescence or early adulthood (Zhu *et al.*, 2014). The disorder consists of groups of conlicting symptoms, divided into three classes including positive, negative and cognitive symptoms (Rollins *et al.*, 2010). Accordingly, these conflicting groups of symptoms have generated considerable debates whether schizophrenia is a single mental disease or group of diseases with related syptoms. Besides, schizophrenia originates from the Greek words, Skhizein, which means 'to split' and Phren meaning 'mind', however, it does not denotes a 'single or 'mixed personality disease, as confused in public perception (van Os and Reininghaus, 2016). Meanwhile, the disease represents 'splitting of mental performance' thus, reflecting a state of behavioural response impairments (Baucum and Don, 2006). Individuals with psychoses are usually more prone to suicidal tendencies, depression, anxiety, aggression, substance abuse, cognitive impairment, oxidative stress, victimization, poverty and increased medical complications, as well as increased emotional stress to parents of schizophrenia patients (Baldessarini, 2001; Ames *et al.*, 2016).

2.1.1. CLINICAL SYMPTOMS OF SCHIZOPHRENIA

According to Rollins *et al.* (2010), symptoms of schizophrenia are basically classified into three main classes of symptoms: positive, negative and cognitive symptoms. The positive symptoms are noted by an excess or alteration of normal behaviour or cognition (e.g., hallucinations and delusions), and are usually distressing experience for the patient" and often associated with hyperactivity (Rollins *et al.*, 2010). These behavioural symptoms are often episodic and the rapid onset contributes to the poor quality of life and social outputs (Green *et al.*, 2000). The major positive symptoms that individuals present with are hallucinations, delusions, disorganized speech and thought, agitation or paranoia (Tandon *et al.*, 2009). Hallucination which involves the indistinct of voices is usually due to the patient's inability to discriminate between external stimuli and inner mental states. It is characterized by a marital state, in which the patient is no longer aware of his own thoughts and purpose. Thus, the volitional power of the individual is

grossly impaired (Baldessarini, 2001). Delusions or false beliefs are attempts by the patient to deal with confusion of reality (Davis *et al.,* 1991; Allan and Robert, 2005). Delusions may be bizarre (e.g., that the patient can control thoughts), but are most commonly persecutory (e.g., imagining that people are trying to harm them), with persecutory delusions occurring in about 64% of patients (APA, 2013). Also common with this disease as positive symptoms are: thought insertion, thought withdrawal, thought broad casting (Davis *et al.,* 1991). Other positive symptoms include stereotyped behaviour, which is reflected as continuous, ritualistic, and functionless motor activity (Morrens *et al.,* 2006). However, the positive symptoms generally respond well to medication (APA, 2000).

The negative symptoms of schizophrenia are impairment of normal emotional responses and normal human qualities, and response to medications remained equivocal (leifker *et al.*, 2009). They usually include flat affect or poor emotion, avolition or abulia (lack of motivation or drive), alogia (poverty of speech), anhedonia (lack of pleasure), social withdrawal and behavioural preservation (Rollins *et al.*, 2010). These symptoms are likely to be stable and continue for the course of the illness (Rollins *et al.,* 2010). Negative symptoms are known to largely mediate more to low quality of life and functional deficits (Leifker *et al.,* 2009). Persons with higher negative symptoms usually possess history of weak motivation before the manifestation of disease, and the effects of treatments is often poor (Rollins *et al.,* 2010). Furthermore, cognitive symptoms usually involving alterations in executive functions, spatial and non spatial memory deficits, and poor attention filtering ability, can be ongoing (Combs and Muester, 2007). These symptoms play a significant role in the degree to which everyday tasks become too difficult for the individual (Leifker *et al.,* 2009). To this end, schizophrenia is associated with arrays of behavioural symptoms accompanied with memory impairments that ofter undermine foundamental human belief and judgement (Leifker *et al.,* 2009).

Increasing body of evidence shows the relevance of negative and cognitive symptoms in the behavioural phenotype of schizophrenia due to the clinical inefficiency of currently available antipsychotic agents in improving these alleviating these symptoms (Meltzer, 2010; Monte *et al.,* 2013). Both negative and cognitive symptoms represent core intrinsic features of the disorder and plays prominent contribution to the functional deficits observed in the day to day activity of schizophrenia patients (APA, 2013). However, negative and cognitive symptoms are often preceded by the manifestation of robust schizophrenia-like episodes and continue in the presence of antipsychotic medications (Leifker *et al.,* 2009; APA, 2013).

2.1.2. SUBTYPES OF SCHIZOPHRENIA

According to the Diagnostic and Statistical Manual of Mental Disorders-4 (DSM-IV), schizophrenia can be classified into the following sub-types, although the developers of DSM-V published May 18, 2013 are recommending they be dropped from the new classification, and that schizophrenia should be seened as one mental disorder with an array of different symptoms (APA, 2000), due to "limited diagnostic stability, low reliability and poor validity" (Grohol and John, 2013) (Figure 2.1).

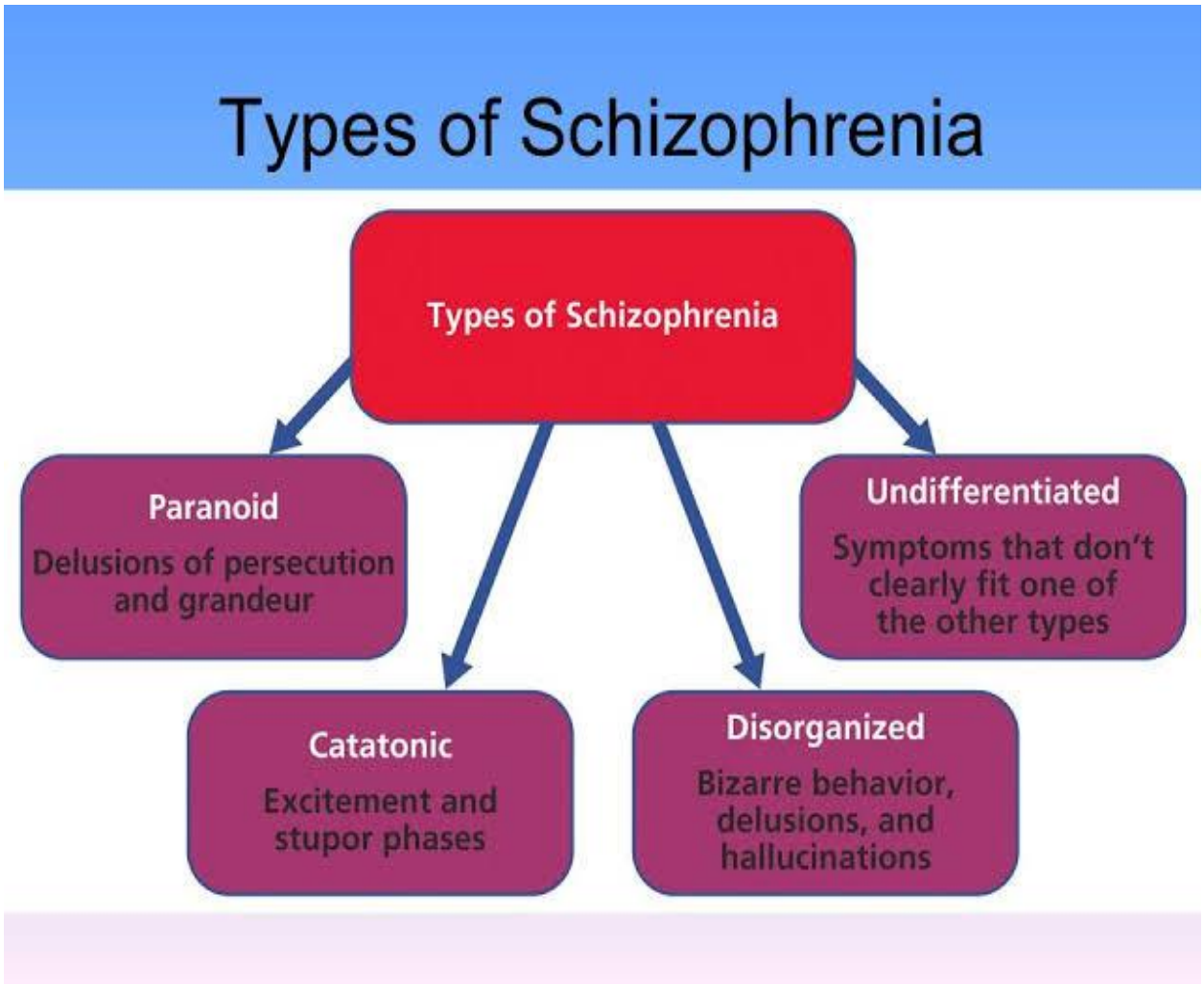


Figure 2.1: Diagrammatic grouping of the different types of schizophrenia (American Psychiatric Association, 2013**).**

2.1.3. EPIDEMIOLOGICAL PROFILE OF SCHIZOPHRENIA

Schizophrenia is among the most devastating and economically expensive medical disorders, ranked by the World Health Organization as one of the top ten illnesses contributing to the global burden of mental disease (APA, 2013; Altamura *et al.,* 2014). Moreover, earlier studies on prevalence suggest that at any given time, the disability adjusted life years (DALYs) of schizophrenia affects about 1% of the World population (McGrath *et al.,* 2015; van Os and Reininghaus, 2016). Despite the believe that schizophrenia occurs at equal rates World-wide, its epidemiological profile varies across the world (APA, 2013), with countries, and at the local and neighborhood levels (McGrath *et al.,* 2015); with higher rate in most African countries compared to Europe (e.g., England) or America (e.g., U.S.A.) (McGrath *et al.,* 2015). Oceania has the highest impact of schizophrenia, followed by Middle East and East Asia, and Japan, Australia and United States showed low impact (McGrath *et al.,* 2015). The difference occurring between DALY rates arises based on variations in availability of medical treatment, as the during of mental illness may be related to higher DALY values in unmedicated relative to medicated state (van Os and Reininghaus, 2016). However, with only a few possible exceptions, the severity of the economic burden and negative impacts of the prevalence present with psychotic disorders are notably similar among all neighborhoods and cultures (Altamura *et al.,* 2014; van Os and Reininghaus, 2016).

Schizophrenia typically occurs earlier and in 1.3-1.5 folds more in males than in females (Picchioni and Murray, 2007). Males most frequently have their first episode during their early twenties, whereas with females it is usually during their late twenties to early thirties (APA, 2000) – the peak ages of onset ranges between 20-28years for males and 26-32 years for females (Cascio *et al.,* 2012). However, onset in childhood, middle-or old age is much rare (Kumra *et al.,* 2001; Kirkbride *et al.,* 2007). Meanwhile, the delayed onset of schizophrenia in women raises interesting possibility about the pleiotropic pathogenesis of the disease; thus, raising the notion about the "different form" of schizophrenia that males and females are vulnerable to (Picchioni and Murray, 2007). One possible explanation for the delayed or less occurrence of schizophrenia in female sex may be partially due to the compensatory protective role of estrogen in women against the development and severity of the disease (Kulkarni *et al.,* 2001).

2.2 PATHOGENESIS OF SCHIZOPHRENIA

Although the causes of schizophrenia are not entirely understood, various factors that include biological factors, stress, sex, ethnicity, psychosocial deficiencies, structural brain abnormalities, decreased brain antioxidant defense system (oxidative stress), and neuroimmune alterations have been linked to the illness (Monji *et al.*, 2009, 2013; da Silva *et al.*, 2017). The cause of this illness is likely multifactorial; that is multiple pathophysiological abnormalities may play a role in producing the similar but varying clinical symptoms of the disorders (Pogarell *et al.*, 2012).

2.2.1 THE GENETIC ETIOLOGY OF SCHIZOPHRENIA

Schizophrenia genetic studies have been found to be associated with multiple genes rather than a single gene (Sekar *et al.*, 2016). In fact, majority of the gene variants highly related to schizophrenia are critically linked with dopaminergic neurotransmissions, including the catechol-o methyltransferase gene (*COMT*) (gene that regulate synaptic dopamine availability), neuregulin 1 (*NRG1*) (genes that regulate neuronal migration and synaptagenesis), dystrobrevin binding protein 1 or dysbindin (DTNBP1) (gene that regulate glutamate and dopamine neurotransmissions) (Harrison and Owen, 2003; Sekar *et al.*, 2016), nicotinic neurotransmission (α7-nicotinic receptor polymorphisms) and disrupted-in-schizophrenia 1, DISC1) (gene that regulate neurogenesis and cognition) (Harrison and Owen, 2003). Several of the other genes are also critically implicated in brain developments and functions such as reelin development, or regulation of more ubiquitous brain transmitters such as glutamate or γ-aminobutyric acid (GABA) (Lewis *et al.*, 2008). Moreover, a high number of supportive evidence has stimulated high level of interest in the genetic variants in schizophrenia. However, they are less common and may likely not account for the majority of occourence of schizophrenia. Remarkably, majority of the genes that have been identified are linked to dysfunctional dopamine transmission however, the main functional importance to dopamine neurotransmission remains yet unknown (Harrison and Owen, 2003; Sekar *et al.*, 2016).

Environmental or Epigenetic Risk Factors for Schizophrenia

A high number of epigenetic risk factor play prominent role in the pathology of schizophrenia (Monji *et al.,* 2009). Factors of environmental and social differences including migration, unemployment, loss of loved ones, lack of close friends and child abuse are all implicated in the vulnerability risk for schizophrenia, which lacks by genetic risk models (Selten *et al.,* 2007). Preclinical investigation using animal models of social withdrawal and subordination (Kirkbride *et al.,* 2007) observed that these factors contribute to alteration in dopaminergic system. Also, other factors that could contribute to early life alteration in dopamine activity include infected pregnancy or obstetric complications (Cannon *et al.,* 2002). Supportive evidence from animal models revealed that abnormal developmental activity during or after pregnancy may cause prolong activity of mesostriatal dopaminergic transmission (Selten *et al.,* 2007; Baharnoori *et al.,* 2013). Moreover, prenatal exposure to infectious agents including toxins also increases mesolimbic dopamine concentrations thereby, causing behavioural disinhibition (Baharnoori *et al.,* 2013). Maternal separations have also been shown to cause prenatal and postnatal stress, and thus increase dopamine release and metabolism (Pogarell *et al.,* 2012). A number of psychoactive substances such cocaine, amphetamine etc also increase the risk of schizophrenia. This is because psychoactive ingredient of cannabis has been reported to enhance mesolimbic dopamine activity through the activation of cannabinoid receptors (Pogarell *et al.,* 2012) (Figure 2.2).

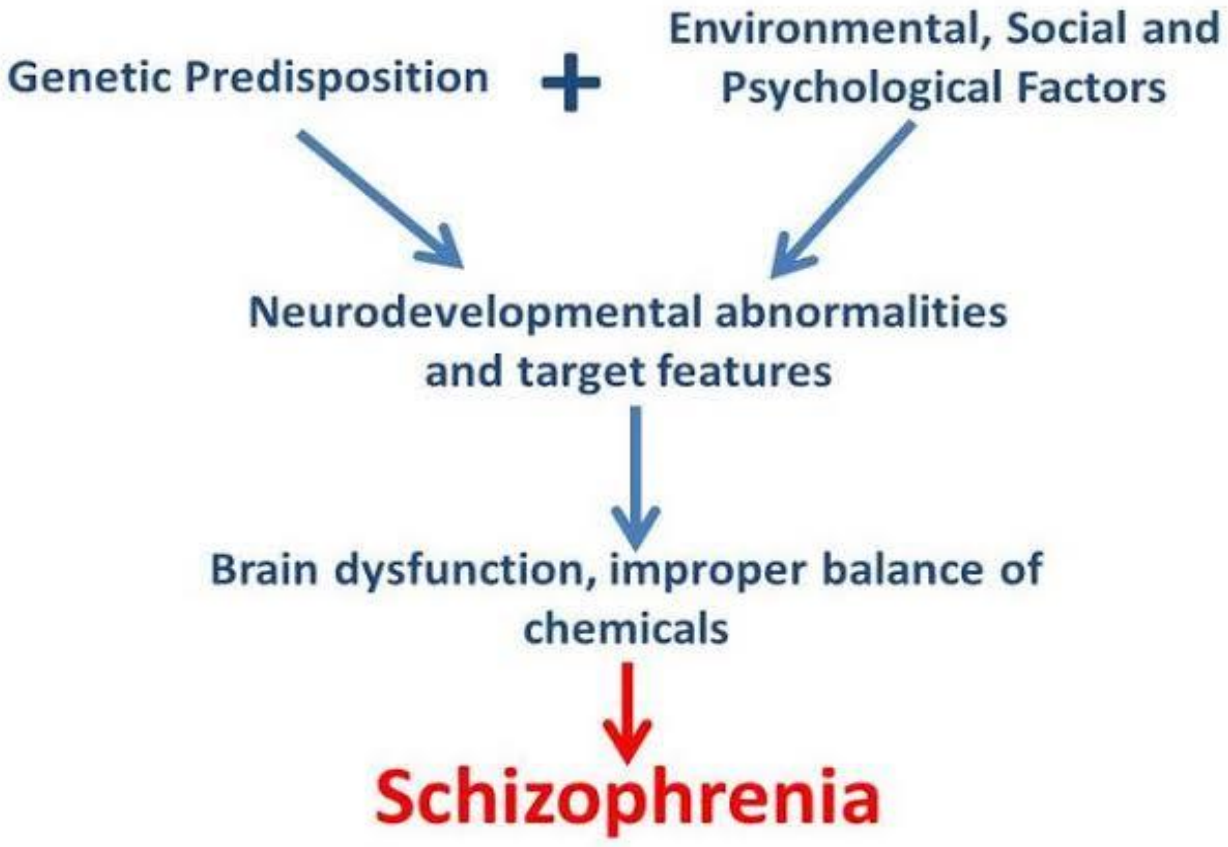


Figure 2.2: Diagrammatic relationship of the different risk factor of schizophrenia (Thomas and Inset, 2010**)**

2.2.2 NEURODEVELOPMENTAL THEORY OF SCHIZOPHRENIA

The neurodevelopmental theory of schizophrenia states that the pathological conditions that causes schizophrenia takes place in the middle stage (second trimester) of gestation prior to the manifestation of symptoms at adult stage (Lewis and Levitt, 2002). Evidence for this has been provided by the abnormal neuronal migration demonstrated in most studies of schizophrenic brains (Harding, 1992). This "schizophrenic lesson" may result in abnormalities in cell shape, position, symmetry and connectivity, and functionally to the development of abnormal brain circuits (Lewis and Levitt, 2002).

Based on this concept, pre- or perinatal animal models of schizophrenia, suggests that various obstetric complications (Cannon *et al.,* 2002) such as genetic, ischaemic, haemorrhagic conditions may cause alteration in neurodevelopmental process and neuronal prunning (Cannon *et al.,* 2002). Meanwhile, these effects which are prominently associated with histomorphological changes and perhaps, gliosis are have been revealed to poorly represent the molecular basis of the disease (Zornberg *et al.,* 2000).

However, a possible explanation for this mechanism of action for delayed onset of schizophrenia according to this hypothesis is the death of a large population of neurons produced in the young brain at maturity (Harding, 1992). Notably, during peak childhood neuronal development, the density of the prefrontal cortical synaptic neurons decreases by an average of 35% by adulthood. It is the process of altered selective neuronal pruning during adulthood that precipitates schizophrenia disease in early life (Zorngerg *et al.,* 2000), which therefore suggests that perinatal or postnatal errors stimulates the deranged cytoachitectural neurodevelopmental molecular and behavioural phenotypes. This is congruent with the neural studies from the magnetic resonance spectroscopy analysis in young schizophrenia patients (Stanley *et al.,* 1995).

2.2.3 NEUROCHEMICAL HYPOTHESES OF SCHIZOPHRENIA

Despite intensive research, the etiology of schizophrenia remains far from being understood. However, the evolution of biological concept for the pathogenesis of chronic mental illness was stimulated by the observations that certain psychoactive compounds modulate mood or behaviour and mimic most of the symptoms of schizophrenia (Davis *et al.,* 1991). Extensive studies have shown the involvement of these compounds in producing behavioural alterations, through the modulation of brain biogenic amine pathways (Baldessarini and Tarazi, 2001). The discovery of substances that could block the actions of these psychoactive agents, thereby alleviate the symptoms of psychosis further engenders the formulation and attraction of the formulation of the different biological hypothesis of mental illness (Baldessarini and Tarazi, 2001).

A) THE DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA:

Dopamine is produced in the substantia nigra and ventral tegmental regions of the brain, and dopamine alterations are related to schizophrenia (Davis *et al.,* 2001). Dopaminergic projections are divided into the nigrostriatal, mesolimbic and mesocortical systems. Impairments in the dopamine system result from dopamine dysfunctions in the substantia nigra, ventral tegmental region, striatum, prefrontal cortex and hippocampus (Pogarell *et al.,* 2012). Dopamine hypothesis of schizophrenia was first proposed by Van Possum (Van, 1967), attributing the symptoms of schizophrenia (like psychosis) to altered and hyperactive sub-cortical dopaminergic neurotransmissions particularly in the substantia nigra and ventral tegmentum, as well as decreased activity in the cortical brain areas (Chatterjee *et al.,* 2012a). However, the "revised dopamine hypothesis" proposes hyperactive dopamine transmission in the mesolimbic areas and hypofunctional dopamine transmission in the cortical brain areas, which is responsible for the positive, and partly responsible for the negative and cognitive symptoms of schizophrenia patients (Pogarell *et al.,* 2012) (Figure 2.3).

The dopamine hypothesis was further reinforced by the high risk of drug-induced psychosis among substances that directly increases synaptic dopamine levels including amphetamine, apomorphine, cocaine and antiparkinsonic drugs, such as L-dopa (Carlsson, 1978), and because all classical antipsychotic drugs (phenothiazines, thioxanthenes, and butyrophenones) has a similar principal mechanism for the blockade of central dopamine receptors (Davis *et al.,* 1991). Moreover, positron emission tomography (PET) study has revealed that drug-naive schizophrenic patients displayed greater straital dopamine released than controls when administered amphetamine (Davis *et al.,* 1991). Accordingly, evidence has shown that the severity of drug-induced schizophrenia in animals is directly related to the concentration of dopamine release and the blockade by antipsychotics (Davis *et al.,* 1991).

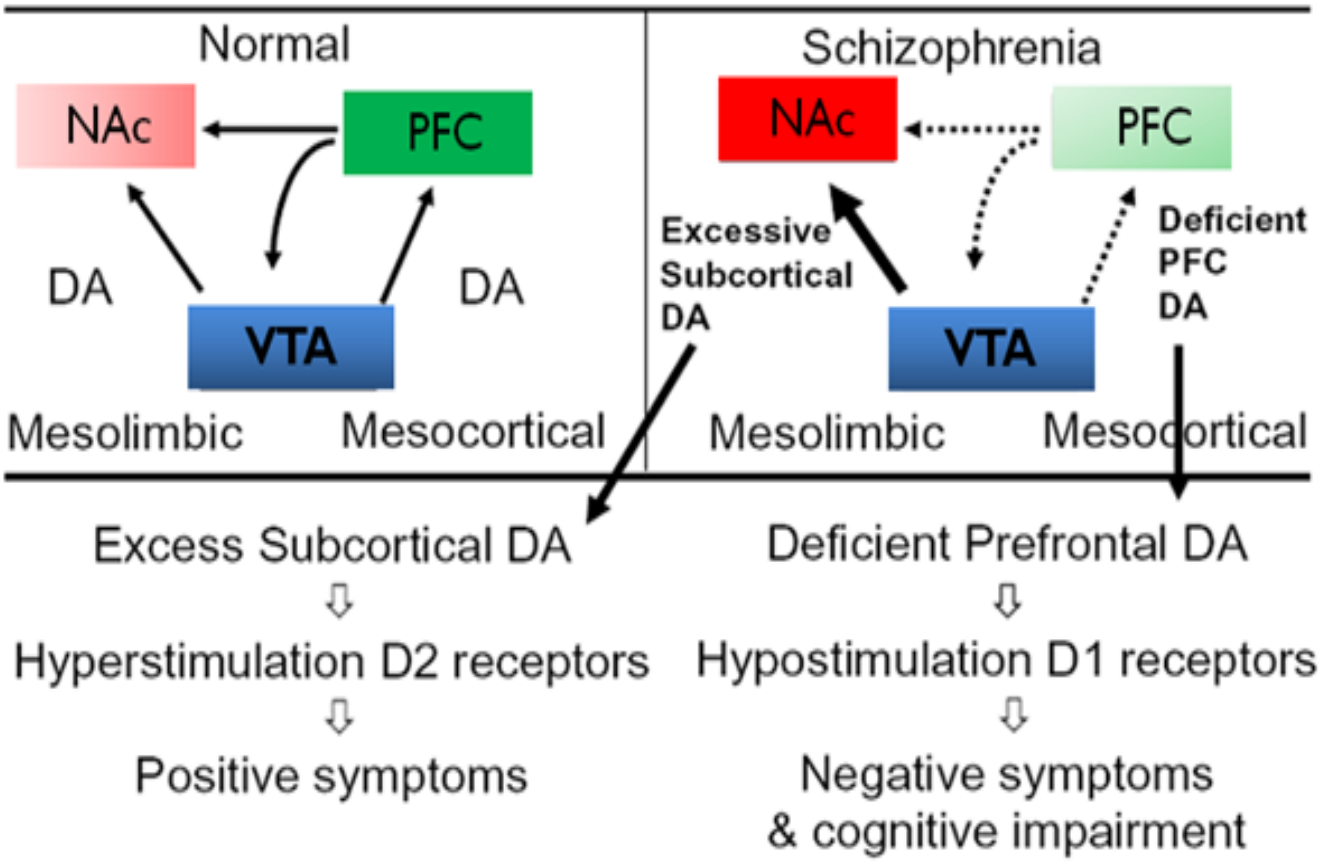


Figure 2.3: Dopamine imbalance hypothesis of schizophrenia. DA: dopamine, NAc: nucleus accumbens, PFC: prefrontal cortex, VTA: ventral tegmental area (Pogarell *et al.*, 2012**)**

B) GLUTAMATE HYPOTHESIS OF SCHIZOPHRENIA:

According to Krystal *et al.*, (1994), a current hypothesis that is receiving a great deal of interest is the glutamate hypothesis. Glutamate is the major excitatory neurotransmitter in the brain (Olney and Farber, 1995). Glutamate receptors are grouped into two main classes including ionotropic receptor [e.g., *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxyl-5-methylisoxazole-4-proprionic acid (AMPA) and kainate subtypes] and metabotropic receptors [e.g., metabotropic glutamate (mGlu) subtypes 1-8] (Krystal *et al.*, 1994). While ionotropic receptors modulates glutamatergic neurotransmission via regulation of ions, metabotropic receptors controls neurotransmissions via activation of G protein-coupled synaptic transduction mechanisms (Olney and Farber, 1995). Studies have shown that NMDA receptors which is a heterogenous complex of different subunits consisting of NR1, NR2A-D and/or NR3A-B (Javitt

and Zukin, 1991), are involved in the regulation of learning and memory, and are therefore also implicated in disease conditions associated with learning and memory (Monte *et al.,* 2013).

It has been suggested that blockade of the ionotropic receptor, NMDA is responsible for a generations of events that mimics the appearance of psychosis and schizophrenic-like symptoms in both human and experimental animals (Krystal *et al.,* 1994). This hypothesis postulates that schizophrenia is associated with decreased glutamatergic transmission in the hippocampal glutamate-mediated apparent pathways and cerebral dysfunction in the hippocampus and its target areas, especially the anterior cingulated cortex (Krystal *et al.,* 1994). This is based on the evidence that noncompetitive NMDA receptor antagonists such as phencyclidine and ketamine, produces behavioural deficits in normal humans tightly resembling schizophrenic symptomology (Javitt and Zukin, 1991). The NMDA antagonist indirectly acts to stimulate dopamine availability by decreasing the glutamate-dopamine pathway. This is based on the evidence that NMDA receptor stimulation is involved in the inhibition of mesolimbic dopamine release, but facilitates mesocortical dopamine release (Krystal *et al.,* 1994). Thus, suggesting that glutamatergic abnormalities may be involved in the aetiology of schizophrenia (Coyle *et al.,* 1996).

Moreover, support for this hypothesis includes research which has shown that several feature of the illness cannot be explained by previous hypothesis alone, such as dopamine hypothesis (Featherstone, 2008) (Figure 2.4). Since glutamatergic deficiency has been shown to interact with dopamine (Tiedtke *et al.,* 1990), the effect that antagonist of the NMDA glutamate receptor can have on this and other neurotransmitter systems may provide a useful model for explaining schizophrenic symptomatology. Moreover, ketamine infusion studies in animal and healthy volunteers demonstrate that decreases NMDA function results in a picture that more accurately encompasses the positive, negative and cognitive symptom of schizophrenia (Krystal *et al.,* 1994; Javitt and Zukin, 1991; Chindo *et al.,* 2012; Chatterjee *et al.,* 2012a).

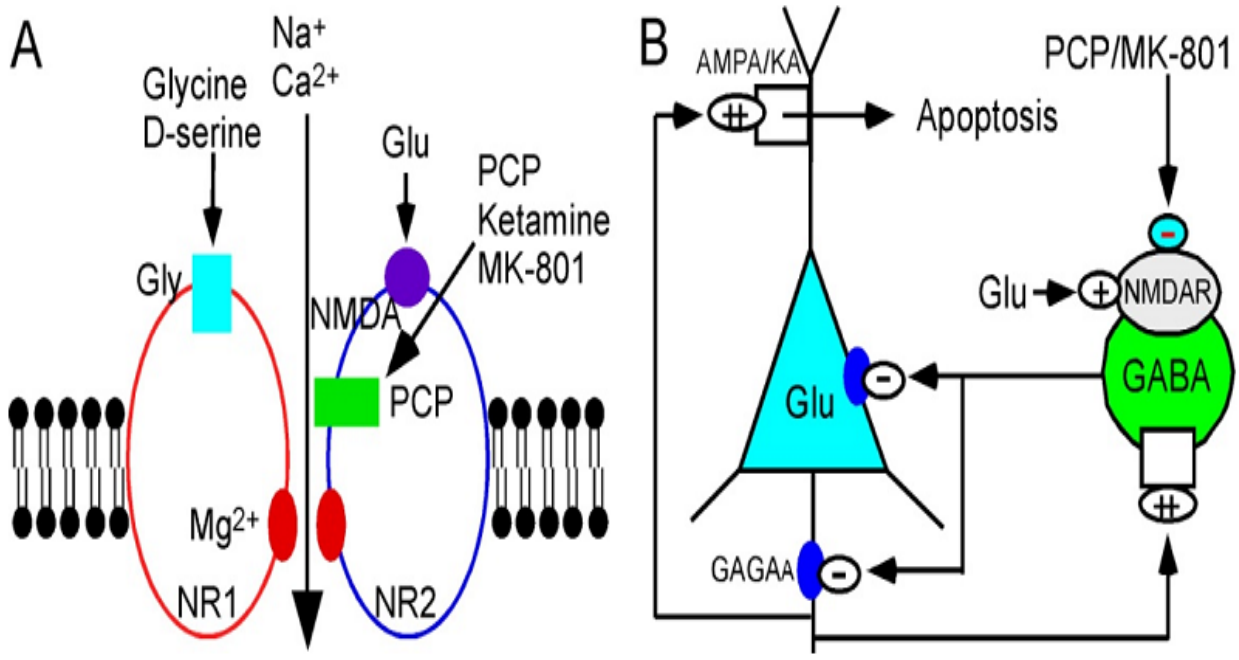


Figure 2.4: Hypothesis of NMDA receptor hypofunction: A, Schematic diagram of NMDA receptor complex. B, NMDA receptor hypoactivity and glutamate neurotoxicity. PCP/MK801 ⇒ NMDA receptor hypofunction on GABAergic neurons ⇒ disinhibition of pyramidal neurons ⇒ more glutamate release ⇒ AMPA/KA receptors excessively stimulated ⇒ excitotoxic damage (Featherstone, 2008**).**

Mechanisms of NMDA receptor internalization and its implication in schizophrenia

The NMDA receptors are located in the postsynaptic densities (PSD, cytoskeletal specializations that include the scaffolding protein complex and other signaling proteins) (Lisek *et al.*, 2017). The PSD link the receptor to kinases and phosphatases. These protein complexes play important role in intracellular trafficking and synaptic delivery of NMDA receptor (Köhr *et al.*, 1994; Lisek *et al.*, 2017). The NMDA receptor subunits NR1 and especially NR2 confer most of the biophysical and pharmacological properties to this receptor (Mouri *et al.*, 2007). One of the main mechanisms of regulation of the NMDA receptor is the balance of phosphorylation in the intracellular C-terminal domain of these subunits. While phosphorylation of the NR2B subunit of the NMDA receptor facilitates the suppression of clathrin-mediated endocytosis of these receptors, dephosphorylation of this subunit triggers NMDA receptor internalization. Controlling the phosphorylation levels of NMDA receptor signaling is an important mechanism of

glutamatergic receptor-dependent synaptic plasticity (Mouri *et al.,* 2007). Mechanisms for the rapid internalization of the NMDA receptor might explain the hypofunction of this receptor in the pathophysiology of schizophrenia (Coyle *et al.,* 1996).

As previously mentioned, the dephosphorylation of the NMDA receptor NR2 subunit may produce internalization of the NMDA receptor through a clathrin-dependent mechanism (Köhr *et al.,* 1994). For example, the binding of neuregulin-1 (NRG1) to the ERBB4 receptor produces dephosphorylation of the NR2A subunit, leading to an altered downstream signaling of the NMDA receptor (Köhr *et al.,* 1994). NRG1 is one of the four proteins of the neuregulin family that act on the family of epidermal growth factor receptors. NRG1 induces proliferation, migration, differentiation and apoptosis in different cell types during neurodevelopment (Köhr *et al.,* 1994; Mei and Xiong 2008). Another mechanism for the augmented internalization of the NMDA receptor in schizophrenia might involve the over-activation of phosphatases in the NMDA receptor downstream signaling (Mei and Xiong 2008). Serine/threonine Phosphatase PP2B, also known as calcineurin, is a neuron-enriched phosphatase that is involved in the regulation of synaptic plasticity and NMDA receptor neurotransmission (Mei and Xiong 2008). Relevant to this, PP2B dephosphorylates and activates striatal-enriched tyrosine phosphatase, which induces dephosphorylation of the NR2B subunit, promoting internalization of the NMDA receptor (Köhr *et al.,* 1994). Interestingly, calcineurin knockout mice displayed an increased locomotor activity, decreased social interaction, and impaired attention and working memory function, which further reinforces the influence of deranged glutamatergic involvement in the eatiology of the disease (Köhr *et al.,* 1994).

C) GABA HYPOTHESIS OF SCHIZOPHRENIA:

GABA is a major inhibitory neurotransmitter within the brain. Decreased GABAergic system has been proposed to be an important feature in the pathogenesis of schizophrenia (Lewis *et al.,* 1999). The hypothesis is reinforced from the alteration in GABA neurotransmission in the cortical and sub-cortical brain regions of schizophrenia patients (Lewis *et al.,* 1999; Lewis, 2000). Moreover, evidences from preclinical and clinical showing that the balance between excitatory and inhibitory systems are closely dependent on the levels of synaptic interactions between cortical and sub-cortical inhibitory controls of GABAergic neutransmission in the middle layer of prefrontal cortex against the excitatory dopaminergic terminals of the pyramidal neurons; thus, bringing a balance between excitatory and inhibitory neurotransmission (Lewis *et*

al., 1999; Lewis, 2000). In addition, the cerebral cortex generates coherent synchronized gamma (ϒ) frequency (30-80 Hz) oscillations during conscious brain activity (Didier, 2008). Alteration in cognition related coherences of ϒ oscillations between cortical areas due to alteration in cortical excitation and inhibition have also been correlated as a major functional abnormality in schizophrenic patients (Spencer *et al.,* 2010). This is because ϒ frequency oscillations essentially depends upon parvalbumin expressing GABAergic interneurons, which are involved in recurrent perisomatic inhibition of pyramidal cells, which are specifically affected in schizophrenic patients (Lewis *et al.,* 2008).

Furthermore, the number of GABAergic interneurons that express NMDA receptors has been increasingly reported to be decreased in schizophrenic patients (Lewis *et al.,* 2000, 2008). These and other findings (Olney and Farber, 1995; Didier, 2008) strongly support the hypothesis that GABAergic- and NMDA receptor-dependent reduced recurrent inhibition is involved in the pathophysiology of schizophrenia (Figure 2.4). Accordingly, evidence of poor cortical GABA uptake (Lewis *et al.,* 2000, 2008), enhanced postsynaptic binding of $GABA_A$ receptor in upper layers of cingulated cortex (Benes *et al.,* 1992), downregulation, and reduced cellular expression of glutamic acid decarboxylase 67 (GAD_{67}) messenger RNA and parvalbumin (PV) in the prefrontal cortex (Lewis *et al.,* 2008; Volk *et al.,* 2000) provides supportive evidences for GABAergic derangement in the pathophysiology of schizophrenia. Thus, relevant to this, GABAergic dysfunction may disorganize the simultaneous firing patterns of cortical neurons, which may be responsible for the information-processing, attention deficits and response inhibition impairment observed in schizophrenic patients (Lewis *et al.,* 2000, 2008; Didier, 2008).

D) SEROTONIN (5-HYDROXYTRYPTAMINE, 5-HT) HYPOTHESIS OF SCHIZOPHRENIA:

Serotonin is a monoamine neurotransmitter which has also been linked with the pathogenesis of schizophrenia (Harrison and Burnet, 1997), arising from the testimonies of pleiotropic mechanisms of action of second generation antipsychotic drugs such as risperidone, clozapine (Meltzer and Matsubara, 1989). However, the findings that lysergic acid diethylamide (LSD), a hallucinogenic drug produces visual hallucination, which is not a feature of schizophrenia limited the endophenotypic properties of the serotonergic hypothesis of this disease (Boyer, 1990). However, the findings that atypical antipsychotic agents alleviate the negative symptoms

of schizophrenia further revitalized the involvement of serotoninergic abnormalities in the pathogenesis of schizophrenia (Chatterjee *et al.,* 2012a; Chindo *et al.,* 2012). Moreover, NMDA receptor antagonists have been reportedly shown to elevate extracellular brain levels of serotonin in the prefrontal cortex (Chatterjee *et al.,* 2012a). Notably, attenuation of 5–HT_{2A}–receptor-dependent serotonergic neurotransmissions by 5–HT_{2A}–receptor antagonists such as risperidone and clozapine has been shown to contribute to the clinical efficacy of atypical antipsychotics (Meltzer, 1996; Chatterjee *et al.,* 2012a; Chindo *et al.,* 2012). Interestingly, hallucinogens are known to induce their psychobehavioural effects via modulation of 5–HT_{2A}–receptor system (Abi-Dargham *et al.,* 1997).

Although hallucinogens induce their psychobehavioural effects via modulation of 5–HT_{2A}–receptor system, evidence showed that there is relatively little evidence for the alteration of serotonergic system in the pathophysiology of the disease (Chatterjee *et al.,* 2012a). Indeed, the effect of LSD on the psychobehaviour related to schizophrenia remains elusive (Chatterjee *et al.,* 2012a). Moreover, chronic exposure of LDS to human and rodents causes behavioural tolerance. Also, hallucinogens including LSD produce visual hallucinations rather than auditory, as exhibited by schizophrenia patients (Braff and Geyer, 1980). These two limitations thus endanger the face validity of the LSD model of schizophrenia. Although apart from the existing face and predictive validities of the LSD model, the etiological validity remains limited in relation to other neurotransmitter models including dopamine and glutamate (Geyer and Moghaddam, 2002).

2.3 ANIMAL MODELS OF SCHIZOPHRENIA

Experimental paradigms used in the evaluation of psychotic behaviours comprises of both tests for specific behaviour or complete syndrome of the disease (Geyer and Moghaddam, 2002). Studies are carried out on disease conditions because of the face, construct and predictive validity of the model relatively to the clinical effects. Accordingly, psychiatric animal model is an experimental set-up used to simulate representive psychiatric clinical conditions (Geyer and Markou, 1995). Appropriately, only dependent and clinically relevant signs and symptoms are used, while others are evaluated using dependent measure based on disease progression (Geyer and Moghaddam, 2002).

2.3.1 BEHAVIOURAL PHENOTYPIC CHARACTERIZATION OF ANIMAL MODELS OF SCHIZOPHRRENIA

The validity of an animal model of psychiatric disease can be measured using several behavioural, cellular and anatomical triats (the phenotypy of the model) (Geyer and Moghaddam, 2002). Although behavioural phenotype has been the main marker of observation for schizophrenia, studies have shown that some of the behavioural indices including horizontal motor activity do not completely represent the full behaviour of the animal, rather only provides quantitative measure of the inhibitory effects on dopaminergic transmission by neuroleptics (Geyer and Moghaddam, 2002).

1) LOCOMOTOR ACTIVITY

One of the most widely used behavioural measures of drug-induced positive symptoms of schizophrenia is changes in locomotor activity (hyperlocomotion) (Chatterjee *et al.,* 2012a). Increase in locomor activity by pro-psychotic agents has been the main stay of schizophrenia studies relative to reduced locomotor activity by antipsychotic agents (Monte *et al.,* 2013). The use of hyperlocomotion as behavioural test for schizophrenia-like positive symptom is based on the observation that dopamine agonists e.g., amphetamine, increases locomotor activity as seen in schizophrenia patients and this increase in locomotor activity attenuated by antipsychotic agents including the typical and atypical antipsychotic drugs (Geyer and Moghaddam, 2002). Examples of some of the doses of psychostimulants used to induce hyperlocomotor activity in mice include: 1 mg/kg of amphetamine (Omogbiya *et al.,* 2013); 10 mg/kg of ketamine (Yamamoto *et al.,* 1997), 0.4 mg/kg for Mk-801 (Leite *et al.,* 2008) and 2 mg/kg for phencyclidine (Sams-Dodd, 1999).

2) STEREOTYPED BEHAVIOURS

Stereotyped behaviours are behaviours which manifest as repetitive, ritualistic, and functionless motor behaviours (Morrens *et al.,* 2006). Stereotypy in psychosis is a positive symptom that often presents in schizophrenia characterized of repetitive and functionless movements, postures, or utterances proposed to be resulting from the hyper-activation of the dopamine D_2 receptors in the striatum (Costall *et al.,* 1982). It has been noted that normal individuals administered with amphetamine, apomorphine, ketamine and phencyclidine exhibit stereotyped-like behaviours

such as rocking and head shaking which are similar to what patients with schizophrenia may experience (Morrens *et al.,* 2006). These symptoms are also observed in animal models of the illness, when administered with ketamine, or amphetamine at higher doses than that needed for hyperactivity (Geyer and Moghaddam, 2002). It has been shown that ketamine induces stereotyped behaviours such as circling, head movements, intermittent sniffing, chewing, intense licking when administered either acutely (Yamamoto *et al.,* 1997) or chronically (Chatterjee *et al.,* 2011).

3) AFFECTIVE FLATTERING

Affective flattening also referred to as behavioural despair, is one of the earliest negative symptoms to occur in schizophrenia disease (Porsolt *et al.,* 2010; Chindo *et al.,* 2012). The immobility induced by forced swimming has been suggested to reflect a state of despair or a lowered affective state in the rodent, which used an index of negative symptom of schizophrenia (Porsolt *et al.,* 2010, Chatterjee *et al.,* 2012a; Chindo *et al.,* 2012). Phencyclidine and ketamine increases the immobility in the forced swim test in the mouse, particularly after repeated treatment (Chatterjee, 2011; Chindo *et al.,* 2012).

4) PREPULSE INHIBITION (PPI)

Prepulse inhibition, which is also known as habituation, is the inhibition of the response to a startling impus (stimulus) followed by the occurance of a weaker impus. Abnormalities in PPI are seen in patients with schizophrenia (Gayer and Moghaddam, 2002). Accordingly, increasing lines of evidence have repeatedly indicated impaired PPI in the pathophysiology of schizophrenia partially due to altered sensorimotor gating sub-fields for motor and cognitive functions (Geyer and Markou, 1995). Animal model using a direct or indirect dopaminergic agonist models have revealed that impaired PPI activity is associated with the pathophysiology of schizophrenia (Geyer and Markou, 1995; Monte *et al.,* 2013). Accordingly, apomorphine or ketamine induced PPI have been reported to directly reflect the behavioural response impairments observed in schizophrenia patients (Gayer and Moghaddam, 2002; Monte *et al.,* 2013). However, studies have shown that the ability of antipsychotic drugs to inhibit PPI deficits induced by apomorphine, phencyclidine or ketamine in experimental animals have been suggested to be partially linked to D_2 receptor blocking activity and clinically relevant effects seen in schizophrenic patients (Geyer and Markou, 1995; Monte *et al.,* 2013).

2.3.2 PHARMACOLOGICAL MODEL OF SCHIZOPHRENIA

According to Geyer and Moghaddam, (2002), the understanding of the involvement of various neurotransmitters in the pathophysiology of schizophrenia has helped in the design of different pharmacological model of schizophrenia-like symptoms in experimental animals. It is also called drug-induced animal model and the most common animal model of schizophrenia for evaluating potential antipsychotic drugs (Bourin *et al.*, 1986). There are several established pharmacological models for assessing psychosis in experimental animals; these include the stereotype-induced behavioural paradigm, enhanced immobility in forced swim test (FST) paradigm, aggregated toxicity test paradigm, cataleptic test paradigm, ptosis-induced paradigm (Yamamoto *et al.*, 1997; Geyer and Moghaddam, 2002; Chindo *et al.*, 2012). However, there are different classes of pharmacological animal model that can be used to any of these paradigms. These include:

A) DOPAMINE-AGONIST MODELS:

The antagonisms of amphetamine-induced hyperactivity, and apomorphine- or amphetamine-induced stereotyped behaviour are well known animal models employed in the test for compounds with antipsychotic activity (Bourin *et al.*, 1986). They have been extensively exploited by researchers especially in the field of behavioural pharmacology in the detection or discovery of compounds with antipsychotic properties. The ability of antipsychotic drugs to attenuate the hyperactivity and stereotypy has being ascribed to the interference with dopaminergic system (Bourin *et al.*, 1986; Davis *et al.*, 1991). Although all classical antipsychotics inhibit amphetamine-induced hyperactivity, and apomorphine- or amphetamine-induced stereotypies, atypical antipsychotics, e.g., clozapine or rispeondone have been found to be less effective against stereotypies (O'Neill and Shaw, 1999). This finding could reflect clozapine's supposed preferential action on D_2 receptors in the limbic system. Because extra-pyramidial symptoms (EPS) are thought to result from decreased dopamine activity in the striatum, a preferential action of a novel substance against dopamine-agonist induced hyperactivity this might represent a first indicator of a lower propensity to induce EPS in patients (Leite *et al.*, 2008).

B) GLUTAMATE ANTAGONIST MODELS

The induction of NMDA receptor hypofunction and the production of behavioural deficits in healthy humans similarly to schizophrenic symptoms by noncompetive NMDA receptor antagonists including phencyclidine and ketamine have consistently supported the involvement of dysfunctional glutamatergic neurotransmission in the pathogenesis of schizophrenia (Javitt and Zukin, 1991; Krystal *et al.,* 1994). The remarkable similarity of phencyclidine-simulated behaviours with diversed arrays of symptoms (positive, negative and cognitive symptoms) associated with schizophrenia include the use of ketamine and phencyclidine in pharmacological models of schizophrenia preclinically and clinically (Krystal *et al.,* 1995; Chatterjee *et al.,* 2012a, 2012b; Monte *et al.,* 2013; da Silva *et al.,* 2017). The intriguing aspects of glutamatergic model unlike the monoamine-derived models includes the evidence that ketamine induces behavioural hyperactivity in rodents via mechanisms partially related to indirect increased dopamine release and therefore, their psychobehavioural effects are not inhibited by first generation antipsychotic agents (Gayer and Markou, 1995; Geyer and Moghaddam, 2002). Also, the behavioural disinhibition caused by ketamine in normal humans are significantly blocked by second generation antipsychotic drugs however, these effects are not blocked by the first generation drugs (Geyer and Moghaddam, 2002). Thus, suggesting that the effectiveness of this model in the development of antispsychotic agents with possible atypical-like mechanism of action (Gayer and Markou, 1995).

Some of the animal model of schizophrenia that can be induce pharmacologically through glutamatergic blockade using antagonists include phencyclidine- or ketamine-induced hyper-locomotion (Yamamoto *et al.,* 1997), phencyclidine- or ketamine-induced stereotyped behaviour (Yamamoto *et al.,* 1997), -social withdrawal (Sams-Dodd, 1995), -social recognition memory impairment (Gao *et al.,* 2009), -enhanced behavioural despair in forced swim test (FST) (Chindo *et al.,* 2012) and -cognitive dysfunction (Monte *et al.,* 2013).

MECHANISMS OF ACTION OF KETAMINE-INDUCED SCHIZOPHRENIA-LIKE BEHAVIOURS

Ketamine is a short acting dissociative anaesthetic agent with pharmacological effects similar to phencyclidine (Jentsch and Roth, 1999). Ketamine acts primarily by interfering with glutamatergic activity by binding non-competitively to the same receptor site as phencyclidine in the NMDA receptor in channel complex, and this action accounts for most of its effects (Anis *et al.,* 1983). However, it is known to directly interact with a variety of other sites like the voltage sensitive calcium (Ca^{2+}) channels, and opioid, monoaminergic and muscarinic receptors to varying degrees (Lahti *et al.,* 1995). Like phencyclidine, it is a non-competitive NMDA receptor antagonist that is known to be responsible for its strong psychomimetic effects in humans and rodents and also known to enahnce psychosis in schizophrenia patients (Krystal *et al.,* 1995; Jentsch and Roth, 1999). The NMDA antagonist act indirectly to stimulate dopamine availability by decreasing the glutamate-dopamine pathway. Moreover, NMDA receptor stimulation is involved in the tonic inhibition of mesolimbic dopamine release, while facilitating enhanced mesocortical dopamine release (Krystal *et al.,* 1994). This means that ketamine induces hyperdopaminergic state in the mesolimbic system and hypodopaminergic state in the mesocortical system to produce all groups of schizophrenic (positive, negative and cognitive) symptoms of the disease, respectively (Chatterjee *et al.,* 2012a).

Following acute administration, ketamine-induced behavioural (positive symptoms) effects have been partly linked to the blocking effect of NMDA receptors distributed on the GABAergic system in the limbic and sub-cortical brain structures (Duncan *et al.,* 1998; Chatterjee *et al.,* 2015). Thus, resulting to increased neuronal activity (behavioural disinhibition) via increased glutamate and dopamine outflow in the limbic striatal regions (Duncan *et al.,* 1998; Hons *et al.,* 2010), and 5-HT release in the striatum, eventually leading to constitutively active D_2 and 5-HT_2 receptors in the striatal areas translating into positive symptoms of the disease (Chatterjee *et al.,* 2012a) (Figure 2.5).

Moreover, chronic administration of ketamine induces negative symptoms, which results from increased 5-HT-dependent alterations in the cortical and sub-cortical brain areas, leading to enhanced turnover of 5-HT to 5-hydroxyindoleacetic acid (5HIAA) production (Chatterjee *et al.,* 2012a). Also, ketamine induced dopamine turnover in the striatum and cortex possibly enhances 5-HT production and hence, activation of the overall serotonergic system in the cortex

(Chatterjee *et al.,* 2012a). Since serotonin is tightly associated with dopaminergic system, the dorsal raph project serotoninergic inhibitory transmission directly to the substantial nigra, dopaminergic activity decreases in the PFC (Kaplan and Sadock, 1995). Thus, further indicating the ability to induce behavioural deficits closely associated with depressive-like behaviour of the disorder (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013). Furthermore, ketamine is known to induce memory impairment through multiple mechanisms (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013). Accordingly, ketamine acts by interfering with nicotinic cholinergic transmission (Chatterjee *et al.,* 2012a). Acetylcholine plays a prominent role in the initial formation of memory (Hasselmo, 2006). Acetylcholine via α-7nAChR regulates glutamate release which activates the NMDA receptors and activated NMDA receptors are thought to induce the influx of calcium ions by increased activation of calcium–dependent neuronal nitric oxide synthase (nNOS). This action leads to increased concentrations of nitric oxide (NO) and ultimately causing enhanced glutamate release from pre-synaptic cells through multiple mechanisms (Chatterjee *et al.,* 2012a). However, ketamine negatively modulates the nicotinic acetylcholine receptor α-7nAChR of the acetylcholine transmission, resulting in memory impairment (Coates and Flood, 2001). Specifically, the blockade of these receptors, induces increased acetylcholine outflow; thus, activating cholinesterase enzyme that partially interferes with hippocampal memory formation, down regulation of neuronal nitric oxide synthase expression responsible for the NO synthesis, as a retrograde messenger and reduction of NMDA subunit transport to membrane affecting synaptic plasticity (Chatterjee *et al.,* 2012a).

Ketamine, after repetitive administration, also causes cognitive impairment by disinhibion of excitatory circuits in the PFC via activation of superoxide-producing enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (Nox2) through a depolarization induced neuronal production of the cytokine, interleukin -6 (IL-6) (Sallmann *et al.,* 2000; Behrens *et al.,* 2008). This in turn results in high production of ROS such as superoxide (O_2) via increased IL-6/Nox2 activation (Behrens *et al.,* 2009). Thus, leading to redox inactivation of the glutamate transporter, excitatory amino acid carrier 1 and consequently diminishing cystein uptake system and GSH synthesis; as well as glutamate-induced oxidative stress in the brain (Aoyama *et al.,* 2006; Behrens *et al.,* 2008). Consequently, the increased production of ROS results in the loss of population of cortical prefrontal GABAergic phenotype of the calcium-binding protein, parvalbumin (PV) interneurons and GAD_{67} (Behrens *et al.,* 2008, 2009; Koh *et al.,* 2016).

PV is fast spiking-positive inhibitory neurons, representing 5% of central cortical neurons while GAD_{67} is the main enzyme isoform responsible for synthesizing GABA from glutamate in the brain (Benes *et al.,* 2001). PV interneurons helps in the propagation of inhibitory control (gamma oscillations), which plays important role in the control of cognitive functions and information processing around cortical brain regions (Lewis *et al.,* 2008). Accordingly, PV interneuron-induced neuronal inhibition regulates excitatory activity of pyramidal neurons as well as the control of sub-cortical and cortical-dependent higher order functions (Behrens *et al.,* 2008, 2009); which further suggests that ketamine-induced memory impairment may be associated with derangement in the neuronal integrity of cortical pyramidal cells (Gonzalez-Burgos and Lewis, 2008; Koh *et al.,* 2016). Ketamine also induces cognitive (latent learning) deficit by decreasing the phosphorylation of NRI (Ser 897) subunit of NMDA receptor, leading to decreased cellular expressions of NR1 subunit (Mouri *et al.,* 2007). Remarkably, reduced expression of NMDA receptor result in decreased influx of Ca^{2+} that is required for the rapid activation of Calcium/Calmodulin-linked protein kinase II (CaMKII) at the threonine residue (Thr286) in the PFC for cognitive function (latent learning). Thereby causing a dysfunctional prefrontal cortical NMDA-CaMKII signaling (Mouri *et al.,* 2007) that relies on decreased activity of presynaptic dopaminergic system, leading to impaired latent learning (Mouri *et al.,* 2007).

In summary (Figure 2.5), 1) ketamine enhance the outflow of dopamine in the striatum, 2) ketamine inhibits NMDA receptor in the GABAergic neurons leading to increased dopamine and 5-HT release resulting in hyperactivation of 5-HTIA and 5-HT2A receptors, reducing NMDA subunit transport to membrane affecting synaptic plasticity, 3) blockade of these receptors also increases cortical cholinesterase enzyme thus, interfering with hippocampal memory formation, 4) ketamine down-regulates neuronal nitric oxides synthase expression, a key factor in synaptic plasticity, 5) ketamine induces the production of ROS through activation of IL-6/Nox-2 pathway (Behrens *et al.,* 2008; Chatterjee *et al.,* 2012a).

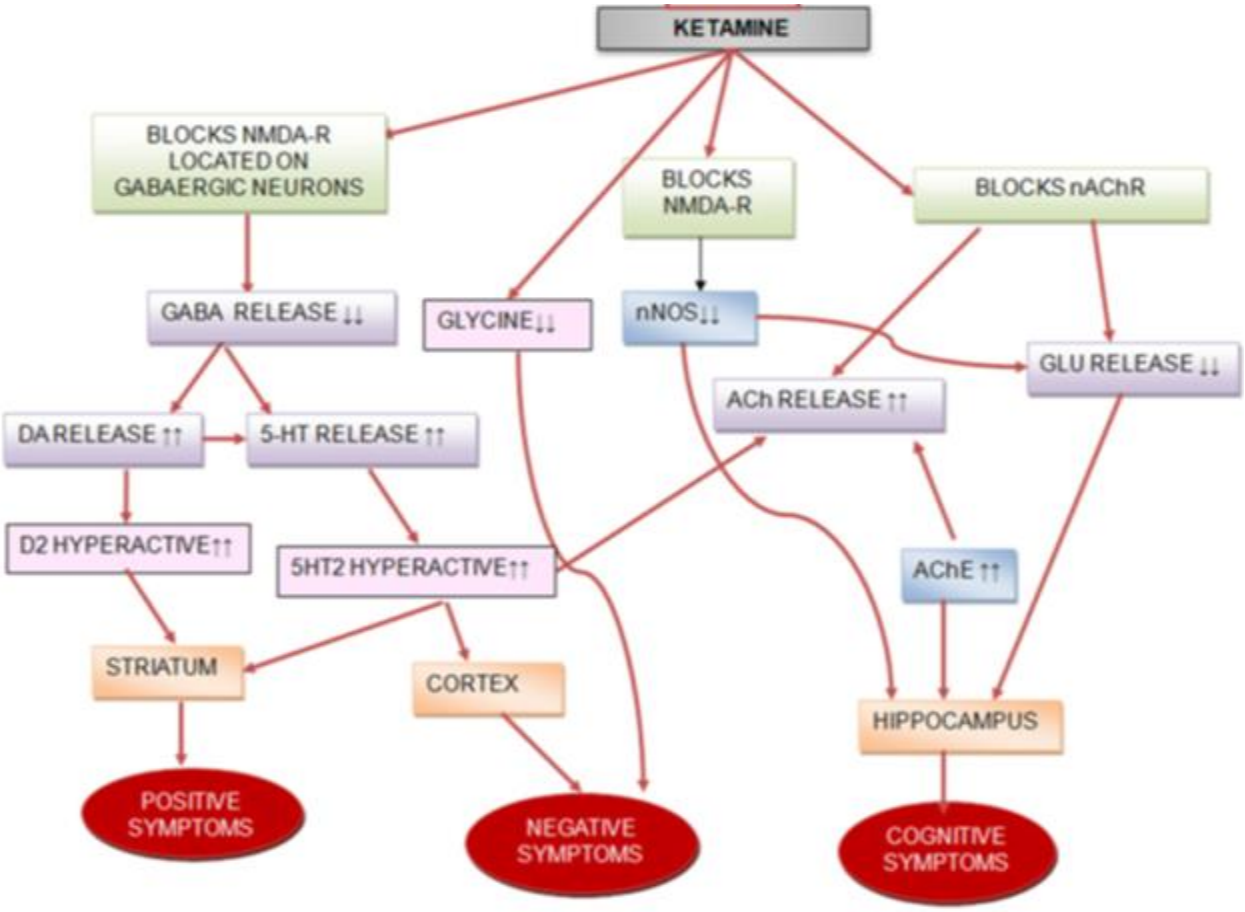


Figure 2.5: Summary of mechanism of action of ketamine in schizophrenia. nNOS = Neuronal nitric oxide synthase, Ach = Acetylcholine, GLU = Glutamate (Chatterjee *et al.*, 2012a).

2.4 TREATMENT OF SCHIZOPHRENIA

Antipsychotic drugs share many pharmacological effects and therapeutic applications (Meltzer, 2010) such as sedation which rapidly manifest early in treatment, although some tolerance typically develops (Kane and Corell, 2010). However, sedation can be of added advantage in the case of psychotic patients with severe agitation. The first generation antipsychotic drugs possess stronger sedative property than the second generation drugs (Meltzer, 2010). Meanwhile, clinically effective doses of atypical show markedly reduced extrapyramidal symptoms (EPS) risk (or nearly absent in the case of quetiapine and clozapine) compared to typical antipsychotic agents (Meltzer, 2010). Excessive D_2 blockade, as is often the case with the use of high-potency typical agents causes motor impairment (e.g. akathisia, catalepsy) and slows mentation (bradyphrenia), and interferes with central reward pathways resulting in patient complaints of ahedonia (Leucht *et al.*, 2009).

2.4.1 CLASSIFICATION OF ANTIPSYCHOTIC DRUGS

Generally, antipsychotic drugs are classified into two major groups: typical and atypical antipsychotic (Meltzer, 1996; Meltzer, 2010).

A) Typical (First-Generation) Antipsychotic Drugs

Typical antipsychotics are categorized into high and low potency, where high potency (e.g. haloperidol) allows for greater schizophrenic symptom relief but has many neurological side effects, including various forms of dyskinesia. Low potency (e.g., chlorpromazine) is less adversely effective; however they still target or possess high affinity for histaminic (H_1), muscarinic (M_1) and alpha-1 (α_1) receptors that cause many of their undesirable effects (sedation, anticholinergic properties, orthostasis (Leucht *et al.*, 2009). They are generally known to produce symptoms similar to Parkinson's disease, tardive dyskinesia, and body rigidity. With yearly exposure, studies showed an increase of 5% in likelihood of developing such adverse neuromuscular effects. With increasing age, these extrapyramidal effects increase in incidence rate (Leucht *et al.*, 2009). This group of drugs includes:

1) **Phenothiazines:** Chlorpromazine, trifluoperazine, thioridazine, fluphenazine,
2) **Butyrophenones:** Haloperidol, trifluperidol, penflurifol, droperidol,
3) **Thioxanthiness:** Chlorprothiexe, thiothixene, flupenthixol.

B) Atypical (Second Generation) Antipsychotics

They are known as atypical antipsychotics for lacking the same incidence of extrapyramidal effects as typical. The typical antipsychotics are the drug of choice in the current market and the first-line drug/treatment against schizophrenia. It is more likely that the atypical antipsychotic drugs are able to be generalized for each patient's usage, as they have several drug profiles that meet the needs of patient better than that of first generation antipsychotics (Meltzer, 2010). However, the trade-off for their high level of efficacy is the increase in metabolic effects (e.g., weight gain, precipitation of diabetes etc) as well as higher cost of production (Kane and Corell, 2010). But, the lesser severity of adverse effects neurologically prompts patients to continue their treatment, with less resulting in relapse (Leucht *et al.,* 2009). They include

1) **Bensizisoxazole:** Risperidone, **4) Thionobenzodiazepam:** Olanzapine
2) **Dibenzodiazephines:** Clozapine **5) Dibenzothiazepine:** Quetiapine
3) **Dihydrocarbostyril:** Aripiprazole, **6) Dibenzoxazepine:** Lozapine

2.4.2 MECHANISMS OF ACTION OF ANTIPSYCHOTICS DRUGS

Despite the current involvement of glutamatergic and cholinergic systems in the antipsychotic effects of new neuroleptic drugs, majority of the clinically effective drugs acts via reduction of dopaminergic neurotransmission (Kane and Corell, 2010). This is because all antipsychotic agents mediate their actions by blocking mainly D_2 dopamine receptor (e.g. haloperidol) or by blocking preferentially D_2 dopamine receptor (Goodman and Gilman, 2012). Although the ability of these drugs to treat all groups of symptoms depends on the blockade of other receptors such as 5-hydroxytryptamine (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2c}), cholinergic (7-αnAch), histamine (H_1) and α–adronoceptors (α_1 and α_2 adrenergic) and muscarinic (M_1), the potency of antipsychotic agents has shown good correlation with their capacity to bind to or block D_2 receptor (Meltzer,

2010; Goodman and Gilman, 2012). Phenothiazines and thioxanthenes also block D_1, D_3 and D_4 receptors but still there is no correlation with antipsychotic potency. Some may show selectivity actions (e.g., the typical have preference for D_2 receptors over those of D_1; while some of the newer agents like sulpiride exhibit high affinity for D_2 receptor) (Goodman and Gilman, 2012). Still, there are some that show equi-affinity for both D_2 as well as 5-HT_2 receptors (clozapine, risperidone, sertidone, and ziprasidone). However, aripiprazole remain the only current drug with D_2 antagonistic and partial D_2 agonistic effect (Baldessarini and Tarazi, 2001).

The reduction in dopaminergic neurotransmission is presently achieved through one of two mechanisms: D_2 antagonism or partial D_2 agonism (Goodman and Gilman, 2012). Blockade of dopaminergic projections to the temporal and prefrontal areas constituting the 'limbic system' and in mesocortical areas respectively, is probably responsible for the antipsychotic action (Leuch *et al.*, 2009). Meanwhile, increasing prefrontal cortical (PFC) dopaminergic activity has been positively correlated to amelioration of negative and cognitive symptoms (Meltzer, 2010). For example, aripiprazole's capacity to stimulate D_2 receptors in brain areas where synaptic dopamine levels are limited (e.g., cortical areas) or reduce dopamine outflow in hyperdopaminergic neurons (e.g., mesolimbic cortex) is thought to be the basis for its clinical effects in schizophrenia (Goodman and Gilman, 2012). However, the reciprocal blockade of striatal D_2 dopamine receptor is responsible for the genesis of extrapyramidal side effects produced by chlorpromazine and related drugs (Kane and Corell, 2010). The low tendency of atypical compounds to cause little or no extrapyramidal side effect is due to their preferential blockade of D_2 dopamine receptors (Leuch *et al.*, 2009; Kane and Corell, 2010) and affinity for 5-HT_2, M_1 and α_1 blocking action, and some are relatively selective for D_4 receptors (Kane and Corell, 2010). Thus, antipsychotic property may depend on a specific profile of action of the drugs on several neurotransmitter receptors (Baldessarini and Tarazi, 2001; Leuch *et al.*, 2009).

2.4.3 PHARMACOLOGICAL PROPERTIES AND ADVERSE EFFECTS OF ANTIPSYCHOTICS

In a psychotic patient, antipsychotic drugs reduce psychotic behaviours associated with positive symptoms as well as ameliorate negative and cognitive symptoms. Specifically, altered thought processes, hallucination, delirium and hightnened fear syndromes related to the positive symptoms are slowly ameliorated. Behavioural disinhibition such as stereotypy and hyperactivity are also attenuated gradually (Goodman and Gilman, 2012). Majority of the first generation antipsychotic drugs including phenothiazines, butyrophenones and thioxanthenes produce similar effects at relatively different doses. However, other first generation drugs such as aliphatic and piperidine produces mild clinical effects but associated with stronger sedation and synergistically potentiates the effects of sedatives and opioids (Kane and Corell, 2010; Goodman and Gilman, 2012).

Extrapyramidal Symptoms (EPSs) is the most serious adverse effect in terms of frequency and reason for patients' non-compliance of antipsychotic drug therapy (Kane and Corell, 2010). Three (3) categories of EPSs usually manifest: dystonic reaction, pseudo-parkinsonism and akathisia. Dystonic Reaction include oculogyric crisis i.e., fixed upward gaze; torticollis i.e., neck twisting; opisthotomus i.e., aching of back; Pseudo-parkinsonism include akinesia-rigidity and immobility, stiffness and slowness, stooped posture, shuffling gail, slow monotonous speech and tremo-regular rhythmic oscillations of extremities especially hand and fingers. Akathisia is manifested as the inability to sit still, constant pacing continuous agitation of restless movement, rocking shifting of waist while standing; sifting of leg (Kane and Correll, 2010). While typical antipsychotic agents have been associated with EPSs, atypical antipsychotics have been found to produced serious metabolic adverse effect such as diabetics, obesity, agranulocytosis, cardiovascular disease like hypertention etc (Meltzer, 2010; Wu *et al.,* 2015; Ames *et al.,* 2016).

2.5 OXIDATIVE STRESS IN SCHIZOPHRENIA

Oxidative stress is the condition arising from imbalance between toxic ROS and antioxidants (Halliwell *et al.,* 2006; Wood *et al.,* 2009). The brain produces increased loads of ROS due to its high levels of metabolic activity (Wood *et al.,* 2009). It is a very active organ of human body weighting only 2% of the body weight but it consumes 20% of body oxygen and 25% of body glucose at rest (Clark and Sokoloff, 1999). Since reactive oxygen species (ROS) produced in any tissue is directly proportional to its oxygen consumption which further increases with intellectual process like thinking, planning and reasoning (Ikonomidou and Kaindi, 2011), the brain is continuously under oxidation/antioxidant process which makes it prone to oxidative damage (Wood *et al.,* 2009). This vulnerability is further enhanced by certain factors including volume neurotransmission and reduction-oxidation (redox) property of dopamine, and redox regulated activity at glutamate receptors (Bókkon and Antal, 2011).

Although a clear mechanism underlying the pathogenesis of schizophrenia remains unknown, oxidative stress as a consequence of aberrant redox control has become an attractive hypothesis for explaining, at least in part, the pathophysiology of schizophrenia (Ng *et al.,* 2008; Do *et al.,* 2009; Zhang *et al.,* 2010). This hypothesis has theoretical appeal, as the brain is considered particularly vulnerable to oxidative damage for several reasons. These include high oxygen consumption, high metabolic acid activity, low levels of protective antioxidant enzymes, a high ratio of membrane surface area to cytoplasmic volume, neuronal anatomical network vulnerable to disruption, high proportion of readily oxidizable membrane polyunsaturated fatty acids (PUFAs), and auto-oxidizable neurotransmitters like dopamine, adrenaline and noradrenaline (Halliwell, 2006; Zhang *et al.,* 2010). Also, the brain is vulnerable to the neurotoxic effects of released excitatory amines (mainly glutamate) and iron, and the activated inflammatory response (Halliwell, 2006). In fact, the increasing body of evidence supports the claim that severity of symptoms of schizophrenia is dependent on the antioxidant levels (Pavlovic *et al.,* 2002). According to Pazvantoglu *et al.,* (2009), it was demonstrated that the severity of symptoms was associated with the decreased antioxidant levels. Moreover, various antioxidant systems have been found to be related to positive, negative and cognitive symptoms, poor premorbid functions and computed tomography abnormalities, because oxidative stress is a strong cellular pathways that alters dopaminergic neurotransmission via changes in D_2 receptor activity (Pazvantoglu *et al.,* 2009) (Figure 2.6).

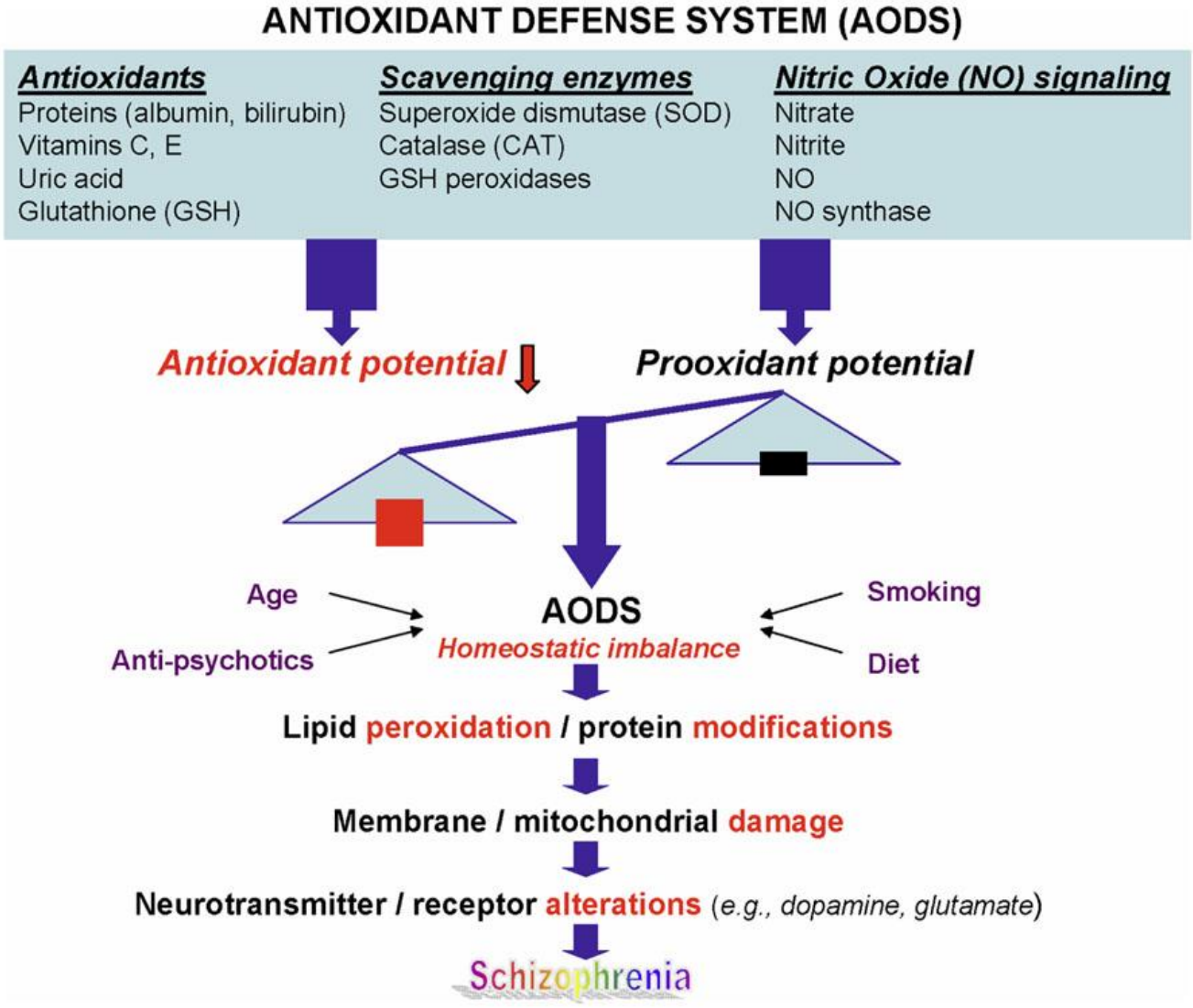


Figure 2.6: Oxidative stress in processes of schizophrenia development (Ravindra *et al.*, 2004**)**

2.5.1 REACTIVE SPECIES OF FREE RADICALS

In every living tissue, biological metabolism is one major source of free radical production. There are different types of reactive species that evolve from biological metabolism. The commonly but dangerous reactive metabolite include reactive oxygen, nitrogen and iron species (ROS, RNS and RIS) (Ravindra *et al.,* 2004; Selva *et al.,* 2011).

A) SUPEROXIDE ANION

This is a potentially toxic unreactive free radical produced majorly in the mitochondria electron transport system (Bókkon and Antal, 2011). Although the superoxide anion is partially stored in the active site of the cytochrome oxidase, other parts of the mitochondria respiratory system readily transports superoxide anions out of their active sites and do not retain them inside the mitochondria (Ravindra *et al.,* 2004). The newly formed anion products may be a product of several enzymatic reactions, as a flavoprotein reaction (xanthine oxidase, aldehyde oxidase, purine oxidase), oxidase and hydroxylase, and they are also involve in non-enzymatic reaction with glutathione (Selva *et al.,* 2011) or riboflavin, as happen in the mitochondria respiratory chain. Superoxide anions are also be produced in the internal structure of the mitochondria via the break down of semiquinones by-product of dopamine (Ravindra *et al.,* 2004).

B) HYDROGEN PEROXIDES (H_2O_2)

This is a reactive oxygen species that is not a free radical, however may easily diffuse through the membranes to more reactive species (Selva *et al.,* 2011). Hydrogen hydroxide can be formed from biological metabolism via a removal of two electrons from oxygen and through the break down of superoxide anion by superoxide dismutase (Ravindra *et al.,* 2004). Other enzyme source of hydrogen peroxide includes xanthine oxide reductase (Selva *et al.,* 2011). Also, another important source of hygrogen peroxide is the autoxidization of neurotransmitters such as dopamine in the sub-cortical brain regoins (Selva *et al.,* 2011).

C) HYDROXYL ($^{\cdot}OH$)

This is the most reactive species, with an average life estimated of about 10^{-9} seconds (Halliwell and Whiteman, 2004). Because of its high reactivity its chemical action is confined to the vicinity of the site of production. It can be formed in vivo as a result of high-energy radiation (x-ray, gamma ray), which can cause hemolytic breakage of water. Uv light does not have enough energy to split a water molecule but it can split oxygenated water into two molecules of hydroxyl radical (Selva *et al.,* 2011). At the biological level, the most important hydroxyl radical formation is the Fenton reaction. Hydrogen peroxide and superoxide radical can form the hydroxyl radical by the Haber-wass reaction (Halliwell, 2006).

2.5.2 ANTIOXIDANT SYSTEMS

In the presence of the oxygen, organisms have been forced to develop mechanisms for their protection against the ROS. Antioxidants are biological substances that are able to compete for oxidizable substrate and inhibit oxidation (Halliwell and Gutteridge, 2007). Antioxidant systems can be divided into enzymatic and nonenzymatic systems (Selva *et al.*, 2011). The enzymatic antioxidants include superoxide dismutase (SOD), glutathione perioxidase (GSH-Px), catalase, reduced glutathione, cytosolic glutathione-s-transperase (GST) and thioredoxin. Non-enzymatic types include vitamins, proteins, and amino acids, which are less reactive but in greater concentration in contrast to the enzymatic types, which have a high reactivity with the ROS, but are in lower concentrations (Selva *et al.,* 2011).

1) ENDOGENOUS ANTIOXIDANT SYSTEMS:

A) SUPEROXIDE DISMUTASE (SOD)

The catalytic role of the SOD was discovered by McCord and Fridovich in 1969. The SOD is an enzyme that catalyzes the reduction of the superoxide anion, which is produced in the body as the resulting product of oxidative phosphorylation, either derived from UV radiation or during inflammation, by transforming the superoxide anion into a product such as hydrogen peroxide that is metabolized easily by catalase to oxygen and water or by glutathione peroxidase to water (Selva *et al.,* 2011). The SOD is present in different forms, such as Copper-zinc SOD (Cu-Zn

SOD) and manganese SOD (Mn-SOD). The Cu-Zn SOD is found in the cytosol and the cell membrane, has a molecular mass of 32 kDa with two identical subunits. The Mn-SOD is located in the mitochondrial matrix (Halliwell and Gutteridge, 2007) and has a molecular mass of 88 kDa with four identical subunits. It acts as a first line of defense in the detoxification of the superoxide anion and seems to be involved in processes of tumor removal or cellular differentiation (Selva *et al.,* 2011).

B) GLUTATHIONE ANTIOXIDANT SYSTEM

The glutathione antioxidant system is formed by reduced glutathione, glutathione reductase (that reduces the oxidized glutathione), and glutathione peroxidase (which along with the reduced glutathione contributes to the elimination of peroxides) (Ayoama *et al.,* 2006). Glutathione (GSH) is the brain's most powerful cellular antioxidant molecule (Ng *et al.,* 2008). It consists of three sub-molecules including glutamic acid, glycine and cysteine, and changes between two oxidative states (reduced monomeric, GSH and oxidized dimeric forms, GSSG). Glutathione serves as a reducer, conjugates to drugs to make them more soluble in water, is involved in the transport of amino acids across cell membranes (Ɣ-glutamyl cycle), is a substrate for the peptide-leukotrienes, serves as a cofactor for some enzyme reactions, and as an aid in the re-organization of protein bridges and glutamate neurotransmission (Selva *et al.,* 2011). Glutathione reductase uses NADPH as the cofactor to reduce GSSG to two molecules of GSH. Therefore, the pentose phosphate pathway is important to produce the NADPH required for glutathione reductase. Glutathione peroxidase is a selenium-dependent enzyme that catalyzes the reduction of H_2O_2 or lipoperoxide (L-OOH) using the reduced glutathione (GSH) (Selva *et al.,* 2011).

C) CATALASE

Catalase (CAT) or hydrogen peroxide oxidoreductase is one of the more abundant enzymes in nature and is widely distributed in the human body. Its activity varies depending on the tissue, highest in the liver and kidney, lowest in connective tissue and the lining, and practically nonexistent in the nervous system (Rukmini *et al.,* 2004). At the cellular level it is located in the mitochondria and peroxisomes, except in erythrocytes, where it is located in the cytosol. This enzyme is a tetrameric metaloprotein of four identical subunits that are held together by noncovalent interactions. Each subunit contains a prosthetic group generated during cellular metabolism. It has two features; the catalytic and the peroxidative (Selva *et al.,* 2011).

The general reaction covers the substrate reduction taking hydrogen atoms from a donor, and the products are the reduced substrate and the oxidized donor. In the catalytic reaction, the donor is another molecule of H_2O_2. This reaction can only be accompanied by the enzyme in its tetrameric form. In the peroxidative reaction, the enzyme can be used as donors of hydrogen to methanol, ethanol, formic acid, and formaldehyde (Rukmini *et al.,* 2004). The CAT acts in the presence of high, concentrations of H_2O_2, and the GSH-Px acts at low concentrations, which shows an inverse correlation in the activity of two enzymes (Selva *et al.,* 2011).

2) EXOGENOUS ANTIOXIDANT SYSTEMS:

Vitamin C is a potent antioxidant agent which is soluble in water. In the aqueous phase, it detoxifies the actions of free radicals prior to the initiation of lipid peroxidation (Wood *et al.,* 2009). The actions of Vitamin C yields a high antioxidant level because it includes the inhibition of the formation of the superoxide radical or nitrosamines during digestion (Mayes, 1997). In addition, it is the agent that reduces the phenoxy radical formed during vitamin E activity (Chao *et al.,* 2002). Vitamins C and E are classified as antioxidant switches because they act by stopping the formation of free radical chain reactions (Bitanihirwe *et al.,* 2011). Vitamin E is a substituted lipid isoprenoid of the tocopherol family with a very potent lipid solubility antioxidant profile (Ng *et al.,* 2008). It is the most powerful lipid soluble, free radical scavenger capable inhibiting lipid peroxidation and oxidative damage of cell membrane PUFAs (Sreedharan *et al.,* 2009; Kapoor and Kakkar, 2012). The activity of vitamin E is one of the first barriers against the peroxidation of the polyunsaturated fatty acids (Do *et al.,* 2009; Zhang *et al.,* 2010). Vitamin E can be found in cereals and high quality vegetable oils (Halliwell, 1994). Furthermore, dietary flavonoids derived from plant sources otherwise known as phytochemicals, are strong antioxidant compounds (Zhang *et al.,* 2010). For example, include polyphenolic agents which are potent antioxidant products, ubiquiteously distributed in several fruits and vegetables sources (Zhang *et al.,* 2010; Kapoor and Kakkar, 2012). They prevent lipid peroxidation and other negative oxidative processes via free radical scavenging mechanisms in several human diseases including neurodegenerative disorders (Sreedharan *et al.,* 2009; Kapoor and Kakkar, 2012).

2.6 NEUROINFLAMMATION: INFLAMMATORY-RESPONSE IN SCHIZOPHRENIA

Inflammation is an important protective mechanism for protection and/or restoration of damaged tissues and a normal reaction of the immune defense system to prevent the deleterious effect of infectious agents (Selva *et al.,* 2011). When an inflammatory response exceeds the acute phase, a process of chronic inflammation is established. Chronic inflammation plays a prominent role in the aethiology of different neurological disorders such as Azheimier's disease, schizophrenia and depression (Müller and Schwarz, 2010a; Monji *et al.,* 2013). Specifically, inflammatory response of the CNS is controlled by the help of the brain's innate immune defense cells otherwise known house cleaners e.g., microglia cells, which is responsible for the house-keeping and scavenging effects of the CNS against neuroimmune pathogens (Ribeiro *et al.,* 2013).

The microglia cells, which are found in macrophages and other immune cells helps in the first-line defense system to scavenge infectious agents, damaged immune cells, plagues and pathological debris (Ransohoff and Perry, 2009). Also, microglia cells are involve in neuroprotective actions via the production of brain-derived neurotrophic factors, for the release of protective immune cells, enzymes/cytokines and pro-oxidants relevant for normal neuronal communication and synaptic activity (Ribeiro *et al.,* 2013; Zhu *et al.,* 2014). However, increased release of inflammatory enzymes/cytokines and pro-oxidant have been linked to hypermicroglia activation, which is associated with several neuropsychiatric diseases (Monji *et al.,* 2009). Moreover, reactive species modulate the release of proinflammatory cytokines including tumor necrosis factor- alpha (TNF-α), interleukine-1 beta (IL-1β), IL-6, IL-8, interferon-beta (IFN-β) and the cell-adhesion molecules, such as the adhesion intercellular–I (ICAM-1); all of which have been found in neuroinflammation (Selva *et al.,* 2011).

The excessive generation of these cytokines may result in the systemic inflammatory response syndrome (SIRS), severe tissue damage, and septic shock (Shimazu *et al.,* 1999). Among the cytokines, TNF-α is believed to be one of the early important mediators of inflammatory reaction. It is increased in some pathogenic conditions and possesses potential toxic effect that results in hypersensitivity reactions with chronic inflammation (Zhu *et al.,* 2014). Seemingly, IL-6 is a cytokine produced by a number of normal and transformed cells, and possess several neuroprogressive features (da Silva *et al.,* 2017). It plays an important role as an endogenous mediator of LPS-induced fever (Lu *et al.,* 1995). Also, IL-1 is a multifunctional cytokine that

participates in various processes including host defense, inflammation and response to injury. It is produced by many cell types, predominantly by macrophages (Monji *et al.,* 2009).

Maternal exposure to infection observed during gestation has been linked with high vulnerability of offspring developing schizophrenia during the aldult life (Brown, 2008; Monji *et al.,* 2013; Zhu *et al.,* 2014). The increased maternal cytokine-associated inflammatory response to infection has been recognized as a crucial link for increased microglia activity, as the identity of the pathogen seems irrelevant (Monji *et al.,* 2013). The mechanism linking maternal immune infection to schizophrenia is recognized following prenatal infection, thereby inducing increased release of pro-inflammatory enzymes that infiltrates the placenta and cause sustained production of IL-1β, IL-6, TNF-α and IFN-β among others, within the fetal cells (Monji *et al.,* 2013). DNA fragmentation may initiate ROS and cytokines production, particularly IFN-β (Monji *et al.,* 2009). Consequently, the alterations in basal ROS and cytokine levels could induce enhanced neuronal energy consumption rate accompanied with reduced cell turn over. Accordingly, the rate of release of these inflammatory/oxidative mediators and associated neuropathological markers determines the manifestation of the symptoms of schizophrenia (Bitanihirwe *et al.,* 2011; Monji *et al.,* 2013).

In line with this, inflammatory responses induced by increased proinflammatory cytokines for example by LPS provide a source of free radicals with the capacity to modify proteins, lipids, and nucleic acids that are potentially toxic to neurons (Carpenter *et al.,* 2007; Bitanihirwe *et al.,* 2011). LPS, an external membrane of Gram-negative bacteria, binds to the toll-like receptor 4 (TLR4) found on the surface of different immune cells of host organisms like monocytes, macrophages, neutrophils and dendritic cells to trigger the release of proinflammatory cytokines (Carpenter *et al.,* 2007; MacDowell *et al.,* 2013). Preclinical studies have shown that prenatal or postnatal treatment with LPS have been used to mimick the psychobehavioural phenotype and neuroinflammatory biology of several neuropsychiatric diseases including schizophrenia (MacDowell *et al.,* 2013; Ribeiro *et al.,* 2013; Abdel-Salam *et al.,* 2015; Jung *et al.,* 2017).

2.6.1 IMMUNE SYSTEM ABNORMALITY: IMPLICATION OF CYTOKINE-EVOKED NEUROTRANSMITTER ALTERATIONS IN SCHIZOPHRENIA

The role of activated neuroinflammation in schizophrenia has been well established and approximately linked to social and cognitive deficits. Moreover, clinical evidences have revealed a tight relationship in the severity of negative and cognitive symptoms, and increased concentrations of cytokines such as IL-1β, IL-6, TNF- α, C-reactive protein in in schizophrenic patients (Monji *et al.,* 2013; MacDowell *et al.,* 2013). Currently, evolving neuroregulatory pathways connecting the involvement of neuroinflammation in the initiation of certain negative symptoms of schizophrenia involves aberration in central metabolism of the tryptophan catabolites, kynurenic acid (KYNA), a neuropathologic NMDA endogenous receptor antagonist (Schwieler *et al.,* 2015). Notably, tryptophan is an important precursor for the synthesis of 5-HT. Decreased 5-HT synthesis has been repeatedly linked to increased diversion of tryptophan to kynurenine production by indoleamine 2,3-dioxygenase via neuroinflammatory mechansims in the pathogenesis of some neurological diseases, thus decreasing the levels of tryptophan bioavailable for 5-HT synthesis (Schwarcz *et al.,* 2012) (Figure. 2.7). Although 5-HT is one of the main neurochemicals involve in the pathogenesis of depression, it has also been implicated in the pathophysiology of schizophrenic negative and cognitive symptoms via several neuropathological mechansims including neuroinflammation due to increased release of pro-inflammatory cytokines (Monji *et al.,* 2009; Schwieler *et al.,* 2015).

Of note, pro-inflammatory cytokines have previously been reported to influence the actions of some receptors and neurotransmitters including dopamine, adrenaline and 5-HT systems (Müller and Schwarz, 2010a; Lykhmus *et al.,* 2016). Specifically, IL-1β for example, has been shown to interact with NMDA receptor, thereby affecting the central control of cortical and sub-cortical neurotransmissions of dopaminergic, 5-HTergic and glutamatergic activities. Moreover, one of the hallmarks of this disease is the hypofunctionality of the NMDA receptor and impaired glutamatergic signaling in relevant to negative and cognitive symptoms (Javitt, 2007; Neill *et al.,* 2010; Chatterjee *et al.,* 2012a). Intriguingly, KYNA has also been linked to increased levels of IL-1β and IL-6, suggesting an inflammatory component may be related to the onset and progression of schizophrenic disease (Monji *et al.,* 2013; Schwieler *et al.,* 2015). Moreover, enhanced production of KYNA and associated changes in the central kynurenine pathway has

been consistently documented in schizophrenic patients, as well as in post-mortem brains of schizophrenic patients (Schwarcz *et al.,* 2012; Schwieler *et al.,* 2015).

Furthermore, KYNA also interfers with the cholinergic system via blockade of alpha-7 nicotinic acetylcholine (α7nCh) receptor (Schwarcz *et al.,* 2012), which provides supportive evidence for involvement with cholinergic system and inflammatory pathway in cognitive symptoms of schizophrenia (Lykhmus *et al.,* 2016). In point of fact, acetylcholine has been recently described to attenuate the release of pro-inflammatory cytokines, like IL-1 or TNFα, by peritoneal monocytes and macrophages in response to bacterial endotoxin—LPS through α7nAChRs (Lykhmus *et al.,* 2016). However, the increase in acetylcholinesterase (AChE) during chronic neuroinflammation decreases acetylcholine levels thus, stimulating enhanced release of pro-inflammatory cytokines and consequently the down-regulation of α7nAChRs (Soreq, 2015). This phenomenon has been called ‘‘Cholinergic Anti-Inflammatory Pathway’’ (Lykhmus *et al.,* 2016) (Figure. 2.7).

Remarkably, repeated administration of ketamine have been found to evoke the upregulation of neuroregulatory tryptophan catabolite including KYNA via increase production of proinflammatory cytokine, IL-6 (Behrens *et al.,* 2009), and/or as a result of infectious state e.g., Lyme disease (Koola *et al.,* 2015), that increases the levels of IFN γ, TNF α or IL1 β (Kegel *et al.,* 2011). Injection of IL-6 into the rat hippocampus has been found to be associated with the induction of IDO (Kim *et al.,* 2012). Notably, a specific inhibitor of KYN aminotransferase II, which reduces brain KYNA levels, prevents ketamine-induced working memory impairments and tends to attenuate hallucinatory like behaviours in primates (Kozak *et al.,* 2014). Thus, increased levels of brain KYNA may be the link between immune activation including aberrant neurotransmissions and behaviour impairments analogous to patients with schizophrenia (Schwieler *et al.,* 2015). Indeed, the negative and cognitive symptoms of this mental disorder have been related to microglial activation and the resulting inflammatory response (Monji *et al.,* 2013).

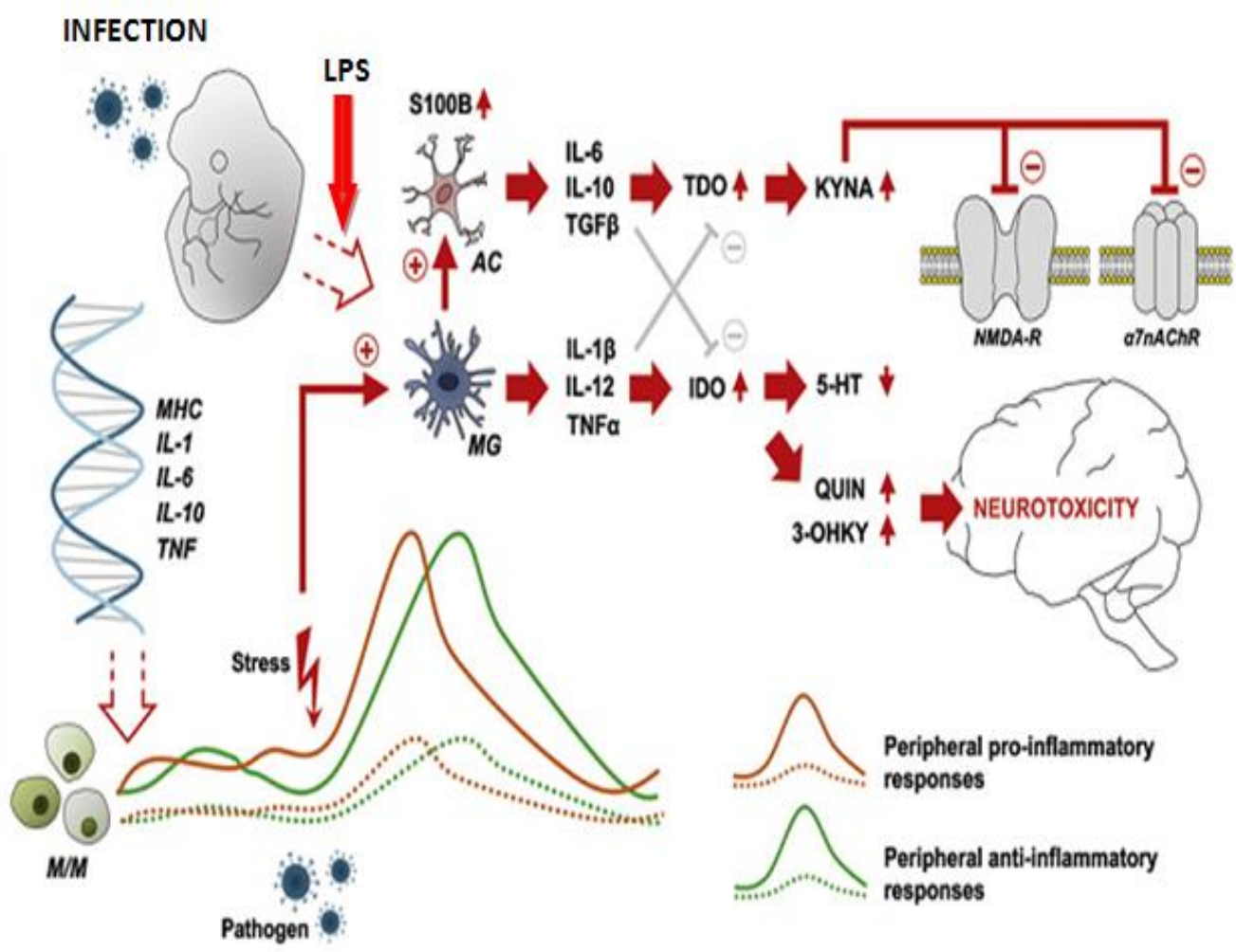


Figure 2.7: The role of activated neuroimmune pathways in the pathophysiology of schizophrenia. MM = Monocytes/Macrophages, MG = Macroglia, LPS = Lipopolysaccharide, Kynurenic acid = KYNA, TDO = Tryptophan 2,3-dioxygenase, IDO = Indoleamine 2,3-dioxygenase, QUIN = Quinolinic acid, 3-OHKY = 3-hydroxykynurenine, NMDA-R = N-methyl-D-arspatete receptor, α7nAChR = α7 nicotinic acetylcholine receptor, IL-1β, 6, -10, -12 = Interleukine-1β, 6, 10, 12, TNF-α = Tumor necrosis factor-α, Major histocompactibility complex, 5-HT = 5-hydroxytryptamine (Schwarcz *et al.*, 2012**).**

2.6.2 IMMUNOMODULATORY EFFECTS OF ANTIPSYCHOTIC DRUGS AND ANTI-INFLAMMATORY TREATMENT STRATEGIES IN SCHIZOPHRENIA

Different neuroleptic drugs produce their antipsychotic effects via modulation of neuroimmune activity (Monji *et al.,* 2013). Preclinical and clinical evidence revealed that chronic treatment with antipsychotic drugs significantly attenuates pro-inflammatory cytokines such as IL-6 while increasing peripheral levels of anti-inflammatory cytokines including IL-10 (Müller and Schwarz, 2010a; MacDowell *et al.,* 2013; Zhu *et al.,* 2014). Moreover, a tight correlation exists between the severity of schizophrenic symptoms versus the concentration of pro-inflammatory mediators (Pazvantoglue *et al.,* 2009; Tsai *et al.,* 2013). However, second generation antipsychotics such as risperidone have reported to significantly reduce the levels of pro-inflammatory molecules more than the first generation antipsychotic drugs like haloperidol (Monji *et al.,* 2013). This difference in anti-inflammatory signaling action between both generation antipsychotic drugs may, at least in part, show the allerged differences in efficacy of second generations over the first generation antipsychotic drugs (Müller and Schwarz, 2010a).

Based on the implication of neuroimmune alteration in the pathogenesis of schizophrenia, the used of psychotropic compounds with neuroprotective and immunomodulatory properties has been encourage in the treatment of schizophrenia (Müller and Schwarz, 2010a). In line with this, the combinations of anti-inflammatory agents with psychotropic activity as add-on therapy with standard antipsychotic agents have been shown to demonstrate favourable outcomes clinically relative to treatments with standard neuroleptic agents alone. Evidence from double-blind, randomized, placebo-controlled studies have shown that combination of anti-inflammatory agents such as minocycline, a tetracycline antibiotic (Levkovitz *et al.,* 2010) and specific cyclooxygenase-2 inhibitors like celecoxib (Müller *et al.,* 2010b) in the management of schizophrenic disease as add-on therapy with known antipsychotic drugs demonstrated beneficial results, which is currently receiving attention for the treatments of the negative and cognitive symptoms schizophrenia (Levkovitz *et al.,* 2010). Furthermore, the use of agents with strong antioxidant activity along with potential immunomodulatory property has also been recommended for use with standard antipsychotic drugs (Lavoie *et al.,* 2008). For example, N-acetyl-cysteine, a thiol antioxidant compound has been reported to modulate dopaminergic and glutamatergic neurotransmissions, enhance neurotrophic and antioxidant activities, as well as inhibit inflammatory processes (Lavoie *et al.,* 2008).

2.7 FLAVONOIDS

Plant derivatives including decoctions, extracts or pure compounds have been shown to provide several avenues for drug development due to their biodiversities in pharmacotherapy (Kapoor and Kakkar, 2012). For example, flavonoids are group of a naturally occouring phytochemicals distributed in fruits and vegetables amongs other plant sources, and are used on daily basis as part of human diets because of their antioxidant property (Kapoor and Kakkar, 2012). Moreover, flavonoids have been known to elecit different beneficial effects based on their potential to modulate antioxidant and neurotransmissions, which may serve as the basis for their neuroprotective and psychotropic properties against neurological diseases (Hall *et al.,* 2005; Gupta *et al.,* 2012). In addition, some investigations have also shown their ability to down-regulate the immune response, an effect that may also contribute to their potential beneficial influence in management of neurodegenerative diseases (Hall *et al.,* 2005). Indeed, supportive evidence have reported that some flavonoid-based compounds such as DA-6034, morin, disomin, chrysin, naringin among several others possess strong psychotropic actions in animals models of neurological disorders (Wang *et al.,* 2005; Gottlieb *et al.,* 2006).

2.8 MORIN

Morin or morin hydrate (C_{15} H_{10} 0_7; 2',4,3',5,7-pentahydroxyflavone) is one of the naturally occuring non-toxic, yellow crystalline bioflavonoids (flavonol), originally isolated from members of the Moraceae family (Kapoor and Kakkar, 2012); abundantly distributed in many fruits and vegetables including onion, seed weeds, mill, almond, red wine, osage orange and other Chinese herbs (Sreedharan *et al.,* 2009; Nandhakumar *et al.,* 2012). It can also be derived as yellow pigment from plants such as old fustic (*Chlorophora tinctoria*, family Moraceae), almond (*Prunus dulcis*, family Rosaceae), and sweet chestnut (*Castanea sativa*, family Fagaceae). Over the years, numerous activities have been assigned to this flavonol (Nandhakumar *et al.,* 2012).

Morin has been reported to possess various pharmacological properties including antioxidant (Sreedharan *et al.,* 2009), antiinflammatory (Galvez *et al.,* 2001), chemoprotective (Li *et al.,* 2016), anticancer (Hyun *et al.,* 2015). Evidence have shown that morin prevented oxidative damages in different human cell lines including erythrocytes, myocytes and hypatocytes due to free-mediated oxidative stress, and safely modulates cancer cell biology (Chen *et al.,* 2017;

Zhang *et al.,* 2018). Moreover, studies have also reported that morin demonstrates chemopreventive and immunomodulatory activities on chemically induced cancer of the tongue in rats (Li *et al.,* 2016), decreases the proliferation of human leukemia cells through inhibition of cell cycle via a mitochondria-mediated apoptotic mechanism (Zhang *et al.,* 2009; Li *et al.*, 2016). However, the pharmacological investigation of morin as novel therapeutic molecule for the treatment of neuropsychiatric disease such as schizophrenia stems from the finding of previous studies showing that morin demonstrated neuroprotective property in some neurological diseases associated with oxidative and immune imbalance (Gottlieb *et al.,* 2006; Chen *et al.,* 2017).

2.8.1 Chemistry of morin

The morin hydrate structure (Figure 2.8) represents an isomeric form of quercetin, both having OH in position 3, a resorcinol moiety, and a carbonyl group in position 4; the only difference between them is the hydroxylation pattern on B-ring, which is meta in morin hydrate but Ortho in quercetin (Marder and Paladini, 2002). Although quercetin is regarded as one of the flavonoid with the highest antioxidant potential because it has all those groups, as well as an ortho hydroxylation pattern on B-ring, studies have also demonstrated a higher effectiveness of morin hydrate facing certain oxidative processes (Janeiro *et al.,* 2004). The chemical structure of morin and other bioflavonoids can be distinguished by the presence of two aromatic rings connected by c-pyrone ring where polar hydroxyl groups are bind at various positions. These hydroxyl groups are suggested to be responsible for the free radical scavenging properties shared by morin and other naturally occurring bioflavonoids (Subash and Subramanian, 2009).

Figure 2.8: Structure of morin (Al-Numair *et al.,* 2014**)**

2.8.2 Pharmacological activities of morin

An increasing number of studies have showed that morin possess different pharmacological activities, including cardioprotective, antidiabetic mellitus, anticancer and anti-inflammatory activities. These effects include:

A) Anti-oxidant activity

Animals generates free radicals in their body whether knowingly or unknowlingly. The increasing rise in the involvements of oxidative stress in the pathogenesis of several human diseases has increased their importance as relevant target site for drug discovery in different diseases **(**Nandhakumar *et al.,* 2012**)**. Morin exhibits several biological effects including antiinflammation, antidiabetic, anticataracts, anticancer, antiamyliodogenic and neuroprotective properties via its antioxidant activity. It has also been found to attenuate liver dysfusion and cardiovascular diseases, as it has been reported to suppress ischeamic reperfusion induced organ alteration (Hillwell, 1994; Santos *et al.,* 1998). Nandhakumar *et al.,* (2012) reported that, morin supplementation to cancerous rats significantly attenuated oxidative stress and brought the decreased of SOD, CAT, and GPx activities to normal levels. Also, morin demonstrated antioxidative effects in different human cells including erythrocytes, myocytes, hepatocytes and endothelia cells via free radical extenuation, and safely modulates cancer cell biology (Subash and Subramanian, 2009). In addition to reducing oxidative stress, morin lowered [Ca2+]i induced by excitotoxic insults and thus contribute to diminish neuronal demise, all of which might a protective mechanisms against neurological disorders (Gottlieb *et al.,* 2006; Chen *et al.,* 2017).

B) Anti-inflammatory activity

Free radicals, nitric oxide, leuckotrienes are involved in the production of inflammatory mediators in the intestinal inflammatory conditions (Galvez *et al.,* 2001). Morin ameliorated rats imparted with colitis by single injection of colonic instillation of 30 mg of the hapten trinitrobenzenesulphonic acid dissolved in 0.25 mL of 50% ethanol. The colitic rats were treated with morin hydrate 25 mg/kg orally for 4 weeks. Morin Hydrate showed the beneficial effect on 4th week following colitis insult, both macroscopically and microscopically (Galvez *et al.,* 2001). The anti-inflammatory activity of Morin Hydrate have been shown to be due to inhibition

of synthesis of most important cytokine interleukine-1β and decreased in the malondialdehyde levels and nitric oxide synthase and free radicals involved in the inflammatory cascade (Galvez *et al.,* 2001).

C) Effect on central nervous system

Increasing lines of evidences have reportedly shown that neuroactive flavonols are positive modulators of central GABAergic neurotransmissions (Marder and Paladini, 2002; Wang *et al.,* 2005), one of the hypofunctional neuropathological substrate implicated in schizophrenia thus, leading to the theorem that they may induce sedative effects against behavioural disinhibition associated with schizophrenia (Gonzalez-Burgos and Lewis, 2008). Moreover, drugs enhancing the function of alpha-2 subunit containing $GABA_A$ receptors such as benzodiazepine-like drugs (Wolkowitz and Pickar, 1991; Nunes *et al.,* 2012), are currently being sought to reduce downstream glutamate release (Lewis *et al.,* 2008). Accordingly, both naturally occurring and synthetic flavonols have been shown to bind to $GABA_A$ receptor with high affinity and to exert anxiolytic-like, sedative, and tranquilizing effects in rodents (Marder and Paladini 2002; Wang *et al.,* 2005). Moreover, investigations of some naturally occurring and synthetic bioactive flavonols suggest that they enhance GABAergic neurotransmissions to alleviate certain behavioural hyperactivity (Campbell *et al.,* 2004; Hall *et al.,* 2005). For example, an investigation has shown that some flavonols including 6-bromo-3-nitroflavones and 6-bromoflavone with potent anxiolytic property similar to diazepam are selective and competitive agonist with the $GABA_A$ receptor antagonist, flumazenil for the benzodiazepine omega receptor found in the rat brain (Griebel *et al.,* 1999). Upon this background, a study has also shown that morin inhibited behavioural impairments and mitigates glutamate-mediated neuronal loss and free radical generation partially due to continuous activation of glutamatergic system (Gottlieb *et al.,* 2006).

D) Cardioprotective activity

Cardiovascular Diseases (CVD) are the chief mortality causes worldwide. Myocardial infarction (MI) is one of the CVD disorder, as morin hydrate shows cardiovascular benefit qualities in isoproterenol-induced myocardial infarction in rats due to its free radical scavenging activity attributed by the polyphenolic group (Al-Numai *et al.,* 2012). Subash and Subramanian (2009) showed that by the administration of morin hydrate (30 mg/kg) by oral administration offered the

protection against hyperammonemia by acting through reducing of blood pressure, oxidative stress and by increasing the antioxidant system in ammonium-chloride induced hyperammonemic rats induced by at dose of 100 mg/kg/b.w i.p. Elsewhere, Al-Numair *et al.,* (2012) showed also that pretreatment of morin hydrate (20, 40 and 80 mg/kg, respectively) daily for a period of 30 days, decreases the levels of cardiovascular markers in the serum while increasing the levels in the cardiovascular system. However, all adenosine based activity in the heart were reduced.

E) Anti-cancer activity

For decades, increasing studies focused the potential anticancer activity of morin hydrate, in various kinds of cancers. For example, morin hydrate, inhihited inflammatory action following trinitrobenzoic acid-induced inflammation of the colon in rats (Galvez *et al.,* 2001), exhibited the chemoprotective effect of chemically produced rat tongue carcinogenesis (Li *et al.,* 2016), and inhibit the phorbol-ester induced transformation of rat hepatocytes (Hsiang *et al.,* 2005). Morin hydrate inhibits the peroxisome proliferated activator receptor induced keratinocyte differentiation by inhibiting the lipoxygenase pathway (Thuillier *et al.,* 2002). Also, morin decreases the proliferation of human leukemia cells through inhibition of cell cycle via a mitochondria-mediated apoptotic mechanism (Zhang *et al.,* 2009; Li *et al.,* 2016); thus, suggesting the possible in vitro and in vivo anti-cancer property of morin against neoplastic diseases (Hyun *et al.,* 2015; Li *et al.*, 2016).

F) Xanthine oxidase and uricosuric activity

Pathological production of gouty arthritis occurs due to over production or decreased excretion of purine metabolic end product i.e. uric acid (Liote, 2003). Some natural herbs were exhibited the xanthine oxidase inhibitor activity (Yu *et al.,* 2006; Wang *et al.,* 2010) along with other types of mechanisms which were helpful in the treatment of gouty arthritis and hyperuricemia related disorders. Morin hydrate, which is a natural Chinese herb, is having the xanthine oxidase inhibitory activity along with other type of mechanisms involved in its anti-rheumatic disorders, inhibits the urate reabsorption at the brush border of proximal renal tubule membrane vesicles (Wang *et al.,* 2010). By using oxonate-induced hyperuricemic rat model, morin hydrate showed inhibition of uricosuric activity (Wang *et al.,* 2010).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1.0. Experimental animals

Animals (Swiss male mice, 6 weeks old weighing beween 20-25 g) were collected from the Main Animal House, University of Ibadan. Five animals were accomodated per plastic cage (42 x 30 x 27 cm) with a 12-hr light and 12-hr dark cycle, relative room temperature (25 ± 1°C) and humidity of 60 ± 5%. They were given free access to pelleted rodent food and water *ad libitum* throughout the experimental period. They were made to acclimatize to the environment, food and water for a minimum of 1 week prior to biginning of the experiments. The experimental procedures of the experiments were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (Ethical approval number: UI-ACUREC/App/12/2016/01), in strict compliance with the National institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

3.1.1 Materials / Apparatus

Plastic cages (42 x 30 x 27 cm), open field chamber (50 cm × 50 cm × 40 cm), Plexiglas (transparent) observation chambers (29 x 18 x 12 cm; 20 × 20 × 23 cm; 35 x 30 x 23 cm; 60 x 50 x 40 cm), Y-maze apparatus, social interaction test chamber, forced swim test transparent glass cylinder, horizontal plane wood (wood-block measuring High-6 cm; Width-4 cm; Length-16 cm), rota rod apparatus (Ugo basile, Model57625), digital camera, electronic digital weighing balances (Ohaus), syringes (1, 2, 5, 10, 20 mL), stopwatches, centrifuge (ATKE) (Anke TGL-16G, Nanjing, China), water bath (Equitron), spectrophotometer (752N INESIA, China), micropipettes and tips (250, 500, 1000 µL), microplate reader (Molecular Devices, Sunnyvale, CA), pH meter (EDT instruments), test tubes, eppendorf tubes, tube racks dissecting kits and boards, electrical homogenizer, pistol and mortar, 70% ethanol, GraphPad Software version 5, Inc. La Jolla, CA 92037 USA.

3.1.2 Drugs and chemicals:

Morin, haloperidol, risperidone, apomorphine, lipopolysaccharide (*Escherichia coli* serotype, 055:B5), L-Arginine, L-Nitro arginine methyl ester (L-NAME), propranolol, yohimbine, prazosine, atropine, phenylchloropiperazine (PCPA), metergoline, cyproheptadine and sulpiride

were all purchased from Sigma-Aldrich, St. Louis, MO, USA. However, ketamine hydrochloride was obtained from Rotex Medica, Germany. Methylene blue was purchased from BDH Chemicals Ltd., Poole, England. Trichloroacetic acid (TCA) (Burgoyne Burbidges and Co., Mumbai, India), thiobarbituric acid (TBA) (Guanghua Chemical Factory Co. Ltd., China), tris-Potassium chloride (KCl) (Sigma-Aldrich, St. Louis, MO, USA), acetic acid (Sigma-Aldrich, St. Louis, MO, USA), sodium bicarbonate ($NaHCO_3$) (BDH Chemicals Ltd., Poole, England), sodium carbonate (fisons, Loughborough Leics, England), sodium chloride (NaOH) (J.T Baker Chemicals Co., Philipsburg, N.J., USA), hydrogen peroxide (H_2O_2) (BDH Chemicals Ltd., Poole, England), adrenaline (epinephrine) (Sigma-Aldrich, St. Louis, MO, USA), Ellman Reagent [5′, 5′-Dithiobis- (2-nitrobenzoate) DTNB] (Sigma-Aldrich, St. Louis, MO, USA), neurotransmitter (dopamine, glutamate, serotonin) Enzyme-linked immunosorbent assay (ELISA) kits (Abnova, Germany), tumor necrosis factor-alpha (TNF-α) ELISA kit (Abcam, England), interleukin-6 (IL-6) ELISA kit (Abcam, England), nuclear factor kappa-B (NF-κB) monoclonal antibody immunohistochemical (IHC) kit (Santa Cruz, Germany), cyclo-oxygenase-2 (COX-2) monoclonal antibody IHC kit (Santa Cruz, Germany), inducible nitric oxide synthase (iNOS) monoclonal antibody IHC kit (Santa Cruz, Germany), nicotinamide adenine denucleotide phosphate oxidase-2 (Nox-2) monoclonal antibody IHC kit (Santa Cruz, Germany), brain derived neurotrophic factor (BDNF) polyclonal antibody IHC kit (Thermo Fisher scientific, USA), glutamic acid decarboxylase-67 (GAD_{67}) (Biorbyt, UK) were used in the study.

3.2 Drug preparations and treatments:

Morin (MOR), risperidone (RIS), haloperidol (HLP), apomorphine (APO) and lipopolysaccharide (LPS) were dissolved in normal saline, while ketamine (KET) was diluted in normal saline. Mice were give intraperitoneal (i.p.) injection of MOR (25, 50 and 100 mg/kg), HLP (1 mg/kg), RIS (0.5 mg/kg), APO (2 mg/kg), KET (10, 20, 30 mg/kg), LPS (100 µg/kg), or vehicle (VEH) (normal saline, 10 mL/kg). Doses of morin (25, 50 and 100 mg/kg) used in this study were chosen based on the findings from preliminary investigations (Olonode *et al.*, 2017). The doses of HLP and RIS used in this study were adopted from previous studies (Monte *et al.*, 2013; Omogbiya *et al.*, 2013). HLP and RIS, which served as positive controls were used for the purpose of comparison, because KET-induced schizophrenia-like behaviours has been shown to be more responsive to atypical antipsychotic drug such as RIS (Becker and Grecksch, 2004).

3.3 EXPERIMENTAL DESIGN / STUDY:

The study was divided into two main studies: acute and chronic studies. The acute study consisted of acute behavioural tests predictive of human psychosis as well as interaction studies with neurotransmitter blocker(s) to seek for possible mechanisms of action. Accordingly, acute studies include the following paradigms: spontanous motor activity, APO-induced stereotypy, acute KET-induced stereotypy; KET-induced hyperactivity (hyperlocomotion); KET-induced enhanced immobility (behavioural despair) in forced swim test, drug-induced ptosis, and catatonic tests (wood block catalepsy and rota rod tests). Also, interaction studies with dopaminergic, GABAergic, serotonergic, adrenergic, cholinergic and nitrergic neurotransmitter receptor blocker(s) using open field, Y-maze and forced swim tests were carried out. In line with this (acute study), mice were allotted into six (6) or seven (7) treatment groups (n = 5/group) depending on the model as listed above. Group 1, which served as normal control received vehicle (10 mL/kg, i.p), group 2 was administered APO or KET, groups 3-5 were pretreated with morin (25, 50 and 100 mg/kg, i.p.), while groups 6 and 7 received HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) as positive controls respectively 30 min prior to behavioural tests.

The chronic study consisted of behavioural tests, neurochemical, and molecular as well as cellular evaluations to determine the underlying possible mechanisms of action of the antipsychotic property of morin following repeated administrations of KET, or KET plus LPS respectively. Accordingly, KET (10 days)-induced schizophrenia-like behaviour, oxidative, nitrergic stress and cholinergic alterations in the whole brain; preventive and reversal of KET (14 days)-induced schizophrenia-like behaviours and neurochemical (neurotransmitter, neurotrophic, oxidative, nitrergic stress and histomorphology) alterations in specific brain (striatum, prefrontal cortex and hippocampus) regions were investigated. Furthermore, LPS-induced neuroimmune activation in combination with KET (14 days)-induced schizophrenia-like behaviour in the striatum, prefrontal cortex and hippocampus were also evaluated. Twenty four (24) hours after the last treatments (except for LPS plus KET study, which was 2 hr after the last administration), behavioural assessments were performed as previously described (Chatterjee *et al.,* 2012b; Monte *et al.,* 2013) with brief modification, i.e.: (a) hyperlocomotor and stereotypy activity (representing *positive symptoms*), (b) Y-maze, novel object recognition and social recognition memory tests (representing *cognitive symptoms*) (c) social interaction and forced swim tests (representing *negative symptoms*), and (d) wood block catalepsy and rota rod tests (catatonic

tests representing *catalepsy and motor coordination*). All behavioural tests were carried out between 8:30 a.m.-1:00 p.m. each day. Immediately after the behavioural tests, mice in the respective treatment groups were sacrificed under ether anaesthesia and the whole brains were rapidly removed, weighed and/or dissected and weighed, homogenized and centrifuged. Thereafter, supernatants were collected accordingly for different biochemical assays. However, mice brains for histology and immunohistochemical studies were perfused via intracardiac perfusion, dissected and fixed with 10% phosphate buffered formaldehyde respectively.

3.3.1 ACUTE STUDIES:

3.3.1.1 Effect of morin on spontaneous motor activity using open field Test (OFT)

The behavioural profile (locomotor activity or line crossing activity) of mice administered with MOR were evaluated individually in the open field test chamber. The open field test apparatus consists of square box (50 cm × 50 cm × 40 cm) with white covering on every side of the wall. The floor of the apparatus which is equally divided into 16 squares (10 cm × 10 cm) was marked black lines. The embodiment consists of opaque Plexiglas sides having one transparent side for observation. Prior to commencement of the test, mice were made to acclimatize in the experimental room to reduced anxiety and randomly divided into various treatment groups (n = 5). Group 1, which served as control received intraperitoneal (i.p.) injection of vehicle (10 mL/kg), groups 2-4 received MOR (25, 50 and 100 mg/kg, i.p.), group 5 received HLP (1 mg/kg, i.p.) and group 6 was treated with RIS (0.5 mg/kg, i.p.). Thirty minutes after treatment, each mouse was placed directly from the home cage at the center of the Plexiglas open field test apparatus and spontaneous motor activity was dependent on number of line crossings for 5 min. After each experiment, the test box was sanitized with ethanol (70%) to erase olfactory odour from test box (Brown *et al.*, 1999).

3.3.1.2 Effect of morin on apomorphine-induced stereotypy

The antipsychotic effect of MOR on stereotyped behaviours, consisting of repetitive, ritualistic and functionless motor behaviour (Morrens *et al.,* 2006), was evaluated using APO-induced stereotyped behaviour (representing *positive symptoms*) as previously described (Taïwe *et al.,* 2012). It manifests itself in animal models as: sideways movement of the head, intermittent sniffing, intense chewing and intense licking behaviours of animals (Omogbiya *et al.,* 2013). Mice were randomized into seven (7) treatment groups (n = 5/group). Groups 1 and 2, which served as normal and negative controls were pre-treated with vehicle (10 mL/kg, i.p) respectively, groups 3-5 were pre-treated with MOR (25, 50 and 100 mg/kg, i.p.), while groups 6 and 7 received HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) as positive controls respectively. Thirty (30) minutes thereafter, each animals from groups 2-7 were given single APO (2 mg/kg, i.p.) injection and immediately placed in a transparent Plassiglass chamber measuring 20 cm × 20 cm × 23 cm, and stereotype behaviours were scored for 2 min at 5, 10, 20, 30, 40, 50 and 60 min time interval after APO injection as described by Amos *et al.,* (2003). Stereotype behaviours were observed as: 0 representing absence of stereotype behaviour; 1 = presence of stereotype movements of the head; 2 = intermittent sniffing; 3 = chewing; while 4 represents intense licking. After each mice session, the observation chamber was cleansed with ethanol (70%) to erase previous mouse odour.

3.3.1.3 Effect of morin on ketamine-induced stereotypy

Ketamine-induced stereotypy (representing *positive symptoms*) was also use to assess the effect of MOR on stereotypy behaviour (Yamamoto *et al.,* 1997). The mice were randomly distributed into six (6) experimental groups (n = 5/group). Groups 1 and 2, which served as normal and negative controls was pre-treated with vehicle (10 mL/kg, i.p) respectively, groups 3-5 were pre-treated with MOR (25, 50 and 100 mg/kg, i.p.), group 6 received HLP (1 mg/kg, i.p.) while group 7 was pre-treated with RIS (0.5 mg/kg, i.p.) as positive controls. Thirty (30) minutes thereafter, animals from groups 2 to 7 were given single KET (10 mg/kg, i.p.) injection and transferred to a Plassiglass observation box measuring 20 cm × 20 cm × 23, and stereotype behaviours were evaluated as previously stated in the APO model, for 2 min at 5, 10, 20, 30, 40 and 50 min after KET injection, and total stereotypy score(s) were calculated as described by Amos *et al.,* (2003). Thereafter each mouse session, the Plassiglass box was cleansed with ethanol (70%).

3.3.1.4 Effect of morin on ketamine-induced hyperlocomotion

The effect of MOR on KET-induced hyperlocomotion (representing *positive symptoms*) was assessed as described by Yamamoto *et al.,* (1997). The mice were randomized into seven experimental groups (n = 5/group). Groups 1 and 2 were given vehicle (10 mL/kg, i.p.) (Group 2 serving as negative control), groups 3, 4 and 5 were pre-treated with MOR (25, 50 and 100 mg/kg, i.p.), group 6 was pre-treated with HLP (1 mg/kg, i.p.) and group 7 received RIS (0.5 mg/kg, i.p.). Thereafter (30 min), animals in groups 2-6 were additionally given single KET (10 mg/kg, i.p.) injection. Immediately, mice were singly transferred to the open field arena (50 cm × 50 cm × 40 cm). The numbers of lines crossing activity were counted for 5 min using a stopwatch.

3.3.1.5 Effect of morin on ketamine-enhanced immobility in forced swim test

The effect of MOR was evaluated on KET-enhanced immobility in forced swim test paradigm (representing *negative symptoms*), was assessed as described by Chindo *et al.*, (2012). The reduction in the immobility time serves as a specific and selective index of antidepressant activity (Weiner *et al.*, 2003) that can be used to alleviate the negative symptoms of schizophrenia. Mice were randomly divided into seven (7) experimental groups with 5 animals per group. Group 1 was pre-treated with vehicle (10 mL/kg, i.p.) while groups 2-7 were pre-treated with KET (30 mg/kg, i.p./day) for 5 days. One hour after the last administration with KET (pretest session), each mice were exposed to water at 25 °C to a depth of 15 cm contained in a standard transparent glass cylinder (Height = 24 cm, Diameter = 13 cm) and was made to swim for a period of 5 min for habituation. Twenty four (24) hours thereafter, group 2, which served as control was treated with vehicle (10 mL/kg, i.p.), groups 3, 4 and 5 were treated with MOR (25, 50 and 100 mg/kg, i.p.), while groups 6 and 7 received HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) respectively. Thirty (30) minutes later, test session was performed and mice were forced to swim again in the same swimming specifications of water as described above. Animals swimmed for 6 min and the duration of immobility which represent behavioural despair i.e., is the time animals float passively on the water was recorded for a period of 5 min with a stopwatch after discarding the first 1 min of swimming activity. Thereafter each swimming session, mice were removed from the cylinder of water, dried with hand towel and aerated in open space before returning the mice to their home cages.

3.3.1.6 Effect of morin on ptosis induction

The effect of MOR on drug-induced ptosis was evaluated based on the dropping of the eyelid (ptosis), representing a specific index of monoamine depletion as described by Bourin *et al.*, (1983). The test apparatus used was a transparent observation chamber (20 cm × 20 cm × 23 cm). Mice were divided into six treatment groups (n = 5/group). Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), groups 2, 3 and 4 were adminstered MOR (25, 50 and 100 mg/kg, i.p.), however groups 5 and 6 received HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) respectively as positive controls. All animals were kept in individual ptosis transparent observation chambers (20 cm above the bench top) immediately after treatment to allow for stable assessment of ptosis. Thirty (30) minutes thereafter, drug-induced ptosis on each mouse was inspected. The degree of ptosis of each animal was evaluated and recorded at 30, 60 and 90 min (that is 30 min after drug treatment). The degree of ptosis was rated according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarter closed; and 4, completely closed. The results obtained were compared with control group treated with vehicle (10 mL/kg, i.p.).

3.3.1.7 Cataleptogenic effect of morin in mice

The cataleptogenic effect of MOR (acute) on catalepsy test, as an index of extrapyramidal side effect was investigated using the wood block catalepsy (Chatterjee *et al.,* 2012b). Mice were randomly divided into six experimental groups (n = 5/group). Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), MOR (25, 50 and 100 mg/kg, i.p.) was given to groups 2, 3 and 4 respectively, group 5 was treated with HLP (1 mg/kg) and group 6 received RIS (0.5 mg/kg, i.p.) for 30 min before testing for catalepsy. Thereafter, each animal was gently placed on the upper surface of a standing wood block (H = 6 cm; W = 4 cm; L = 16 cm). This test was carried out at 30, 60 and 90 min time intervals following drug administration. The descent latency (DL) was defined as the time it took the mouse to descend from the wood block. Animal is considered cataleptic if it remains on the block for more than 60 sec.

3.3.1.8 Effect of morin on motor co-ordination in mice

The effect of MOR (acute) on motor coordination was assessed using rota rod test (Arruda *et al.*, 2008). The rota rod test apparatus (Ugo basile, Model57625), which was 50 cm above the table top consists of horizontal metal rod coated with rubber, divided into 6 divisions, which allows for testing of 6 mice simultaneously, and attached to a speed motor. The minimum and maximum speed of the rota rod consists of 10 and 25 rpm respectively with an acceleration speed of 12 sec. Animals were first habituated to the rotating rod at a slow speed. Following 3 trials of animals with relevant speed, mean latency of mice to fall from the rotating rods were used as an index of motor performances. Rota rod test was conducted for each mouse for duration of 2 min on the roller rods. The performance time and number of fall(s) from the test rod of each animal were recorded with a stopwatch and scored with a counter, respectively. Thereafter, the roller rods were cleansed with ethanol (70%) to remove residual odour.

Mice were grouped into 12 experimental groups (n = 5/group). Group 1 received vehicle (10 mL/kg, i.p.) treatment, while groups 2, 3 and 4 were administered MOR (25, 50 and 100 mg/kg, i.p.), HLP (1 mg.kg, i.p.) and RIS (0.5 mg/kg, i.p.) were given to mice in groups 5 and 6 respectively. However, group 7 was treated with KET (10 mg/kg, i.p.) only, and groups 8-10 were pre-treated with MOR (25, 50 and 100 mg/kg, i.p.) 30 min before KET (10 mg/kg, i.p.) treatment. Group 11 was pre-treated with HLP (I mg/kg, i.p.) prior to treatment with KET (10 mg/kg, i.p.) 30 min later, and group 12 was given RIS (0.5 mg/kg, i.p.) followed by KET (10 mg/kg, i.p.) 30 min later. After treatment with vehicle or KET, the test was evaluated for 2 min each by placing the mice on the rotating rod. The time of performance and number of fall(s) from the rotating rods of each mouse were recorded (Arruda *et al.*, 2008).

3.3.2 INTERACTION STUDY WITH NEUROTANSMITTER RECEPTOR BLOCKER(S) ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

3.3.2.1 Involvement of dopaminergic receptor blockade (e.g., haloperidol, sulpiride) on the activity of morin

The effect of haloperidol (HLP, dopamine D_2 receptor antagonist, 0.2 mg/kg, i.p.) or sulpiride (SUL, dopamine D_2 receptor antagonist, 50 mg/kg, i.p.) pre-treatments on mechanism of action of the antipsychotic-like activity of MOR (100 mg/kg, i.p.) against the positive, negative or cognitive symptoms was assessed using the open field, Y-maze and forced swim tests in mice (Aderibigbe *et al.*, 2010; Amarala *et al.*, 2012; Chindo *et al.*, 2012). Mice were grouped into four (4) treatment groups. Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), group 2 was treated with HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) 15 min prior to treatment with MOR (100 mg/kg, i.p.). Thirty minutes thereafter, animals were evaluated for interaction effects (antagonism) on open field test (representing *positive symptom*), Y-maze test (representing *cognitive symptom*) and forced swim test (representing *negative symptom*).

A) Effects of haloperidol or sulpiride on the antipsychotic-like activity of morin on locomotor activity using open field test

The effect of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) pre-treatment on the antipsychotic-like activity of MOR (100 mg/kg, i.p.) on locomotor activity based on number of line crossing(s), using the open field test (Brown *et al*. 1999). The increase in locomotion (number of line crossings) with the co-administration of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) and MOR (100 mg/kg, i.p.) served as a specific index of the involvement of dopaminergic pathway in the ability of MOR to relieve positive symptom. Thirty (30) minutes after the administration of all drugs, the experiment (locomotor activity) was performed as described in session 3.3.1.1.

B). Effects of haloperidol or sulpiride antagonism on the activity of morin on cognitive function using Y-maze test

The effect of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) pre-treatment on the antipsychotic-like activity of MOR on cognitive function based on spontaneous alternation performance (cognitive searching behaviour) using the Y-maze test (Casadesus *et al.*, 2006). The decrease in percentage correct alternation with the co-administration of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) and MOR (100 mg/kg, i.p.) served as a specific index of the involvement of dopaminergic pathway in the ability of MOR to treat cognitive symptom. The apparatus consists of three identical arms (labeled A, B, C), measuring 33 × 11 × 12 cm in which each arms are separated symmetrically at 120°. Each mouse was placed in the Y-maze apparatus at the end of arm A and allowed to explore all the three arms freely for 5 min, taking the following parameters: the number of arm visits and sequence (alternation) of arm visits visually. Alternation behaviour was defined as consecutive entries into all three arms (i.e., ABC, CAB or BCA but not ABA, BAB, CBC etc) (Casadesus *et al.*, 2006). The percentage alternation, which is a measure of spatial working memory, was calculated using the following formula: (Total alternation number/Total number of entries – 2) X 100. After each test session, the maze was cleansed with ethanol (70%).

C). Effects of haloperidol or sulpiride antagonism on the activity of morin on behavioural despair using forced swim test

The effect of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) pre-treatment on the antipsychotic-like activity of MOR (100 mg/kg, i.p.) on behavioural despair (negative symptom) was evaluated, based on the duration of immobility using the forced swim test with brief modicifcation (Chindo *et al.,* 2012). The increase in the duration of immobility with the co-administration of HLP (0.2 mg/kg, i.p.) or SUL (50 mg/kg, i.p.) and MOR (100 mg/kg, i.p.) served as a specific index of the involvement of dopaminergic pathway in the ability of MOR to treat negative symptom. Thirty (30) minutes after treatments with all drugs, the experiment was performed as described in session 3.3.1.5

3.3.2.2 Effect of GABAergic receptor inhibitor (e.g., flumazenil) on the activity of morin

The effect of flumazenil (FMZ, $GABA_A$ receptor antagonist, 2 mg/kg, i.p.) pre-treatments on the antipsychotic activity of MOR against positive (locomotor activity), negative (behavioural despair) or cognitive (cognitive function) symptoms was evaluated using the open field, Y-maze and forced swim tests as described in session 3.3.2.1 (A, B and C) (Ayoka *et al.,* 2006; Aderibigbe *et al.,* 2010; Chindo *et al.,* 2012). Mice were grouped into four (4) treatment groups. Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), group 2 was treated with FMZ (2 mg/kg, i.p.), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with FMZ (2 mg/kg, i.p.) 15 min before treatment with MOR (100 mg/kg, i.p.). Thereafter (30 min), animals were evaluated for interaction effect (antagonism) as described above on the open field, Y-maze and forced swim tests.

3.3.2.3 Involvement of 5-HT receptor (e.g., metergerline, cyproheptadine, para-chlorophenylalanine) on the activity of morin

The effect of metergoline (MTG, non-selective 5-HT_1 and 5-HT_2 receptor antagonist, 100 mg/kg, i.p.), cyproheptadine (CYP, non-selective 5-HT_2 receptor antagonist, 0.5 mg/kg, i.p.), or para-chlorophenylalanine [PCPA, 5-HT synthesis inhibitor (tryptophan hydoxylase inhibitor), 100 mg/kg, i.p. once daily for 3 days) pre-treatments on the antipsychotic activity of MOR against positive (locomotor activity), negative (behavioural despair) or cognitive (cognitive function) symptoms were evaluated using the open field, Y-maze and forced swim tests as previously

described (session 3.3.2.1(A, B and C)) (Aderibigbe *et al.*, 2010; Chindo *et al.*, 2012; Amarala *et al.*, 2012). Mice were grouped into four (4) treatment groups. Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), group 2 was treated with MTG (100 mg/kg, i.p.), CYP (0.5 mg/kg, i.p.) or PCPA (100 mg/kg, i.p. once daily for 3 days), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with MTG (100 mg/kg, i.p.), CYP (0.5 mg/kg, i.p.), or PCPA (100 mg/kg, i.p. once daily for 3 days) 15 min before treatment with MOR (100 mg/kg, i.p.). Thereafter (30 min), animals were evaluated for interaction effect (antagonism) as previously described on the open field, Y-maze and forced swim tests.

3.3.2.4 Effects of adrenergic receptor (e.g., yohimbine, prazosine, propranolol) on the activity of morin

The effect of yohimbine (YHB, α_2-adrenergic antagonist, 1 mg/kg, i.p.), prazosine (PRA, α_1-adrenergic receptor antagonist, 1 mg/kg, i.p.), or propranolol (PRO, nonselective β-adrenergic receptor antagonist, 0.2 mg/kg, i.p) pre-treatments on the antipsychotic activity of MOR (100 mg/kg, i.p.) against positive (locomotor activity), negative (behavioural despair) or cognitive (cognitive function) symptoms were assessed using the open field, Y-maze and forced swim tests as described in session 3.3.2.1 (A, B and C) (Aderibigbe *et al.*, 2010; Chindo *et al.*, 2012; Ayoka *et al.*, 2006). Also, mice were grouped into four (4) treatment groups. Group 1, which served as normal control was treated with vehicle (10 mL/kg, i.p.), group 2 was treated with YHB (1 mg/kg, i.p), PRA (1 mg/kg, i.p.) or PRO (0.2 mg/kg, i.p.), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with YHB (1 mg/kg, i.p), PRA (1 mg/kg, i.p.) or PRO (0.2 mg/kg, i.p.) 15 min before treatment with MOR (100 mg/kg, i.p.). Thirty minutes thereafter, animals were evaluated for interaction effect (antagonism) as previously described on the open field, Y-maze and forced swim tests.

3.3.2.5 Influence of cholinergic receptor (e.g., atropine) on the effect of morin

The effect of atropine (ATP, muscarinic cholinergic receptor antagonist, 0.5 mg/kg, i.p.) pre-treatment on the antipsychotic-like activity of MOR (100 mg/kg, i.p.) against positive (locomotor activity), negative (behavioural despair) or cognitive (cognitive function) symptoms was determined using the open field, Y-maze and forced swim tests as previously described (session 3.3.2.1(A, B and C)) (Ayoka *et al.,* 2006; Aderibigbe *et al.,* 2010; Chindo *et al.,* 2012). Mice were grouped into four (4) treatment groups. Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), group 2 was treated with ATP (0.5 mg/kg, i.p), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with ATP (0.5 mg/kg, i.p) 15 min prior to treatment with MOR (100 mg/kg, i.p.). Thereafter (30 min), animals were evaluated for interaction effect (antagonism) as previously described on the open field, Y-maze and forced swim tests.

3.3.2.6 Role of nitrergic system (e.g., L-arginine, methylene blue, L-Nitro arginine methyl ester) on the activity of morin

The effect of L-arginine (L-ARG, nitric oxide precursor, 750 mg/kg, i.p.), L-Nitro arginine methyl ester (L-NAME, non-specific nitric oxide synthesis inhibitor, 50 mg/kg, i.p.), or methylene blue (MTB, neuronal nitric oxide synthase inhibitor, 3.75 mg/kg, i.p.) pre-treatments on the antipsychotic-like activity of MOR (100 mg/kg, i.p.) against positive, negative or cognitive symptoms were evaluated using the open field, Y-maze and forced swim tests as described in session 3.3.2.1 (A, B and C) (Aderibigbe *et al.,* 2010; Chindo *et al.,* 2012; Zhang *et al.,* 2013). Mice were grouped into four (4) treatment groups. Group 1, which served as normal control received vehicle (10 mL/kg, i.p.), group 2 was treated with L-ARG (750 mg/kg, i.p), L-NAME (50 mg/kg, i.p.) or MTB (3.75 mg/kg, i.p.), group 3 received MOR (100 mg/kg, i.p.), while group 4 was pre-treated with L-ARG (750 mg/kg, i.p), L-NAME (50 mg/kg, i.p.) or MTB (3.75 mg/kg, i.p.) 15 min prior to treatment with MOR (100 mg/kg, i.p.). Thirty minutes thereafter, animals were evaluated for interaction effect (antagonism) as previously described on the open field, Y-maze and forced swim tests.

3.3.3 CHRONIC STUDIES:

3.3.3.1 Effect of morin on sub-chronic treatment of ketamine-induced schizophrenia-like behaviours and neurochemical alterations in mice

The effect of MOR on KET-induced schizophrenia-like behaviours, oxidative, nitrergic and cholinergic alterations was performed according to the method previously described by Chatterjee *et al.*, (2012a) with modifications. Mice were grouped into 12 experimental groups (n = 5/group). Group 1 was received vehicle (10 mL/kg, i.p.) treatment, while groups 2, 3 and 4 were administered MOR (25, 50 and 100 mg/kg, i.p.), HLP (1 mg.kg, i.p.) and RIS (0.5 mg/kg, i.p.) were given to mice in groups 5 and 6 respectively. However, group 7 was treated with KET (10 mg/kg, i.p.) only, while groups 8-10 were pre-treated with MOR (25, 50 and 100 mg/kg, i.p.) 30 min before KET (10 mg/kg, i.p.) treatment. Group 11 was pre-treated with HLP (I mg/kg, i.p.) prior to treatment with KET (10 mg/kg, i.p.) 30 min later and group 12 was given RIS (0.5 mg/kg, i.p.) followed by KET (10 mg/kg, i.p.) 30 min later for 10 days, respectively.

Twenty four (24) hours after the last treatment with all the drugs on days 9, 10 and 11, behavioural assessment were performed as previously described (Chatterjee *et al.*, 2012a, 2012b) with brief modifications, i.e.: (a) open field test (representing *positive symptoms*) on day 9, (b) Y-maze test on day 9 and novel object recognition test on days 9 and 10 (representing *cognitive symptoms*), (c) social interaction test on day 10 and forced swim test on day 11 (representing *negative symptoms*), and (d) wood block catalepsy and rota rod tests (catatonic tests representing *extrapyramidal symptoms*) listed above were carried out as described above. However, novel object recognition and social interaction tests were carried out as described below.

3.3.3.2 Preventive and reversal effects of morin on ketamine-induced schizophrenia-like behaviours and neurochemical alterations in mice

The preventive and reversal effects of MOR on KET-induced schizophrenia-like behaviours, neurotransmitter, neurotrophic, oxidative, nitrergic and cholinergic alterations was evaluated as described by Monte *et al.*, (2013) with modifications. In the preventive protocol, mice were randomized into 5 experimental groups (n = 5/group). Group 1, which served as normal control was given vehicle (10 mL/kg, i.p.), group 2 received vehicle (10 mL/kg, i.p.) and served as negative control, group 3 received MOR (100 mg/kg, i.p.), while groups 4 and 5 were administered HLP (1 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.), serving as positive controls respectively, for 14 days. Between the 8^{th} and 14^{th} day of treatment, the animals in groups 2-5 additionally received a daily dose of KET (20 mg/kg, i.p.) or vehicle 30 min after MOR, HLP, or RIS administrations respectively.

In the reversal protocol, sub-chronic treatment of psychotic episodes was induced. Mice were grouped into 5 groups (n = 5). Group 1 received vehicle (10 mL/kg, i.p.), while groups 2-5 received one daily injection of KET (20 mg/kg, i.p.) or vehicle 10 (mL/kg, i.p.) for 14 days. Thereafter, from the 8^{th} to 14^{th} day day of treatment, group 2 was treated with vehicle (10 mL/kg, i.p.) as negative control, group 3 was treated MOR (100 mg/kg, i.p.), group 4 received HLP (1 mg/kg, i.p.), and group 5 received RIS (0.5 mg/kg, i.p.) additionally once daily with a 30 min interval between treatments.

Twenty four (24) hours after the last treatment with all the drugs on days 13, 14 and 15, behavioural assessment were performed as previously described (Monte *et al.*, 2013) with modifications, i.e.: (a) locomotor activity (open field test) and stereotypy behaviour (representing *positive symptoms*) on day 13, (b) Y-maze test on day 13 and novel object recognition test on days 13 and 14 (representing *cognitive symptoms*), and (c) social interaction test on day 14 and forced swim test on day 15 (representing *negative symptoms*). However, novel object recognition and social interaction tests were carried out as described below.

3.3.3.3 Effect of morin on lipopolysaccharide and ketamine-induced schizophrenia-like behaviours and neuroimmune alterations in mice

The effect MOR on LPS-induced neuroimmune activation and KET-induced schizophrenia-like behaviour (KET-enhanced LPS-induced neuroimmune alterations) was evaluated in adolescent mice. In the repeated treatment of inflammatory response, a modified developmental protocol of prepubertal endotoxeamia, was simulated with LPS [purified lyophilized *Escherichia coli* endotoxin (serotype, 055:B5; 0.1 mg/kg, i.p.)] (Abdel-Salam *et al.*, 2015; Réus *et al.*, 2017), and the psychotic episode was induced using the sub-chronic treatment of KET (20 mg/kg, i.p.) (Monte *et al.*, 2013). Mice were allocated into five (5) experimental groups (n = 5/group). Group 1 was treated with vehicle (10 mL/kg, i.p.), group 2, which served as negative control, received intraperitoneal injection of LPS (0.1 mg/kg, i.e., 100 µg/kg), group 3 received MOR (100 mg/kg, i.p.) plus LPS, and groups 4 and 5 were treated with HLP (1 mg/kg, i.p.) plus LPS, and RIS (0.5 mg/kg, i.p.) plus LPS respectively, once daily for 14 days. From the 8^{th}-14^{th} day of treatment, animals in groups 2-5 additionally received a daily dose of ketamine (20 mg/kg, i.p.) or vehicle 30 min after MOR, HLP, RIS or LPS administration.

Two (2) hours after the last treatment with all the drugs on day 14, behavioural assessment were performed as earlier described above in previous section (da Silva *et al.*, 2017), i.e.: (a) hyperlocomotor activity (open field test) and stereotypy behaviour (representing *positive symptoms*), (b) Y-maze test, novel object recognition test and social recognition memory (representing *cognitive symptoms*) and (c) social interaction test and forced swim test (representing *negative symptoms*) were carried out as described above. However, novel object recognition and social interaction tests were carried out as described below.

3.3.4 Other behavioural tests

3.3.4.1 Novel Object Recognition Test (NORT)

The effect of MOR was also assessed as an index for the non-spatial cognitive dysfunction commonly associated with schizophrenia according to the method previously described (Zhu *et al.,* 2014). NORT was conducted in an observation chamber (60 x 50 x 40 cm) with discriminated objects (A and B) identically sized (4.5 cm diameter and 11.5 cm height) blue squared cylinders, whereas object C had a red triangular cylinder. The NORT test consists of two sessions; the training session and test session. The animals were videotaped in both training and test sessions. A preceding 5 min of acclimatization to the experimental set-up was carried out to reduce the contribution of anxiety and stress on the outcome. The two identical objects (object A and B) were symmetrically fixed to the floor of the box with a distance of 8 cm from the walls and 34 cm from each other. The training session was carried out by placing each mouse in the middle of the two identical objects (A and B) on the opposite sides of the observation chamber. Each animal was allowed to explore the objects in the box for 5 min. After the training, the animals were immediately returned to their home cages for an interval of 24 hr, and the box and objects were cleaned with 70% ethanol to avoid instinctive odorant cues from previous animal during the training phase. An animal was considered to be exploring the object when its head was facing the object (the distance between the head and object is an approximately 1 cm or less) or was touching or sniffing the object. Test sessions were carried out 24 hr after the pretest session. In the test session, object B used during training session was replaced with a new object (object C), which was novel to the mice. Mice were placed back to the box and allowed to freely explore objects A and C for 5 min, and the time spent(s) exploring each object (object A and C) was recorded. Also, the box and objects were cleaned with 70% ethanol to avoid instinctive odorant cues from previous animal during the test phase. The discrimination index (DI), which was used as a measure of non-spatial working memory function, was calculated according to the equation: $DI = Tn - Tf / Tf + Tn$; where Tn and Tf represents the time (T) spent (during a 5 min observation period) exploring novel (n) and familiar (f) objects, respectively.

3.3.4.2 Social interaction Test (SIT)

The effect of MOR on social behaviour (preference) was evaluated using the social interaction test apparatus as described by Monte *et al.*, (2013). The test chamber consists of a 60 × 40 cm Plexiglas box divided into three chambers (A, B and C). Mice moved between chambers through a small opening (6 × 6 cm) in the dividers. Iron restraining cages were placed in each of the two side chambers (A and C). Test (experimental) mouse was placed in the central start chamber (chamber B) and allowed 5 min of exploration time in all chambers. At the end of the 5 min exploration time, the test mouse was removed, and an unfamiliar, same-sex probe mouse from the same experimental group was introduced in one of two restraining cages, in chamber A, while chamber C was without mice. Thereafter, the test mouse was placed back into chamber B and allowed to explore between chambers A (containing probe mouse) and chamber C (without mouse) in the social test box. The time spent(s) exploring chamber A and C was measured with different stopwatches and the social preference was defined as follows: (% exploration time in the social chamber) − (% exploration time in the opposite chamber).

3.3.4.3 Social recognition memory assessment

The effect of MOR on social recognition memory was assessed by Thor and Holloway's method (Thor and Holloway, 1982) as described by Gao *et al.,* (2009). The test is based on the increased tendency of an adult mouse to investigate an unknown juvenile mouse at first exposure and the decrease in the duration of investigation upon second exposure (fixed inter-trial intervals). The decrement in investigation time between the first and second exposure is used as an indication of social recognition memory. Social memory test (SMT) was conducted in an observation chamber measuring 29 x 18 x 12 cm. The SMT test consists of two exposure sessions: the pretest (training; first exposure) session and test (second exposure) session. Prior to the first exposure, group housed adolescent (experimental mice) and juvenile (non-experimental social interacting mice) mice were brought to the observation room to acclimate to the environment for at least 1 hr. The training session was carried out by placing each experimental mouse into an observation chamber (29 x 18 x 12 cm) and allowed to habituate to the test environment for 15 min. Following the 15 min acclimatization period, a non-experimental juvenile mouse was placed into the same observation chamber with the adolescent experimental mouse 10 cm apart for 5 min. The duration of the social investigatory behaviours, which include direct contact or sniffing, following, nosing, grooming, pawing or generally inspecting anybody part of the novel juvenile mouse within 1 cm^2, within the 5 min period was scored by a trained observer. After the first exposure (training session), the juvenile animals were immediately returned to their home cages for an interval of 30 min (for short term memory) and 24 h (for long term memory; consolidation of social recognition memory). The re-exposure (test sessions) were carried out 30 min and 24 h after the pretest session between the same adolescent and juvenile pair; mice were 10 cm apart in initial placement position, and was conducted exactly like the first exposure. The time spent(s) by the adolescent experimental mice investigating the both juvenile familiar mice at inter-trial intervals were measured with stopwatches. Thereafter, the social recognition memory was defined as a decrement in the investigation time between the first and second (fixed inter-trial intervals) exposure (Gao *et al.,* 2009). The test box was cleaned with 70% ethanol to avoid instinctive odorant cues from previous animal to the next.

3.3.5 PREPARATION OF BRAIN TISSUES FOR SPECTROPHOTOMETRIC AND ENZYME-LINKED IMMUNOSORBENT BIOCHEMICAL ASSAYS

Immediately after the behavioural tests, mice (n = 3) in the respective groups were sacrificed under ether anaesthesia and the brains were rapidly removed. The whole brains in section 3.3.3.1 were weighed and immediately homogenized with 5 mL of 10% w/v phosphate buffer (0.1M, P^H 7.4); While the whole brains from sections 3.3.3.2 and 3.3.3.3 were weighed and dissected into specific brain regions [prefrontal cortex (PFC), striatum (ST) and hippocampus (HC)] on a cold ice tray at 4 ^{o} C. Thereafter, the PFC, ST and HC were singly homogenized with 5 mL of 10% w/v phosphate buffer (0.1M, P^H 7.4) respectively. Each brain tissue homogenates were centrifuged at 10,000 g for 10 min at 4 ^{o}C, the pellet was discarded and the supernatants were immediately separated into various portions for the different spectrophotometric and ELISA assays. The preventive and reversal of KET-treated mice (sections 3.3.3.2) were used for neurotransmitter (dopamine, glutamate and 5-HT) ELISA assays; whereas, LPS plus KET-treated mice (sections 3.3.3.3) were used for spectrophotometric myeloperoxidase (MPO) and ELISA inflammatory cytokines (TNF-α and IL-6) assays. However, other spectrophotometric biochemical assays such as antioxidant (glutathione, superoxide dismutase, catalase, malondialdehyde), nitric oxide, acetylcholinesterase assays and protein levels were carried out in all brain samples including whole (sections 3.3.3.1,) and specific brain regions (ST, PFC and HC) from sections 3.3.3.2 and 3.3.3.3, respectively.

3.3.5.1 Estimation of brain glutathione (GSH) concentration

The GSH concentration was measured by the method described (Jollow *et al.,* 1974), which was based upon the development of a relatively stable (yellow) colour when 5′, 5′–dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. Aliquots of brain of whole and specific brain regions (striatum, prefrontal cortex and hippocampus) supernatants (0.4 mL) of each mouse in the respective treatment groups were added to 0.4 mL of 20% trichloroacetic acid (TCA) and mixed by a gentle swirling motion and then centrifuged in a cold (4°C) centrifuge at 10,000 rpm for 20 min. Then, 0.25 mL of the supernatant was added to 2 mL of 0.6 mM DTNB and the final volume of the solution was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). Absorbance was read at 412 nm against blank reagent - [2 mL of 0.6 mM DTNB + 1 mL phosphate buffer (0.2 M, pH 8.0)] using a spectrophotometer. The concentration of reduced GSH in the brain tissues were expressed as nanomoles per gram tissue (nmol/mg protein).

3.3.5.2 Determination of brain superoxide dismutase (SOD) activity

The levels of SOD activity of whole and specific brain regions (striatum, prefrontal cortex and hippocampus) were determined by the method described by Misra and Fridovich (1972). This method is based on the inhibition of superoxide dependent adrenaline auto-oxidation in a spectrophotometer adjusted at 480 nm. Briefly, aliquot of brain supernatants (0.2 mL) of the diluted samples were added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette (Blank) was made up of 2.5 mL buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of distilled water. Then, increase in absorbance at 480 nm was monitored at 60 s intervals for 3 min. Superoxide dismutase activity was expressed as units of adrenaline consumed per minute per mg protein (Misra and Fridovich, 1972).

3.3.5.3 Determination of brain catalase (CAT) activity

The CAT activity of whole and specific brain regions (striatum, prefrontal cortex and hippocampus) were determined by the method of Sinha (1971), which was based on the disappearance of hydrogen peroxide (H_2O_2) in the presence of an enzyme source (catalase). Briefly, brain supernatants (1 mL) of each treatment groups were added to 5 mL of phosphate buffer (pH 7.0) and 4 mL of H_2O_2 solution (800 µmoles). The reaction mixture was mixed by a gentle swirling motion at room temperature. Then, 1 mL of this portion of the reaction mixture was collected and added into 2 mL dichromate/acetic acid reagent. The absorbance was measured using spectrophotometric technique at 570 nm and change in absorbance at 60 s interval. The catalase activity was expressed as µmoles of H_2O_2 decomposed per min per mg protein.

3.3.5.4 Estimation of brain level of malondialdehyde (MDA)

The brain levels of MDA, a marker of lipid peroxidation were measured in the whole and also in different brain regions (striatum, prefrontal cortex and hippocampus) (Okhawa *et al.,* 1979). This assay principle is based on the fact that lipid peroxidation generates unstable lipid peroxides. The polyunsaturated fatty acid peroxides produced, generate MDA upon decomposition. Malondialdehyde form a 1:2 adduct with thiobarbituric acid (TBA) that give rise to a pink color product when heated in acidic pH, with a maximum absorbance of 532 nm. An aliquot of 0.4 mL of the sample was mixed with 1.6 mL of Tris-potassium chloride (Tris-KCl) buffer to which 0.5 mL of 30% trichloroacetic acid (TCA) was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80^{o}C. This was then cooled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA concentration was calculated using a Molar extinction coefficient of 1.56×10^5 M^{-1} CM^{-1} and the value was expressed as micromole of MDA per g tissue (µmol/mg protein).

3.3.5.5 Estimation of brain nitrite level

The brain level of nitrite level, a biomarker for nitrergic transmission and consequently, nitrergic stress, was also measured in the whole brain and specifically in the striatum, prefrontal cortex and hippocampus, according to the method described (Green *et al.,* 1981), which was based on nitrite content. Nitrite levels of brain supernatants of each mouse in the respective treatment

groups were measured based on Griess reaction. Briefly, 100 µL of supernatants were incubated with 100 µL of Griess reagent, which consisted of sulfanilamine in 1% H3PO4/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/1%H3PO4/distilled water, 1:1:1:1, at room temperature for 10 min. The absorbance was measured at 550 nm via a UV-spectrophotometer. The standard curve was prepared with several concentrations of $NaNO_2$ (ranging from 0.75 to 100µM) and was expressed as µmol/mg protein.

3.3.5.6 Determination of brain acetylcholinesterase (AChE) activity

Acetylcholinesterase enzyme activity, a marker for cholinergic neurotransmission, was measured in the whole and specific brain regions (striatum, prefrontal cortex and hippocampus) by the Ellman's assay (Ellman *et al.,* 1961) as described by Eduviere *et al.,* (2016). Briefly, aliquots of brain homogenates (0.4 mL) of each mouse in the respective treatment groups were added to 2.6 mL of phosphate buffer (0.1 M, pH 7.4) and 0.1 mL of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). Then 0.1 mL of acetylthiocholine iodide solution was added to the reaction mixture. The absorbance was read using a spectrophotometer at a wavelength of 412 nm and change in absorbance for 10 min at 2 min interval was recorded. The rate of acetylcholinesterase activity was measured by following the increase of colour produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined and the rate of acetylcholinesterase activity was estimated and expressed as µmol/min/mg tissue.

3.3.5.7 Determination of brain myeloperoxidase (MPO) activity

The myeloperoxidase activity was determined in the striatum, prefrontal cortex and hippocampus according to the method described by Bradley *et al*. (1982). Briefly, each brain supernatants were suspended in extraction buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer; pH 6.0) and frozen at -20^{o}C. The process of freeze-thawed and sonication for 10 s cycle was repeated three times. The suspension was finally centrifuged at 15,000 rpm at 4^{o}C for 15 min. Myeloperoxidase activity was assayed by adding 0.2 mL of supernatant to 2.8 mL of mixed solution (containing 0.167 mg/mL O-dianisidine in 50 mM potassium phosphate buffer and 0.15 mM H_2O_2). The change in absorbance at 450 nm was monitored over 3 min using UV/VIS Spectrophotometer (INESA). One unit of MPO was defined as that giving a change in absorbance of 0.001 per min and the specific activity expressed as unit of MPO per milligram of protein.

3.3.5.8 Estimation of brain protein level

Protein was measured in all whole and specific brain regions (striatum, prefrontal cortex and hippocampus) supernatants according to the method of Gornall *et al.,* (1949) using the Biuret method. Briefly, 1 mL of the diluted brain supernatant was collected and added to 3 mL of Biuret reagent in triplicate. The mixture was incubated at room temperature for 30 min after which the absorbance was read at 540 nm using distilled water as blank. Bovine serum albumin (1 mg/mL) was used as standard and was measured in the range of 0.01-0.1 mg/mL.

3.3.5.9 Determination of brain dopamine level

Regional brain dopamine levels of the striatum, prefrontal cortex and hippocampus were estimated in mouse brains using ELISA kit (Abnova, Germany; CAT NO KA1887) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Accordingly, brain samples of each treatment groups were extracted using Abnova extraction plate coated with boronate affinity gel, by adding 10 µL of standards, controls and brain supernatant (300 µL) to 250 µL of distilled water, 50 µL of assay buffer (consisting of 1 M hydrochloric acid and a non-mercury preservative) and 50 µL of extraction buffer into the respective wells of the extraction plate. The reaction mixture was incubated for 40 min at room temperature (25°C) on a shaker (approx. 600 rpm). Then, 150 µL of acetylation buffer, 25 µL of acetylation reagent and 175 µL of hydrochloric acid were added to all wells, and then incubated for 25 min at room temperature (25°C) on a shaker (approx. 600 rpm). Thereafter, dopamine enzyme immunoassay was carried out by adding 25 µL of the extracted standards, controls and extracted brain supernatant (50 µL) to 25 µL of the enzyme solution (S-adenosyl-L-methionine) and 25 µL of hydrochloric acid to each well of dopamine microtiter strips, and incubated for 30 min at room temperature (25°C) on a shaker (approx. 600 rpm). Dopamine antiserum (rabbit anti-dopamine antibody, 50 µL) and 100 µL of enzyme conjugate (goat anti-rabbit immunoglobulins, conjugated with peroxidase) were added to reaction mixture in all the wells and incubated for 2 hr and 30 min at room temperature (25°C) on a shaker (approx. 600 rpm). Thereafter, 100 µL of the chromogenic substrate (containing tetramethylbenzidine and hydrogen peroxide) was added into all wells and incubated for 30 min at room temperature (25°C) on a shaker (approx. 600 rpm). Finally, 100 µL of stop solution (0.25 M sulfuric acid) was added to all wells and microtiter plate was shaken to ensure homogenous solution, before the reading was taken at 450 nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader equipped with So ftmax Pro v 5.4 (SMP 5.4).

Thereafter, 4-parameter sigmoid minus curve fit was used to determined unknown concentrations of dopamine in specific brain regions i.e., striatum, prefrontal cortex and hippocampus in ng/mL.

3.3.5.10 Estimation of brain glutamate level

Regional brain glutamate concentrations were estimated in the striatum, prefrontal cortex and hippocampus using ELISA kit (Abnova, Germany; CAT NO KA1894) according to the manufacturer's instructions. Aliquot (100 µL) of brain sample, standards and controls was extracted using Abnova extraction plate by adding 100 µL of diluent to all wells and the reaction mixture was mixed by a gentle swirling motion and incubated for 10 min at room temperature (25°C). Then, 10 µL of NaOH, 50 µL of the equalizing reagent (lyophilized protein), 10 µL of the D-reagent (crossed-linked dimethylsulfoxide) and 75 µL of Q-buffer (Buffered TRIS) were added to 25 µL of extracted standard, controls and regional brain samples in the appropriate wells of the reaction plate, and mixed for 2 hr 10 min at room temperature (25°C) on a shaker (approx. 600 rpm). Thereafter, glutamate enzyme immunoassay was carried out by adding 25 µL of the prepared standards, controls and brain samples to 50 µL of glutamate antiserum (rabbit anti-glutamate antibody) in the appropriate wells of the glutamate microtiter strips and mixed by a gentle swirling motion. Then, the reaction mixture is covered with adhesive foil and incubated for 20 hr (overnight) at room temperature (25°C). After which, content was aspirated from all wells, plate was washed 3x with 300 µL of wash buffer and blotted by tapping the inverted plate on an absorbent surface. Thereafter, 100 µL of enzyme conjugate (goat anti-rabbit immunoglobulins, conjugated with peroxidase) was added to reaction mixture in all the wells and incubated for 30 min at room temperature (25°C) on a shaker (approx. 600 rpm); followed by the addition of 100 µL of the chromogenic substrate (containing tetramethylbenzidine and hydrogen peroxide) into all wells and 30 min incubation at room temperature (25°C) on a shaker (approx. 600 rpm). The reaction was halt by adding 100 µL of stop solution (0.25 M sulfuric acid) to all wells and immediately absorbance was read within 10 min at 450 nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with So ftmax Pro v 5.4 (SMP 5.4). Thereafter, 4-parameter sigmoid minus curve fit was used to determined unknown regional (striatum, prefrontal cortex and hippocampus) brain sample concentrations of glutamate in µg/mL.

3.3.5.11 Determination of brain 5-HT level

The level of 5-HT was estimated in mouse brain regions (striatum, prefrontal cortex and hippocampus) using ELISA kit (Abnova, Germany; CAT NO KA1894) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Briefly, brain samples of each treatment groups were acetylated using Abnova acetylation tubes, by adding 25 µL of standards, controls and brain supernatant to 500 µL of acetylation buffer, 25 µL of acetylation reagent into the respective reaction tubes. The reaction mixture was mixed by a gentle swirling motion and incubated for 15 min at room temperature (25°C). Thereafter, 5-HT enzyme immunoassay was carried out by adding 25 µL of the acetylated standards, controls and brain supernatant to 100 µL of 5-HT antiserum (rabbit anti-serotonin antibody) to each well of 5-HT microtiter strips, and incubated for 30 min at room temperature (25°C) on a shaker (approx. 600 rpm). Thereafter, content was aspirated from all wells, plate was washed 3x with 300 µL of wash buffer and blotted by tapping the inverted plate on an absorbent surface. Then, enzyme conjugate (100 µL) was added to all wells and re-incubated for 15 min at room temperature (25°C) on a shaker (approx. 600 rpm). Thereafter, 100 µL of the chromogenic substrate (containing tetramethylbenzidine and hydrogen peroxide) was added into all wells and incubated for 30 min at room temperature (25°C) on a shaker (approx. 600 rpm). Absorbance of the reaction mixture in the wells was read within 10 min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with So ftmax Pro v 5.4 (SMP 5.4) at 450 nm, after adding 100 µL of stop solution (0.25 M sulfuric acid) to all wells and microtiter plate shaken to ensure homogenous solution. Thereafter, 4-parameter sigmoid minus curve fit was used to determine the concentrations of 5-HT in the striatum, prefrontal cortex and hippocampus in ng/mL.

3.3.5.12 Estimation of brain tumour necrosis factor-alpha (TNF-α)

Specific brain regions (striatum, prefrontal cortex and hippocampus) of TNF-α concentrations were estimated using ELISA MAX^{TM} Deluxe kit (BioLegend, USA; CAT NO 430904) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Briefly, TNF-α enzyme immunoassay was carried out by adding 100 µL of brain samples, standards and controls to each wells of an overnight (18 hr, 4°C) mouse TNF-α capture antibody incubated 96 well plate. After which, plate was sealed with adhesive foil and incubated for 2 hr room temperature (25°C) on a shaker (approx. 500 rpm). Then, 100 µL of biotinylated goat polyclonal anti-mouse TNF-α detection antibody and avidin-horseradish

peroxidase (avidin-HRP) solutions were added to each wells; plate was sealed and incubated for 1 hr 3 min at room temperature (25°C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate [3, 3′, 5, 5′- tetramethylbenzidine (TMB)] was added to each well and incubated in the dark for 15 min at room temperature (25°C) before the addition of stop solution (100 µL) and the absorbance was read at 450 nm within 15 min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4). Thereafter, a log-log logistic 4-parameter curve-fitting was used to determine the regional (striatum, prefrontal cortex and hippocampus) brain concentrations of TNF- α in pg/mL.

3.3.5.13 Determination of brain interleukin-6 (IL-6)

Regional brain IL-6 concentrations were estimated in the striatum, prefrontal cortex and hippocampus using ELISA MAX^{TM} Deluxe kit (BioLegend, USA; CAT NO 431304) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. IL-6 enzyme immunoassay was carried out by adding 100 µL of standards, control and regional brain samples to each wells of an overnight (18 hr, 4°C) mouse IL-6 capture antibody incubated 96 well plate. After which, microplate was sealed with adhesive foil and incubated for 2 hr room temperature (25°C) on a shaker (approx. 500 rpm). Then, biotinylated rat monoclonal anti-mouse IL-6 detection antibody (100 µL) and avidin-HRP (100 µL) solutions were added to each wells, and plates were sealed and incubated for 1 hr 3 min at room temperature (25°C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate (TMB) was added to each wells and incubated in the dark for 20 min at room temperature (25°C). The absorbance was read at 450 nm within 15 min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4), after the addition of 100 µL of stop solution on a shaker to achieve homogenous solutions. A log-log logistic 4-parameter curve-fitting was used to determine the regional concentration of IL-6 in the striatum, prefrontal cortex and hippocampus in pg/mL.

3.3.6 PREPARATION OF BRAIN TISSUES FOR HISTOLOGY (HISTOMORPHOMETRY) AND IMMUNOHISTOCHEMISTRY

As regards histology, histomorphometry and immunohistochemistry, mice (n = 3) in the respective groups were anaesthetized with ether and perfused transcardially with sterile phosphate buffered saline (PBS). Then, the mice were dissected, flushed with normal saline and perfused with 10% buffered formaldehyde. Thereafter, their brains were harvested and fixed with 10% phosphate buffered formaldehyde. The brains were then subjected to the routine method for paraffin wax embedment to obtain paraffin wax embedded tissue blocks. Transverse (sagittal plane) sections (5–6 µm thick) of the striatum (caudate and putamen), prefrontal cortex and CA1 region of hippocampus were obtained with the aid of microtome (Leica, Germany) and the sections were fixed on glass slides.

3.3.6.1 Histological studies and Histomorphometry

The brain sections were processed through the stages of fixation, dehydration, clearing, infiltration, embedding and staining using haematoxylin and eosin to demonstrate general histology of the striatum (caudate and putamen), prefrontal cortex and hippocampus (CA1) regions (Bancroft and Gamble, 2008). Therefore, slides were viewed using Leica DM 500 digital light microscope (Germany) and images captured with Leica ICC50 E digital camera (Germany) connected to a computer interface (MagnaFire). Histomorphometric analyses were done using computerized image analyzer (ApAChE Open Office Tm4 4.0.0. software version). Using an objective lens (x 40) and an ocular lens (x 10), the viable and pyknotic neurons of the cerebral cortex, hippocampus (CA1) and striatum (caudate and putamen) of the brains were observed and counted as circular, cytoplasmic membrane-intact cells, without any nuclear condensation or pyknotic spot. Mice brain atlas (Paxinos and Watson, 2007) with an anatomical explanation was used. The density of pyknotic neurons according to the method described (Taveira *et al.*, 2013), was calculated for in ten different areas of the slides of the striatum (caudate and putamen), prefrontal cortex and CA1 region of the hippocampus in each experimental group. Photomicrograph calibration was done using Micro-Manager (Edelstein *et al.*, 2014).

3.3.6.2 Immunohistochemistry

The expressions of immunopositive cells of glutamic acid decarboxylase 67 (GAD_{67}), nicotinamide adenine denucleotide phosphate oxidase-2 [(Nox-2), gp91 phox subunit] and brain derived neurotrophic factor (BDNF) were carried out in mouse treated with morin on preventive and reversal treatment protocols of KET-induced cellular alterations using immunohistochemistry kits for GAD_{67} (Biorbyt, UK), Nox-2 (Santa cruz, Germany) and BDNF (Thermo Fisher scientific, USA). Also, the expressions of immunopositive cells of cyclooxygenase-2 (COX-2), inducible nitric-oxide synthase (iNOS) and nuclear transcription factor-kappa B (NFκB) were determined in mouse brains treated with LPS plus KET using immunohistochemistry kits [COX-2, iNOS, NFκB (Santa cruz, Germany)] according to the manufacturer's instructions and modified method of Edelstein *et al*. (2014). Briefly, brain tissue sections [striatum (caudate and putamen), prefrontal cortex and hippocampus (CA1) regions] were subjected to the process of deparaffinization and hydration using xylene and graded alcohols (100, 90 and 80%) for 5 min, respectively. The slides were then washed twice with distilled water and incubated with peroxidase block for 5-10 min at room temperature (25°C). Thereafter, tissue sections were rinsed with distilled water, placed in citrate buffer tank and heated in a water bath for 3-5 min for antigen retival. Slides were washed with phosphate buffer saline (PBS) containing 0.02% Tween 20 thrice, before adding protein blocking solution for 5-10 min at room temperature (25°C). Tissue sections were incubated with primary antibody (1:300) for 20-30 min at room temperature (25°C). Slides were then washed with PBS 5-7 times and incubated with one-step horseradish peroxidase (HRP) polymer for 20-30 min at room temperature (25°C). Also, tissue sections were rinsed 5-7 times with PBS containing 0.02% Tween 20 and 2-3 times with distilled water. Few drops of ready to use 3, 3′- diaminobenzidine (DAB) reagent was added on each tissue sections and allowed to incubate for 6-10 min at room temperature (25°C) before washing with PBS 5-7 times and then with distilled water. Then, slides were incubated with hematoxylin for 30-60 s, rinsed with distilled water and allowed to drain before mounting with appropriate mountant. Images acquired using Leica ICC50 E Digital Camera (Germany) connected to a computer interface (MagnaFire).and an Olympus BX-51 Binocular research microscope, and expression of positive cells were analysed using Image J software (NIH, Bethesda, MD, USA) (Edelstein *et al.,* 2014).

3.3.6.3 Golgi staining of pyramidal neurons of the prefrontal cortex

In order to get detailed information on the structure of the pyramidal neuron of the prefrontal cortex, Golgi silver impregnation staining technique was adapted according to the method previously described (Angulo *et al.,* 1996). Accordingly, thereafter 24 hr of perfusion, the perfused brains were immersed in potassium dichromate solution for 5 days (5 changes every 24 hr) and then silver nitrate for 3 days (3 changes every 24 hr). Thereafter, the brain tissues were infiltrated for 30 min in molten wax, embedded in paraffin wax and cooled overnight at 4^{o}C. The paraffin blocks were trimmed and sectioned at 60 µm, transferred into graded series of alcohol (80%, 90%, and two changes of 100%) for 2 min and cleared in xylene for 10 min. Stained brain tissues were thereafter mounted on glass slides using DPX as mountant. Thereafter, slides were viewed using Leica DM 500 digital light microscope (Germany) and images were thereafter captured using Leica ICC50 E digital camera (Germany). The transverse diameters of the soma and dendritic aborization of the pyramidal neurons of the prefrontal cortex were measured and data analyzed using computerized image analyzer (Image J/Micro-Manager 1.4). Photomicrograph calibrations were also done using Image J/Micro-Manager 1.4 (Edelstein *et al.,* 2014).

3.4 STATISTICAL ANALYSIS

The Data were expressed as Mean ± S.E.M. (standard error of mean). The data were analyzed using one-way analysis of variance (ANOVA) or two-way ANOVA followed by Bonferroni *post-hoc* test for multiple comparisons where appropriate using Graph Pad Prism software version 5. A level of statistical significant difference was set at $P < 0.05$ for all tests. The strengths of significance were further shown differently at $P < 0.01$; $P < 0.05$ and $P < 0.001$ respectively.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of morin on spontaneous motor activity (SMA) in the open-field test

The effect of MOR on SMA, as measured by the numbers of line crossing(s) using open-field test is shown in Figure 4.1. Administration of MOR 25 mg/kg, i.p did not reduce SMA when compared to vehicle (10 mL/kg, i.p.)-treated group. On the other hand, treatment with higher doses of MOR (50 and 100 mg/kg, i.p.) significantly ($p < 0.05$) decreased SMA in the OFT in comparison to vehicle-treated group. Similarly, the reference typical and atypical antipsychotic drugs, haloperidol (HLP) (1 mg/kg, i.p.) and risperidone (RIS) (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced SMA in comparison with the vehicle control group (Figure 4.1).

4.2 Effect of morin on ketamine-induced hyperlocomotion

The effect of MOR on KET-induced hyperlocomotion in the OFT is shown in Figure 4.2. Administration of KET (10 mg/kg) significantly ($p < 0.05$) induced hyperlocomotion compared to vehicle (10 mL/kg, i.p.) treated group, as indexed by the increase in the number of line crossings in the OFT. Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) produced a significant decrease in hyperlocomotion by KET (10 mg/kg, i.p.) when compared with KET-treated mice ($p < 0.05$). The standard agents, HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) reduced KET-induced hyperlocomotion in a significant ($p < 0.05$) manner (Figure 4.2).

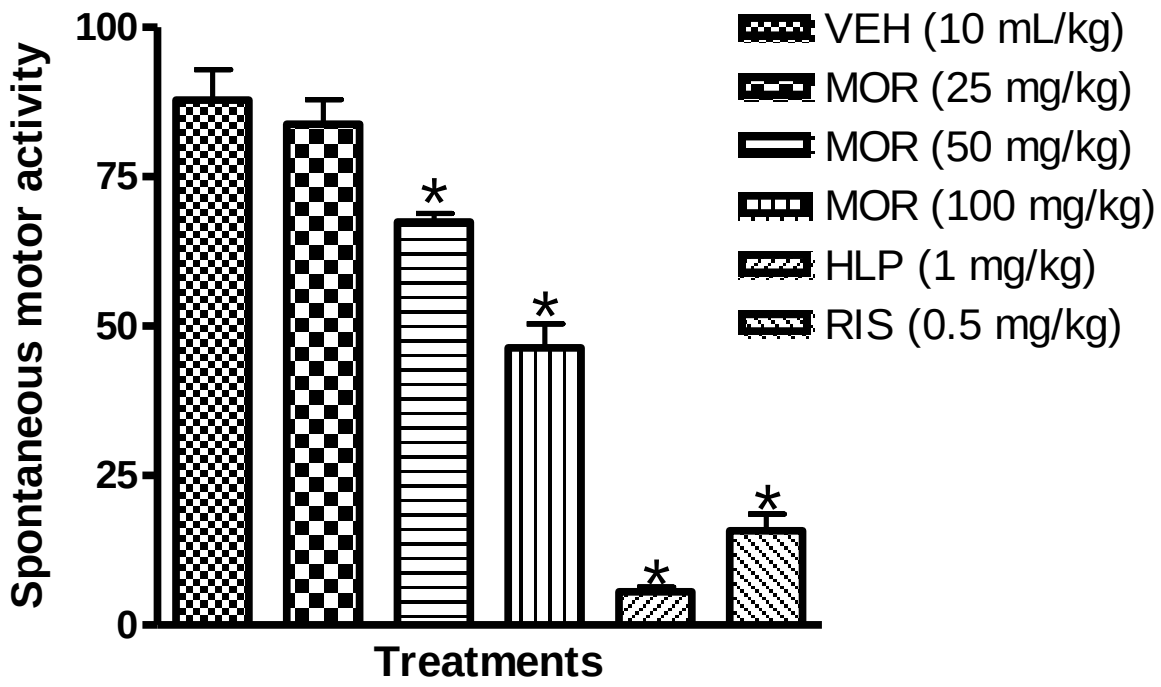


Figure 4.1: Effect of morin on Spontaneous motor activity in open-field test in mice

Bar represents the mean ± S.E.M of 5 animals / group. $^{*}P < 0.05$ compared to vehicle group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

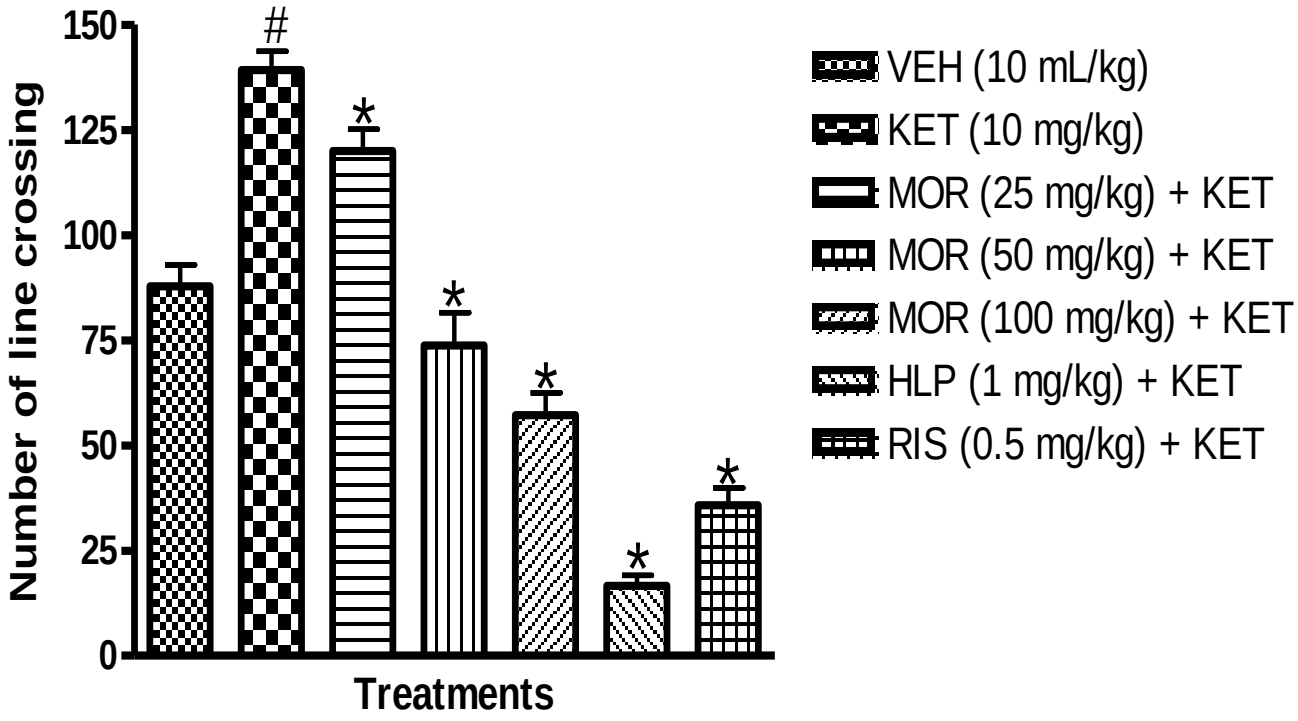


Figure 4.2: Effect of morin on ketamine-induced hyperlocomotion in open field test in mice

Bar represents the mean ± S.E.M of 5 animals / group. $^{\#}P < 0.05$ compared to vehicle group; $^{*}P < 0.05$ as compared to ketamine group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.3 Effect of morin on apomorphine-induced stereotypy

The effect of MOR on APO (2 mg/kg, i.p.)-induced stereotyped behaviour, as measured by the stereotypy count is shown in Table 4.1. Administration of APO (2 mg/kg, i.p.) caused profound stereotyped behaviours in mice in comparison with normal control. Pretreatment of animals with 25 mg/kg, i.p., of MOR produced significant ($p < 0.05$ and $p < 0.001$) reduction in stereotyped behaviours at 50 and 60 min time intervals when compared with APO (2 mg/kg)-treated group. Pretreatment with higher doses of MOR (50 and 100 mg/kg, i.p.) significantly ($p < 0.01$, $p < 0.05$ and $p < 0.001$) reduced stereotyped score from 10-60 min time intervals relative to APO group. HLP (1 mg/kg, i.p.) pre-treated mice demonstrated a marked ($p < 0.001$) inhibition in stereotyped score from 5–60 min time intervals, and RIS (0.5 mg/kg, i.p.) demonstrated significant ($p < 0.05$ and $p < 0.001$) inhibition of stereotyped behaviour from 5–60 min time intervals relative to APO-treated group respectively (Table 4.1).

4.4 Effect of morin on ketamine-induced stereotypy

As shown in Table 4.2, KET (10 mg/kg, i.p.) significantly ($p < 0.05$ and $p < 0.001$) increased stereotypic behavioural activity compared to vehicle control. Pretreatment with MOR (25 mg/kg, i.p.) produced significant ($p < 0.05$ and $P < 0.01$) reduction of KET-induced stereotyped behaviours at 10-40 min time intervals compared to KET (10 mg/kg)-treated group. Pretreatment with higher doses of MOR (50 and 100 mg/kg, i.p.) consistently produced a significant ($p < 0.05$ and $p < 0.001$) inhibition of stereotyped behaviours at 10-50 min time points relative to KET control group. Haloperidol treated group also showed a significant ($p < 0.01$ and $p < 0.001$) reduction in stereotyped score at 5–50 min compared to KET treated group. Furthermore, RIS (0.5 mg/kg, i.p.) significantly ($p < 0.001$) inhibited stereotyped behaviours from 5–50 min time intervals compared to KET control respectively (Table 4.2).

Table 4.1: Effect of morin on apomorphine-induced stereotypy behaviour in mice

Treatments	Stereotypy score (Mean ± S.E.M.) at						
	5 min	10 min	20 min	30 min	40 min	50 min	60 min
VEH (10 mL/kg)	2.22 ± 0.45	2.02 ± 0.56	1.78 ± 0.43	1.55 ± 0.33	1.12 ± 0.21	1.05 ± 0.09	1.34 ± 0.11
APO (2 mg/kg)	4.60 ± 0.67 *	5.40 ± 0.67 #	5.20 ± 0.37 #	5.20 ± 0.66 #	5.60 ± 0.81 #	6.00 ± 0.70 #	5.00 ± 0.31 #
MOR (25 mg/kg) + APO	4.60 ± 0.67	4.60 ± 0.40	4.40 ± 0.60	4.60 ± 0.40	4.00 ± 0.44	4.20 ± 0.48 a	2.00 ± 0.54 c
MOR (50 mg/kg) + APO	4.20 ± 0.48	3.40 ± 0.67 a	3.00 ± 0.63 b	2.60 ± 0.24 c	2.80 ± 0.66 c	2.40 ± 0.40 c	0.00 ± 0.00 c
MOR (100 mg/kg) + APO	2.60 ± 0.60 a	2.20 ± 0.37 c	2.80 ± 0.66 b	2.20 ± 0.20 c	1.20 ± 0.48 c	0.00 ± 0.00 c	0.00 ± 0.00 c
HLP (1 mg/kg) + APO	1.80 ± 0.20 c	2.00 ± 0.00 c	1.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
RIS (0.5 mg/kg) + APO	2.20 ± 0.20 b	2.20 ± 0.20 c	3.00 ± 0.54 b	2.20 ± 0.80 c	1.40 ± 0.60 c	0.00 ± 0.00 c	0.00 ± 0.00 c

Value represents the mean ± S.E.M of 5 animals / group. Data were analyzed by two-way ANOVA, followed by Bonferroni *post-hoc* test: * $p < 0.01$ compared to vehicle VEH group; # $p < 0.001$ compared to VEH control group; a $p < 0.05$ compared to APO group; b $p < 0.01$ compared to APO group; c $p < 0.001$ compared to APO group.

VEH = Vehicle, **APO =** Apomorphine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

Table 4.2: Effect of morin on ketamine-induced stereotypy behaviour in mice

Treatments	Stereotypy score (Mean ± S.E.M.) at					
	5 min	10 min	20 min	30 min	40 min	50 min
VEH (10 mL/kg)	2.22 ± 0.45	2.02 ± 0.56	1.78 ± 0.43	1.55 ± 0.33	1.12 ± 0.21	1.05 ± 0.09
KET (10 mg/kg)	4.40 ± 0.67$^{\$}$	5.80 ± 0.58$^{\#}$	6.80 ± 0.80$^{\#}$	6.80 ± 0.92$^{\#}$	7.00 ± 0.54$^{\#}$	3.00 ± 0.31$^{\$}$
MOR (25 mg/kg) + KET	2.60 ± 0.67	3.20 ± 0.73^{b}	4.60 ± 0.50^{a}	3.80 ± 1.06^{b}	5.80 ± 0.63	2.80 ± 0.37
MOR (50 mg/kg) + KET	2.80 ± 0.48	2.80 ± 0.58^{c}	1.20 ± 0.73^{c}	1.60 ± 0.50^{c}	0.80 ± 0.37^{c}	0.00 ± 0.00^{c}
MOR (100 mg/kg) + KET	3.40 ± 0.40	2.00 ± 0.83^{c}	0.20 ± 0.20^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
HLP (1 mg/kg) + KET	2.20 ± 0.48^{b}	1.00 ± 0.31^{c}	5.40 ± 0.50	3.20 ± 1.24^{c}	0.40 ± 0.24^{c}	0.00 ± 0.00^{c}
RIS (0.5 mg/kg) + KET	0.80 ± 0.20^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.20 ± 0.20^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Value represents the mean ± S.E.M of 5 animals / group. Data were analyzed by two-way ANOVA, followed by Bonferroni *post-hoc* test: $^{\$}p < 0.05$ compared to VEH control group; $^{\#}p < 0.001$ compared to vehicle VEH group; a $p < 0.05$ compared to KET group; b $p < 0.01$ compared to KET group; c $p < 0.001$ compared to KET group.

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.5 Effect of morin on ketamine-enhanced immobility in forced swim test

The effect MOR on KET-enhanced immobility in forced swim test based on immobility time is shown in Figure 4.3. Ketamine (30 mg/kg, i.p.) significantly increased the immobility time in the FST relative to vehicle-treated group. Administration of MOR (25, 50 and 100 mg/kg, i.p.) significantly ($p < 0.05$) dose-dependently reduced immobility time when compared with KET-treated mice. Treatment with the standard atypical drug, RIS (0.5 mg/kg) produced significant decrease in immobility time when compared with KET-treated group. HLP (1 mg/kg, i.p.) did not cause any significant change on KET-enhenced immobility in the FST compared to KET control mice (Figure 4.3).

4.6 Effect of morin on ptosis test

The effect of MOR on ptosis, as measured by the closure of the eye-lid using the ptosis scale at 30^{th}, 60^{th} and 90^{th} min is shown in Table 4.3. Pretreatment of animals with 25 mg/kg, i.p., of MOR induced significant ($p < 0.001$) ptosis at the 90^{th} min compared to vehicle-treated group; higher doses of MOR (50 and 100 mg/kg, i.p.) produced significant ($p < 0.05$ and $p < 0.01$) ptosis induction at the 30^{th} and 60^{th} min respectively, relative to vehicle-treated group. MOR 50 and 100 mg/kg, i.p. produced the same levels of significance ($p < 0.001$) on ptosis at the 90^{th} min compared to vehicle-treated group (Table 4.3). HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) produced significant ($p < 0.001$) ptosis 30^{th}, 60^{th} and 90^{th} min in comparison to vehicle-treated group (Table 4.3).

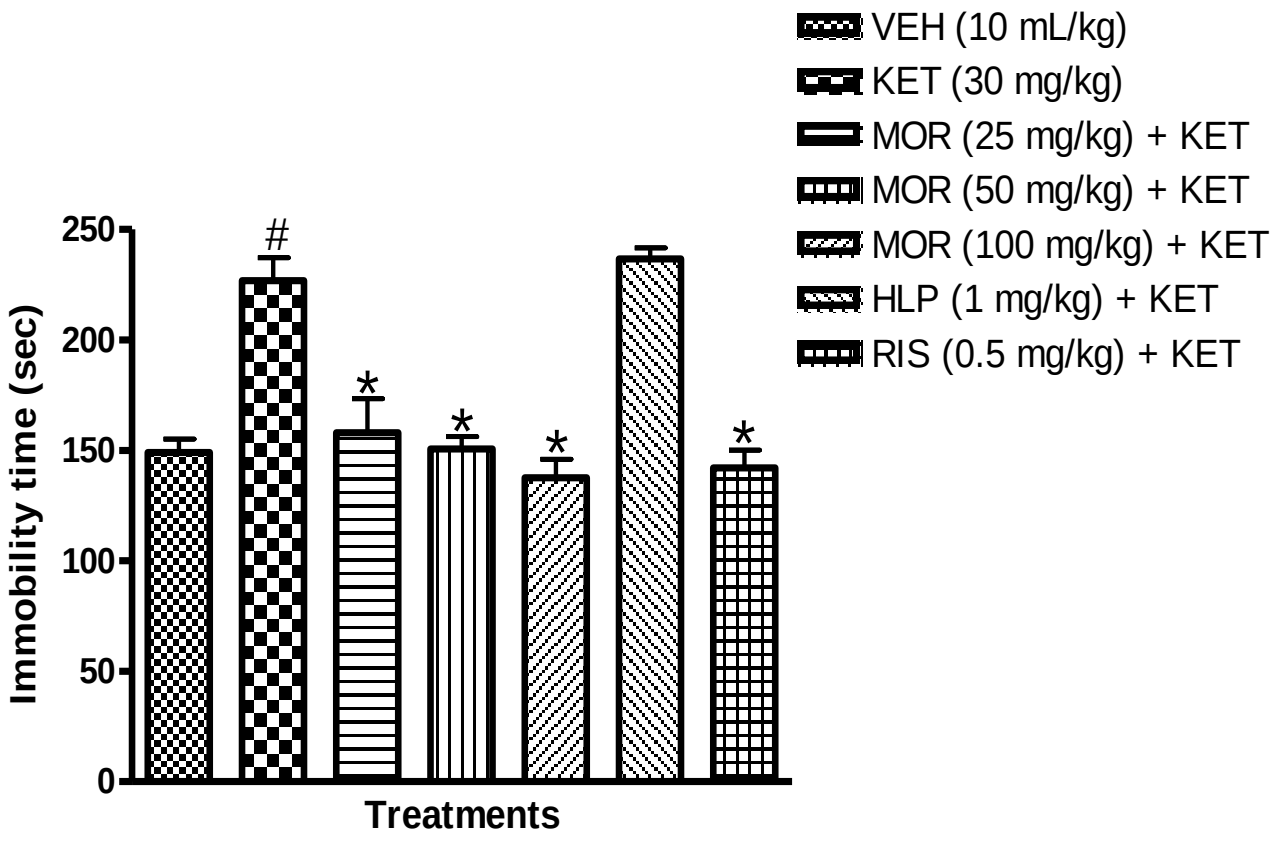


Figure 4.3: Effect of morin on ketamine-enhanced immobility in forced swim test in mice

Value represents the mean ± S.E.M of 5 animals / group. One-way ANOVA followed by Bonferroni *post-hoc* test showed that there is significant effect between various treatment groups. $^{\#}P < 0.05$ as compared to vehicle group, $^{*}P < 0.05$ as compared with ketamine group.

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

Table 4.3: Effect of morin on ptosis test in mice

Treatments	Dose (mg/kg)	30 min	60 min	90 min
VEH	10 mL/kg	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20
MOR	25	0.00 ± 0.00	0.40 ± 0.24	0.80 ± 0.20**
MOR	50	0.80 ± 0.20*	1.20 ± 0.20**	1.40 ± 0.24***
MOR	100	0.80 ± 0.20**	2.0 ± 0.00**	2.40 ± 0.24***
HLP	1	2.40 ± 0.24***	3.60 ± 0.24***	4.00 ± 0.00***
RIS	0.5	2.80 ± 0.20***	3.00 ± 0.00***	3.20 ± 0.20***

Values represent the mean ± S.E.M of 5 animals / group. *P < 0.05 compared to control group, **P < 0.01 compared to control group, ***P < 0.001 compared to control group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.7 Cataleptogenic effect of morin in mice

The effect of MOR (25, 50 and 100 mg/kg, i.p.) on cataleptic behaviour in mice is shown in Figure 4.4. Treatment with both MOR (25, 50 and 100 mg/kg) and RIS (0.5 mg/kg) did not cause any significant ($p > 0.05$) change in DL of mice at 30^{th}, 60^{th} and 90^{th} min time interval when compared to vehicle control group. However, treatment with HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) increased DL at 30^{th}, 60^{th} and 90^{th} min in comparison with vehicle control (Figure 4.4).

4.8 Effects of morin on motor coordination

The effect of MOR on motor coordination, based on the time of performance and number of fall(s) in the rota rod test is shown in Table 4.4. Pretreatment with both MOR (25, 50 and 100 mg/kg, i.p.) did not cause any change in the time of performance and number of falls relative to vehicle control groups. Similarly, RIS (0.5 mg/kg, i.p.) produced no significant effects in the time of performance and number of falls when compared to vehicle control groups. On the other hand, HLP (1 mg/kg, i.p.) pretreatment produced a significant ($p < 0.05$) decrease in the time of performance and increase in the number of falls compared to vehicle-treated mice. KET (10 mg/kg, i.p.) significantly ($p < 0.05$) decreased the time of performance and increased the number of falls of mice from the bar compared to controls (Table 4.4). Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly increased time of performance and decreased the number of falls on the bar in comparison with KET control groups, suggesting increased motoric activity on the roller bar. However, HLP (1 mg/kg, i.p.) did not prevent the effect of KET on the time of performance and number of falls when compared to KET control groups (Table 4.4).

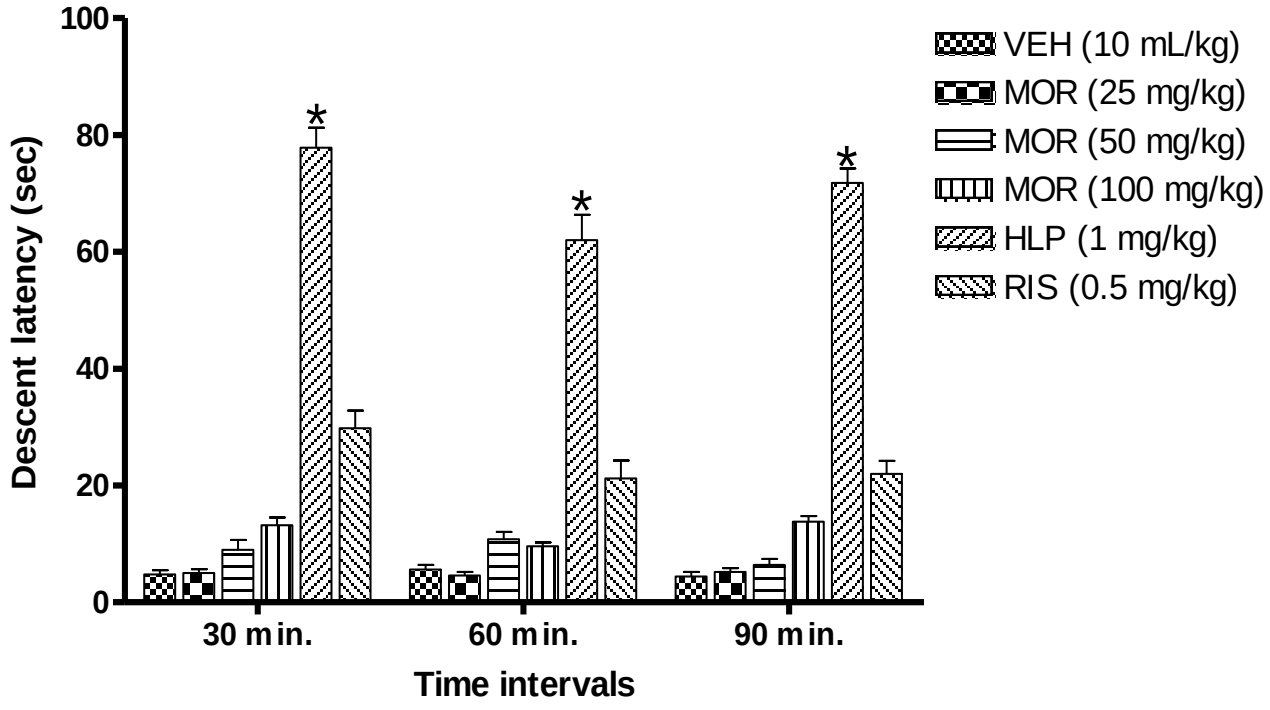


Figure 4.4: Cataleptogenic effect of morin in mice

Bar represents the mean ± S.E.M of 5 animals / group. $^{*}P < 0.05$ compared to vehicle group. Data was considered significant above 60 s (two-way NOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

Table 4.4: Effect of acute administration of morin on the rota rod motor coordination test in mice

Treatments	Time of performance (s)	Number of fall(s)
VEH (10 mL/kg)	119.4 ± 119.4	0.20 ± 0.20
MOR (25 mg/kg)	118.8 ± 1.20	0.20 ± 0.20
MOR (50 mg/kg)	119.0 ± 0.77	0.40 ± 0.24
MOR (100 mg/kg)	117.6 ± 1.50	0.80 ± 0.24
HLP (1 mg/kg)	52.40 ± 3.91*	3.80 ± 0.37*
RIS (0.5 mg/kg)	112.20 ± 4.88	1.40 ± 0.24*
KET (10 mg/kg)	19.40 ± 2.50*	6.20 ± 0.73*
MOR (25 mg/kg) + KET	31.80 ± 2.13#	5.60 ± 0.67
MOR (50 mg/kg) + KET	47.00 ± 4.70#	4.00 ± 0.45#
MOR (100 mg/kg) + KET	50.00 ± 5.80#	3.40 ± 0.24#
HLP (1 mg/kg) + KET	13.20 ± 2.59	6.40 ± 0.40
RIS (0.5 mg/kg) + KET	51.40 ± 5.99#	4.00 ± 0.45#

Values represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group, and #$p < 0.05$ compared to KET (two-way NOVA followed by Bonferroni *post-hoc* test)

VEH = Vehicle, **KET =** Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.9 INVOLVEMENT OF DOPAMINERGIC SYSTEM ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

4.9.1 Effects of haloperidol on the activity of morin

The effect of HLP (dopamine D_2 receptor antagonist) blockade on MOR-induced antipsychotic-like activity on SMA in the OFT, percentage (%) correct alternation behaviour in the YMT and immobility time in the FST are shown in Table 4.5. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) reduced SMA (positive symptom) when compared with vehicle (10 mL/kg) control group; and significantly ($p < 0.05$) increased the in % correct alternation behaviour in the YMT, suggesting memory enhancing activity (cognitive symptom). MOR (100 mg/kg, i.p.) produced a significant ($p < 0.05$) decrease in the duration of immobility in the FST, suggesting decrease in behavioural despair (negative symptom). HLP (0.2 mg/kg, i.p.) given 15 min before treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) blocked and prevented the antipsychotic-like activity of MOR against locomotor activity by increasing the number of line crossings relative to MOR-treatment alone. Thus, suggesting the involvement of blockade of dopamine D_2 receptor transmission by MOR on SMA. HLP (0.2 mg/kg, i.p.) administration prior to MOR treatment caused no significant decrease in the % alternation behaviour in the YMT when compared with MOR-treated group. The combination of HLP (0.2 mg/kg, i.p.) did not block the effect of MOR (100 mg/kg, i.p.) on the reduction in the duration of immobility in the FST in comparison with MOR-treated group. Treatment with HLP (0.2 mg/kg, i.p.) alone decreased locomotor activity in the OFT and increased immobility time in the FST in a significant ($p < 0.05$) manner when compared with vehicle control groups respectively. However, HLP (0.2 mg/kg) did not show any significant effect on the % alternation behaviour in the YMT (Table 4.5).

Table 4.5: Effects of haloperidol on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 6.43	62.80 ± 2.05	142.0 ± 6.27
HLP (0.2 mg/kg, i.p.)	17.40 ± 1.56*	62.20 ± 4.94	204.4 ± 8.69*
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00± 3.17*	108.8 ± 6.39*
HLP + MOR	36.80 ± 4.53#	71.20 ± 4.57	118.2 ± 10.38

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP =** Haloperidol

4.9.2 Effects of sulpiride on the activity of morin

The effect of sulpiride (SULP, dopamine D_2 receptor antagonist) on MOR-induced antipsychotic-like activity on SMA in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown on Table 4.6. Treatment with MOR (100 mg/kg, i.p.) produced a significant ($p < 0.05$) decrease in the number of line crossings (positive symptom) and duration of immobility (negative symptom) in the OFT and FST respectively relative to vehicle (10 mL/kg) control groups. Furthermore, MOR (100 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in % correct alternation behaviour in the YMT (cognitive symptom). However, SULP (50 mg/kg, i.p.) pretreatment prior to MOR (100 mg/kg, i.p.), significantly ($p < 0.05$) antagonized the antipsychotic-like activity of MOR on spontaneous locomotor activity, as evidenced by increased number of line crossings when compared with MOR-treated mice alone, suggesting interference of dopaminergic neurotransmission by MOR on SMA. SULP (50 mg/kg, i.p.) pretreatment prior to MOR (100 mg/kg, i.p.) demonstrated no significant ($p > 0.05$) decrease in the % alternation cognitive behaviour in the YMT when compared with MOR-treated group. Pretreatment with SULP (50 mg/kg, i.p.) caused no significant change in the anti-immobility of MOR in the FST in comparison with MOR-treated group. Moreover, SULP (50 mg/kg, i.p.) alone significantly ($p < 0.05$) decrease in locomotor activity in the OFT and when compared with vehicle control in mice, although did not cause any change in the immobility time in FST and alternation behaviour in YMT (Table 4.6).

Table 4.6: Effects of sulpiride on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
SUL (50 mg/kg, i.p.)	24.20 ± 1.98*	61.60 ± 4.22	112.8 ± 6.20*
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
SUL + MOR	41.60 ± 3.53#	67.60 ± 5.19	122.6 ± 9.24

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **SULP =** Sulpiride

4.10 EFFECT OF GABAergic SYSTEM ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

4.10.1 Effects of flumazenil on the activity of morin

The effect of flumazenil (FMZ, $GABA_A$ receptor antagonist) on the activity of MOR on SMA in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Figure 4.5 (A, B and C). Pretreatment with FMZ (2 mg/kg, i.p.) did not affect % alternation behaviour of MOR in the YMT (Figure 4.5B) compared to MOR treatment. FMZ (2 mg/kg, i.p.) blocked the antipsychotic-like effects of MOR (100 mg/kg, i.p.). However, it significantly ($p < 0.05$) increased locomotor activity in the OFT (Figure 4.5A) and increased the duration of immobility in the FST (Figure 4.5C) relative to MOR-treated mice alone. Treatment with FMZ (2 mg/kg, i.p.) alone significantly ($p < 0.05$) increased SMA in the OFT and duration of immobility in the FST, it did not cause any significant change in % correct alternation behaviour in the YMT when compared with vehicle-treated mice. Administration of MOR (100 mg/kg, i.p.) alone significantly ($p < 0.05$) attenuated SMA in the OFT, increased in % alternation performance in the YMT, and reduced duration of immobility in the FST in comparison with vehicle control groups. Thus, suggesting the influence of GABAergic transmission on MOR-induced antipsychotic-like activity on the positive and negative symptoms (Figure 4.5A, B and C).

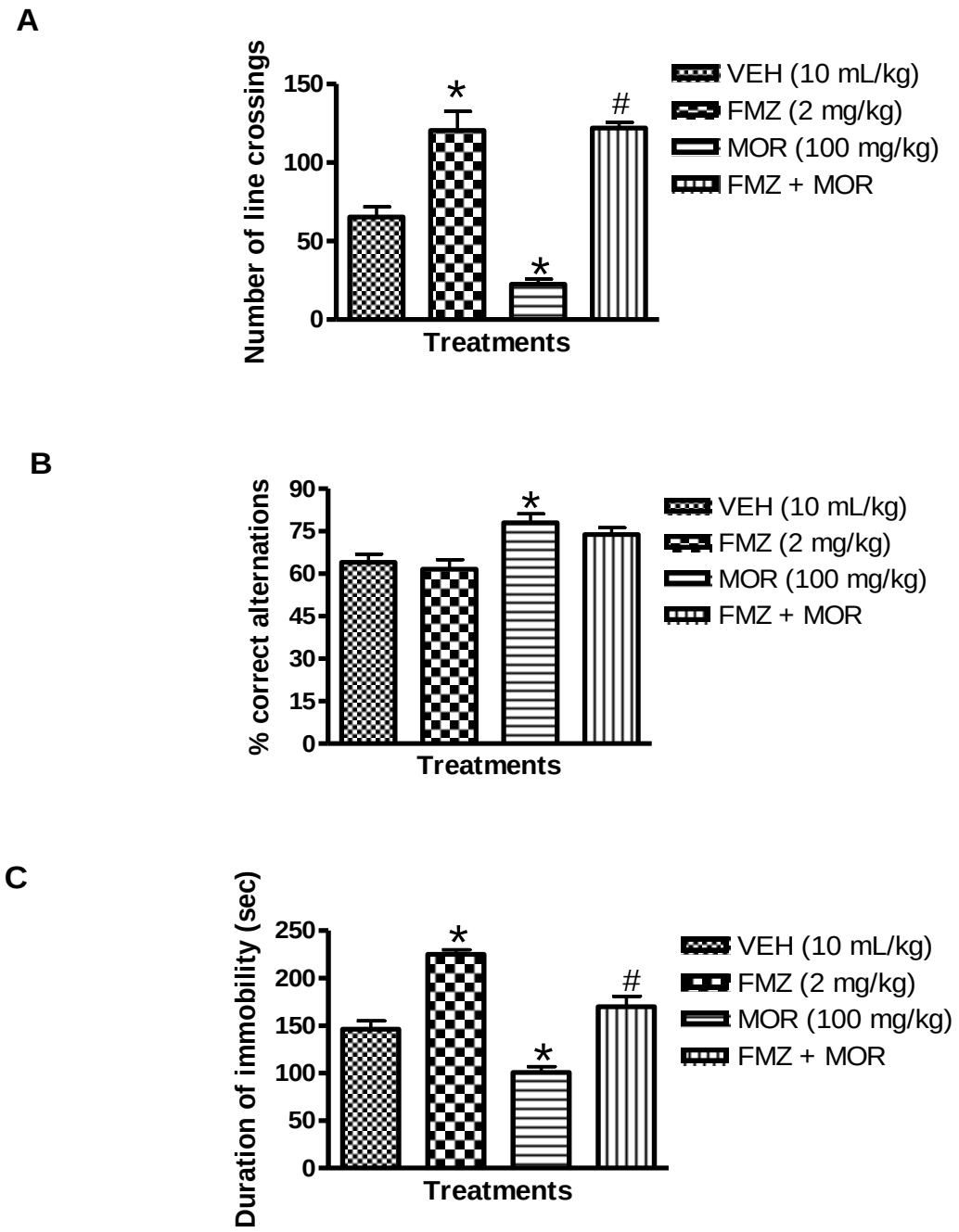


Figure 4.5: Effects of flumazenil on the activity of morin in OFT (A), YMT (B) and FST (C) in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **FMZ =** Flumazenil

4.11 ROLE OF 5-HYDROXYTRYPTAMINERGIC TRANSMISSION ON THE ANTIPSYCHOTIC EEFECT OF MORIN

4.11.1 Effects of metergoline on the activity of morin

The effect of metergoline (MTG, non-selective 5-HT_1 and 5-HT_2 receptor antagonist) on the activity of MOR on SMA in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Table 4.7. MOR (100 mg/kg, i.p.) demonstrated significant ($p < 0.05$) decrease in the number of line crossings (positive symptom) in the OFT when compared with vehicle (10 mL/kg) control group; and MOR (100 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in % correct alternation behaviour in the YMT, suggesting memory enhancing activity against cognitive symptom. MOR (100 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in the duration of immobility in the FST, which also suggests decrease in behavioural despair for negative symptom. MTG (100 mg/kg, i.p.) significantly ($p < 0.05$) decreased spontaneous locomotor activity relative to vehicle-treated group, but did not alter % cognitive searching behaviour and immobility time, as it failed to significantly ($p > 0.05$) affect the % correct alternation and duration of immobility in the YMT and FST when compared with vehicle-treated groups respectively. However, pretreatment with MTG (100 mg/kg, i.p.) 15 min prior to treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) blocked the antipsychotic-like activity of MOR on SMA, by increasing the number of line crossings and duration of immobility in the OFT and FST relative to MOR (100 mg/kg, i.p.) treatment alone. Thus, suggesting the blockade of 5-Hydroxytryptaminergic transmission by MOR against hyperlocomotion and behavioural despair via 5-HT_1 and 5-HT_2 receptors. Nevertheless, MTG (100 mg/kg, i.p.) pretreatment prior to MOR (100 mg/kg, i.p.) treatment did not demonstrated any significant ($p > 0.05$) decrease in the % alternation cognitive behaviour in the YMT when compared with MOR-treated group (Table 4.7).

Table 4.7: Effects of metergoline on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
MTG (100 mg/kg, i.p.)	29.80 ± 4.85*	59.60 ± 4.37	118.0 ± 11.83
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
MTG + MOR	41.20 ± 4.03#	60.60 ± 5.79	219.2 ± 8.21#

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **MTG =** Metergoline

4.11.2 Effects of cyproheptadine on the activity of morin

The effect of cyproheptadine (CYP, 5-HT_2 receptor blocker) on the activity of MOR on SMA in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Figure 4.6 (A, B and C). Treatment with CYP (0.5 mg/kg, i.p.) significantly ($p < 0.05$) enhanced SMA in the OFT (Figure 4.6A), increased duration of immobility in the FST (Figure 4.6C), and decreased % correct alternation behaviour in the YMT (Figure 4.6B) when compared with vehicle (10 mL/kg) control mice. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated the SMA in the OFT and reduced the immobility time in the FST relative to vehicle (10 mL/kg) control group. Also, MOR (100 mg/kg, i.p.) produced an increased in % correct alternation in the YMT in comparison with vehicle (10 mL/kg) treated mice in significant ($p < 0.05$) manner. However, pretreatment with CYP (0.5 mg/kg, i.p.) 15 min prior to treatment with MOR (100 mg/kg, i.p.) reversed MOR-induced antipsychotic-like activity, as it significantly ($p < 0.05$) increased the number of line crossings in the OFT (Figure 4.6A) (positive symptom), reduced the % correct alternation of cognitive performance in the YMT (cognitive symptom) (Figure 4.6B), and enhanced the duration of immobility in the FST (negative symptom) (Figure 4.6C) in mice, thus suggesting the involvement of 5-hydroxytryptaminergic neurotransmission in the antipsychotic-like activities of MOR on the positive, negative and cognitive symptoms through 5-HT_2 receptor (Figure 4.6A, B and C).

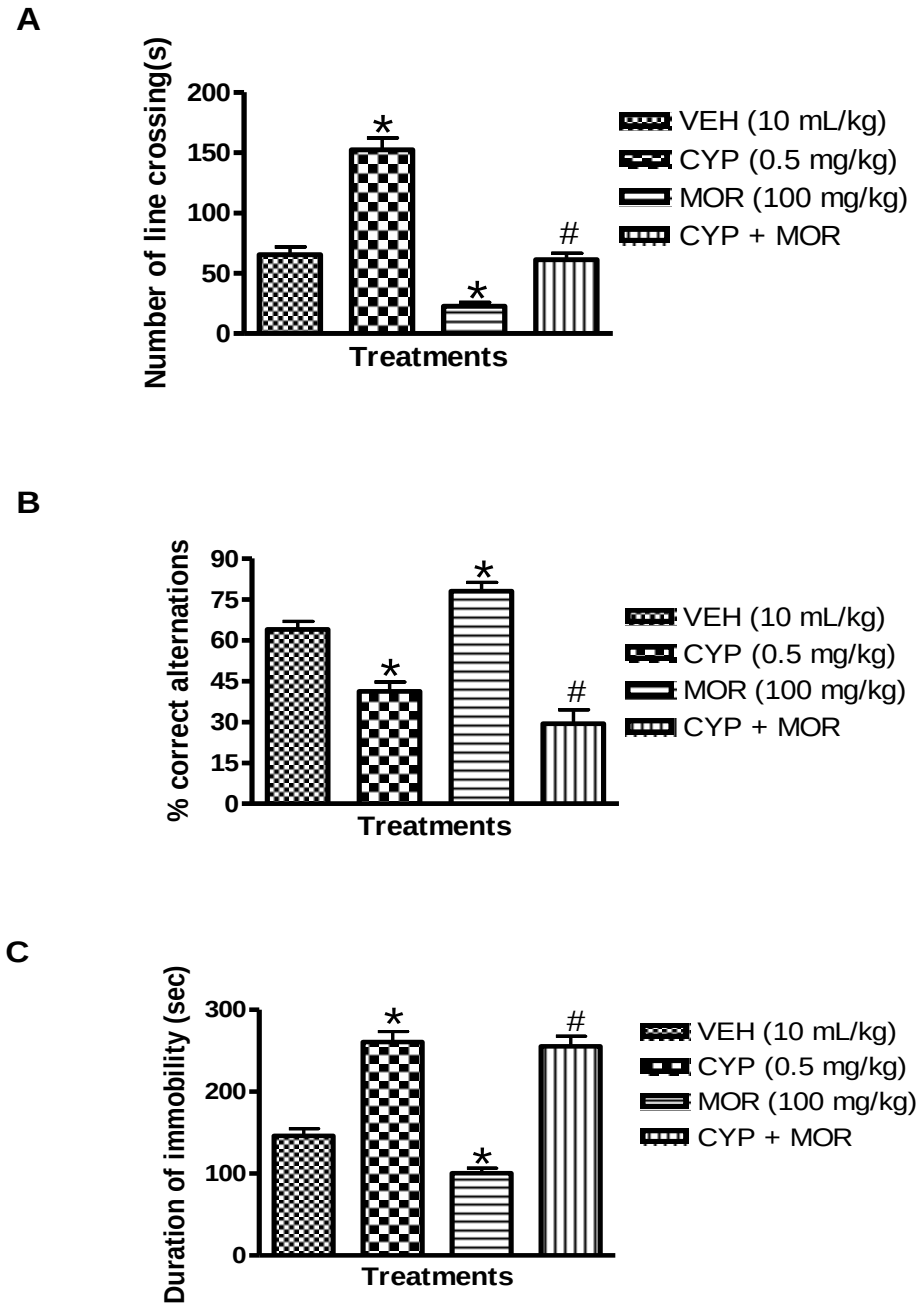


Figure 4.6: Effects of cyproheptadine on the effect of morin in OFT (A), YMT (B) and FST (C) in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **CYP =** Cyproheptadine

4.11.3 Effects of para-chlorophenylalanine on the activity of morin

The effect of para-chlorophenylalanine (PCPA, 5-HT synthesis inhibitor) on MOR on SMA in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Table 4.8. MOR (100 mg/kg, i.p.) produced significant ($p < 0.05$) inhibition of spontaneous motor activity and the duration of immobility in the OFT and FST respectively when compared with vehicle (10 mL/kg) control group. MOR (100 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in % correct alternation behaviour in the YMT in comparison with vehicle-treated group, which further suggest cognitive enhancing activity. PCPA (100 mg/kg, i.p.) given once daily for 3 days caused a significant ($p < 0.05$) decrease in the SMA relative to vehicle treatment. PCPA (100 mg/kg, i.p.) did not increase nor decrease the % correct alternation behaviour or immobility time, as it failed to significantly ($p > 0.05$) affect the % correct alternation and duration of immobility in the YMT and FST respectively when compared with vehicle-treated groups. However, pretreatment with PCPA (100 mg/kg, i.p.) once daily for 3 days prior to treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) increased SMA in the OFT, duration of immobility in the FST, and decreased % correct alternation in the YMT when compared with MOR (100 mg/kg, i.p.) alone, thus suggesting that PCPA pretreatment abolishes the antipsychotic activity of MOR on positive, negative and cognitive symptoms in mice (Table 4.8).

Table 4.8: Effects of para-chlorophenylalanine on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
PCPA (100 mg/kg, i.p.)	29.80 ± 3.05*	60.20 ± 5.34	169.6 ± 10.45
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
PCPA + MOR	72.60 ± 6.40#	60.40 ± 2.99#	164.0 ± 12.78#

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **PCPA** = Para-chlorophenylalanine

4.12 ROLE OF ADRENERGIC SYSTEM ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

4.12.1 Influence of prazosine on the activity of morin

The effect of prazosine (PRA, α_1-adrenergic antagonist) on the activity of MOR on spontaneous motor activity in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Table 4.9. Treatment with PRA (1 mg/kg, i.p.) did not affect % correct alternation in the YMT and duration of immobility in the FST, but it caused a significant ($p < 0.05$) increase in the spontaneous motor activity in the OFT when compared with vehicle-treated mice. However, treatment with MOR (100 mg/kg, i.p.) alone significantly ($p < 0.05$) enhanced % alternation in the YMT in comparison with vehicle-treated group. Moreover, MOR (100 mg/kg, i.p.) alone also produced a significant ($p < 0.05$) decrease in spontaneous motor activity in the OFT and attenuated the duration of immobility in the FST when compared with vehicle-treated mice (Table 4.9). Pretreatment with PRA (1 mg/kg, i.p.) significantly ($p < 0.05$) increased the number of line crossing in the OFT and enhancement of immobility time in the FST when compared with MOR-treated mice. PRA (1 mg/kg, i.p.) did not reverse the activity of MOR on cognitive performance in the YMT when compared with MOR-treated mice, thus suggesting the involvement α_1-adrenergic modulation in the antipsychotic-like activity of MOR against positive and negative symptoms (Table 4.9).

Table 4.9: Effects of prazosine on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
PRA (1 mg/kg, i.p.)	20.20 ± 1.88*	66.60 ± 3.12	193.4 ± 4.56
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
PRA + MOR	41.80 ± 5.07#	81.40 ± 3.16	152.6 ± 9.35#

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **PRA** = Prazosine

4.12.2 Influence of yohimbine on the activity of morin

The effect of yohimbine (YHB, α_2-adrenergic antagonist) on the activity of MOR on spontaneous motor activity in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Figure 4.7 (A, B and C). Treatment with YHB (1 mg/kg, i.p.) did not affect immobility time in the FST (Figure 4.7C) and % correct alternation in the YMT (Figure 4.7B) in comparison to vehicle groups, but it significantly ($p < 0.05$) increased SMA in the OFT (Figure 4.7A) relative to vehicle control. MOR (100 mg/kg, i.p.) administered alone produced a significant ($p < 0.05$) decrease in spontaneous motor activity in the OFT (Figure 4.7A), reduced the duration of immobility in the FST (Figure 4.7C) and enhanced cognitive performance in the YMT (Figure 4.7B) by increasing % correct alternation in comparison to vehicle treatment. However, YHB (1 mg/kg, i.p.) significantly ($p < 0.05$) increased the SMA (number of line crossings) in the OFT relative to MOR-treated mice; and failed to reverse the % correct cognitive performance and duration of immobility of MOR-treated mice (Figure 4.7A, B and C).

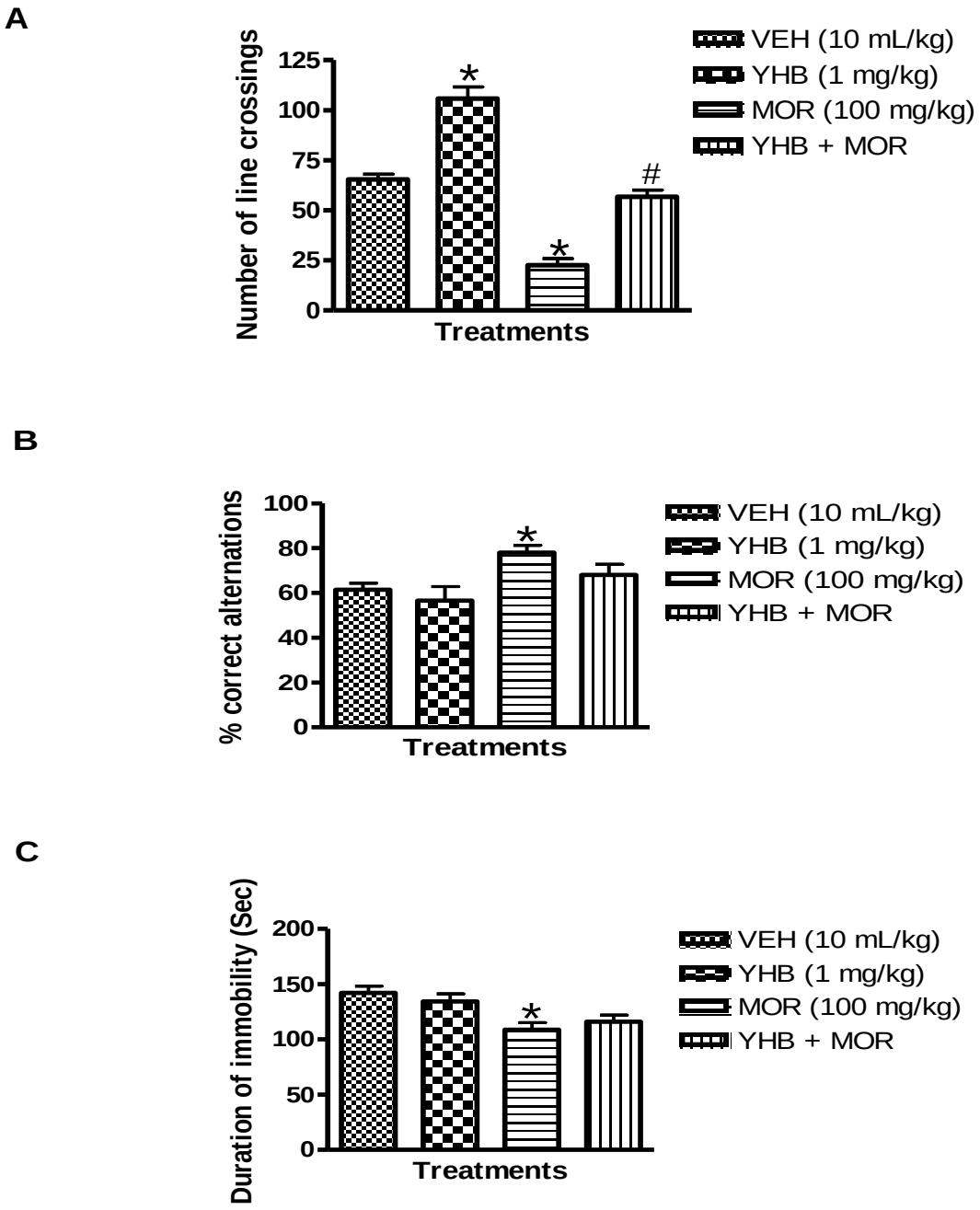


Figure 4.7: Effects of yohimbine on the activity of morin in OFT (A), YMT (B) and FST (C) in mice

Bars represent the mean ± S.E.M of 5 animals / group. *P < 0.05 compared to vehicle group. #P < 0.05 compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **YHB** = Yohimbine

4.12.3 Effects of propranolol on the activity of morin

The effect of Propranolol (PRO, non-selective β-adrenergic antagonist) on the activity of MOR on spontaneous motor activity in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown in Table 4.10. Treatment with MOR (100 mg/kg, i.p.) alone produced a significant ($p < 0.05$) decrease in spontaneous motor activity in the OFT and reduced the duration of immobility in the FST, as well as accentuated the cognitive activity in the YMT by increasing % correct alternation in comparison to vehicle treatment. PRO (0.2 mg/kg, i.p.) alone did not affect immobility time in the FST and % correct alternation in the YMT, but significantly ($p < 0.05$) decreased SMA in the OFT relative to vehicle treated. However, pretreatment with PRO (0.2 mg/kg, i.p.) potentiated the activity of MOR on SMA of MOR, as both significantly ($p < 0.05$) decreased the number of line crossing in the OFT when compared with MOR-treated mice, but no reversal effects were observed in the FST and YMT respectively (Table 4.10).

Table 4.10: Effects of propranolol on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
PRO (0.2 mg/kg, i.p.)	36.80 ± 4.96*	68.00 ± 4.06	162.6 ± 12.37
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
PRO + MOR	11.80 ± 1.39#	69.00 ± 4.45	125.4 ± 9.03

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **PRO** = Propranolol

4.13 ROLE OF CHOLINERGIC SYSTEM ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

4.13.1 Effects of atropine on the activity of morin

The effect of atropine (ATP, muscarinic cholinergic receptor antagonist) on MOR on locomotor activity in the OFT, % correct alternation behaviour in the YMT and immobility time in the FST are shown on Table 4.11. ATP (0.5 mg/kg, i.p.) did not affect duration of immobility in the FST, but caused a significant ($p < 0.05$) increase in the spontaneous motor activity in the OFT and decreased % correct alternation in the YMT when compared with vehicle-treated mice. Treatment with MOR (100 mg/kg, i.p.) alone significantly ($p < 0.05$) decreased motor activity in the OFT, duration of immobility in the FST and enhanced % alternation in the YMT in comparison with vehicle-treated groups (Table 4.12). Pretreatment with ATP (0.5 mg/kg, i.p.) prior to treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) reversed the activity of MOR against spontaneous motor activity (positive symptom) and cognitive performance (cognitive symptom), as indicated by the increase in the number of line crossing in the OFT and decrease in the % correct alternation behaviour in the YMT when compared with MOR-treated mice. Pretreatment with ATP (0.5 mg/kg, i.p.) did not reverse the anti-immobility effect of MOR-treated mice in the FST when compared with MOR (100 mg/kg, i.p.) treatment alone. Thus, suggesting the role cholinergic pathway in the antipsychotic-like activity of MOR (Table 4.11).

Table 4.11: Effects of atropine antagonism on the activity of morin in mice

Treatments	Number of line crossing(s)	% correct alternations	Duration of immobility (sec)
VEH (10 mL/kg, i.p.)	65.40 ± 2.61	62.80 ± 2.05	142.0 ± 6.27
ATP (0.5 mg/kg, i.p.)	113.0 ± 1.23*	42.80 ± 3.44*	143.8 ± 5.39
MOR (100 mg/kg, i.p.)	22.60 ± 3.23*	79.00 ± 3.17*	108.8 ± 6.39*
ATP + MOR	119.0 ± 8.14#	52.20 ± 2.48#	114.6 ± 5.18

Values represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group (ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **ATP** = Atropine

4.14 EVIDENCE FOR THE INVOLVEMENT OF NITRERGIC (NITRIC OXIDE) PATHWAY ON THE ANTIPSYCHOTIC ACTIVITY OF MORIN

4.14.1 Influence of L-arginine, methylene blue and L-NAME on the activity of morin in open field test

The effect of L-arginine (L-ARG, nitric oxide precursor), methylene blue (MTB, specific nitric oxide synthase inhibitor) and L-Nitro arginine methyl ester (L-NAME, nonspecific nitric oxide synthase inhibitor) pretreatments on the activity of MOR on SMA using the OFT is shown in Figure 4.8. Treatment with L-ARG (750 mg/kg, i.p.) alone significantly ($p < 0.05$) increased SMA in the OFT when compared with vehicle (10 mL/kg) treated mice. Treatment with L-NAME (50 mg/kg, i.p.) significantly decreased SMA relative to vehicle-treated mice. MTB (3.75 mg/kg, i.p.) alone did not induce any significant changes in the locomotor activity in comparison with vehicle treatment. Treatment with MOR (100 mg/kg, i.p.) alone significantly ($p < 0.05$) attenuated spontaneous motor activity when compared with vehicle control group. However, the combination of L-ARG (750 mg/kg, i.p.) or MTB (3.75 mg/kg, i.p.) pretreatment and MOR (100 mg/kg, i.p.) treatment significantly ($p < 0.05$) reversed the activity of MOR, by increasing the SMA decreased by MOR when compared with MOR-treated mice. Pretreatment with L-NAME (50 mg/kg, i.p.) significantly potentiated MOR-induced antipsychotic-like effect, by synergistically decreasing SMA in comparison with MOR treatment alone. Meanwhile, the co-administration of L-ARG (750 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.) significantly ($p < 0.05$) increased the SMA in the OFT when compared with L-NAME administration alone, suggesting that L-ARG inhibits the decreased SMA by L-NAME. However, pretreatment with the combination of L-ARG (750 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.) prior to the administration MOR (100 mg/kg, i.p.) showed significant ($p < 0.05$) decrease in SMA in the OFT relative to the combination of L-ARG and L-NAME treatment (Figure 4.8).

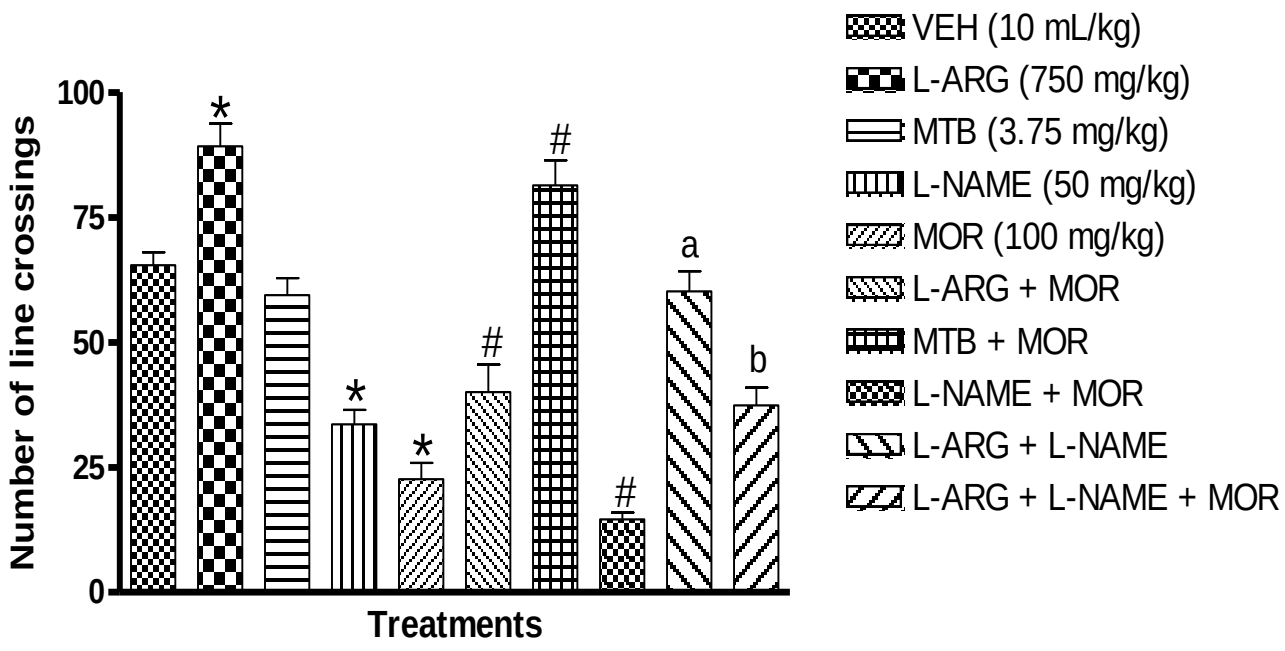


Figure 4.8: Effects of L-ARG, methylene blue and L-NAME on the activity of morin in open-field test in mice

Bars represent the mean of ± S.E.M of 5 animals / group. *P < 0.05 compared to vehicle group. #P < 0.05 compared to MOR-treated group, ^{a}P < 0.05 compared to L-NAME group, ^{b}P < 0.05 compared to L-ARG plus L-NAME group (one-way or two-way ANOVA followed by bonferroni *post hoc* test).

VEH = Vehicle, **MOR** = Morin, **L-ARG** = L-Arginine, **MTB =** Methylene blue, **L-NAME** = L-Nitro arginine methyl ester

4.14.2 Effects of L-arginine, methylene blue and L-NAME on the activity of morin in Y-maze test

The effect of L-ARG, MTB and L-NAME on the activity of MOR on % alternation behaviour in YMT in mice is shown in Figure 4.9. L-ARG (750 mg/kg, i.p.) or L-NAME (50 mg/kg, i.p.) did not show any significant difference from vehicle (10 mL/kg, i.p.) control group. In contrast, MTB (3.75 mg/kg, i.p.) induced significant ($p < 0.0.5$) increase the % correct cognitive alternation in the YMT when compared with vehicle-treated mice. MOR (100 mg/kg, i.p.) produced a significant ($p < 0.0.5$) increase in the cognitive performance relative to vehicle-treated mice. Pretreatment with L-ARG (750 mg/kg, i.p.) or L-NAME (50 mg/kg, i.p.) demonstrated significant ($p < 0.0.5$) reversal of MOR-cognitive enhancement when compared with MOR administration alone, although pretreatment with MTB (3.75 mg/kg) did not show any significant difference from MOR-treated group alone (Figure 4.9). Moreover, the combination of L-ARG (750 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.) produced a significant ($p < 0.0.5$) decrease in % alternation behaviour in comparison with L-NAME (50 mg/kg, i.p.). However, pretreatment with the combination of L-ARG (750 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.) prior to treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.0.5$) reversed the inhibitory effect of L-ARG on L-NAME relative to L-ARG plus L-NAME treatments treated group, which is suggestive of the modulatory activity of nitrergic pathway on the effect of MOR on cognitive enhancement in mice (Figure 4.9).

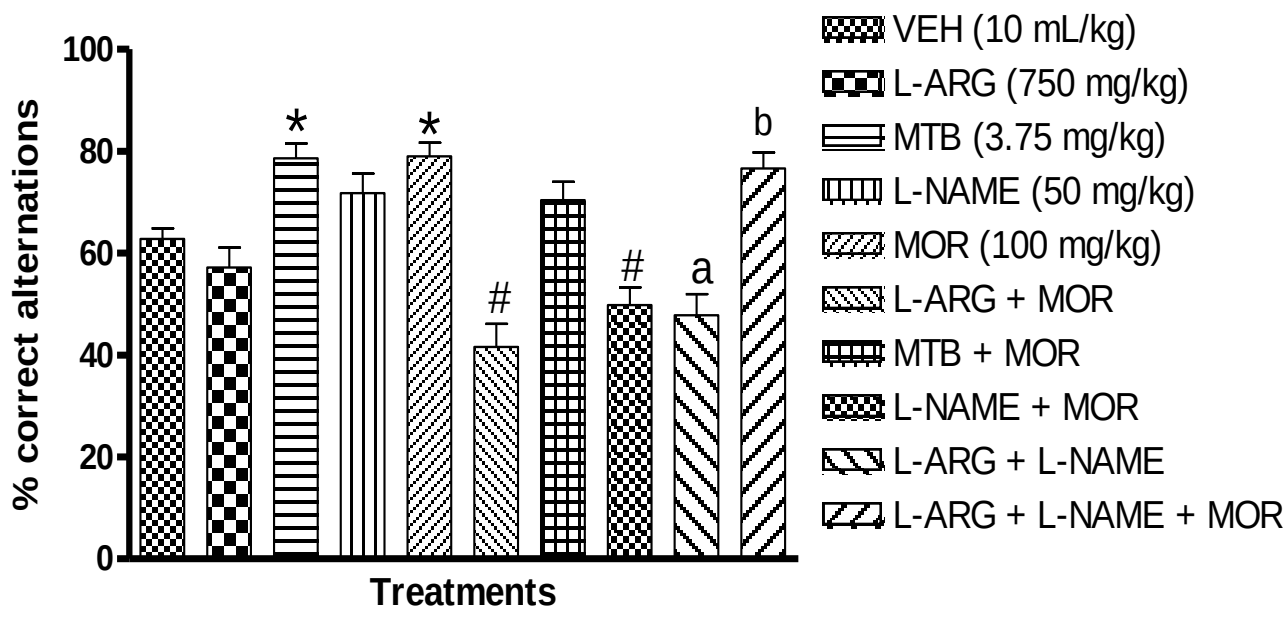


Figure 4.9: Effects of L-arginine, methylene blue and L-NAME on the activity of morin in Y-maze test in mice

Bars represent the mean ± S.E.M of 5 animals / group. *P < 0.05 compared to vehicle group. #P < 0.05 compared to MOR-treated group, ^{a}P < 0.05 compared to L-NAME group, ^{b}P < 0.05 compared to L-ARG plus L-NAME group (one-way or two-way ANOVA followed by bonferroni *post hoc* test).

VEH = Vehicle, **MOR** = Morin, **L-ARG** = L-Arginine, **MTB =** Methylene blue, **L-NAME =** L-Nitro arginine methyl ester

4.14.3 Effects of L-arginine, methylene blue and L-NAME on the activity of morin in forced swim test

The influence of L-ARG, MTB and L-NAME pretreatments on the activity of MOR on immobility time in the FST in mice is shown in Figure 4.10. Treatment with L-ARG (750 mg/kg, i.p.) alone significantly ($p < 0.05$) increased immobility time in the FST when compared with vehicle (10 mL/kg) treated mice. Treatment with either MTB (3.75 mg/kg, i.p.) or L-NAME (50 mg/kg, i.p.) alone did not induce any significant ($p > 0.05$) changes in the duration of immobility when compared with vehicle treatment in mice. MOR (100 mg/kg, i.p.) alone significantly ($p < 0.05$) decreased immobility time when compared with vehicle control group (Figure 4.10). Pretreatment with L-ARG (750 mg/kg, i.p.) significantly ($p < 0.05$) blocked the antipsychotic-like activity of MOR, by increasing the duration of immobility in MOR-treated mice when compared with MOR administration alone. Pretreatment with either MTB (3.75 mg/kg, i.p.) or L-NAME (50 mg/kg, i.p.), significantly potentiated the effect of MOR (100 mg/kg, i.p.) on immobility time in FST, as they both produced significant ($p < 0.05$) decrease in the duration of immobility in mice treated with MOR (100 mg/kg, i.p.) respectively, when compared with MOR treatment alone. On the other hand, pretreatment with L-ARG (750 mg/kg, i.p.) prior to the administration of L-NAME (50 mg/kg, i.p.) significantly ($p < 0.05$) increased immobility times of mice treated with L-NAME (50 mg/kg, i.p.) in the FST when compared with L-NAME-treated mice alone. However, pretreatment with the combination of L-ARG (750 mg/kg, i.p.) and L-NAME (50 mg/kg, i.p.) prior to the administration MOR (100 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in the duration of immobility in the FST when compared with co-administration of L-ARG and L-NAME, which thus suggests the modulation of nitrergic pathway in the antipsychotic activity of MOR on behavioural despair in FST (Figure 4.10).

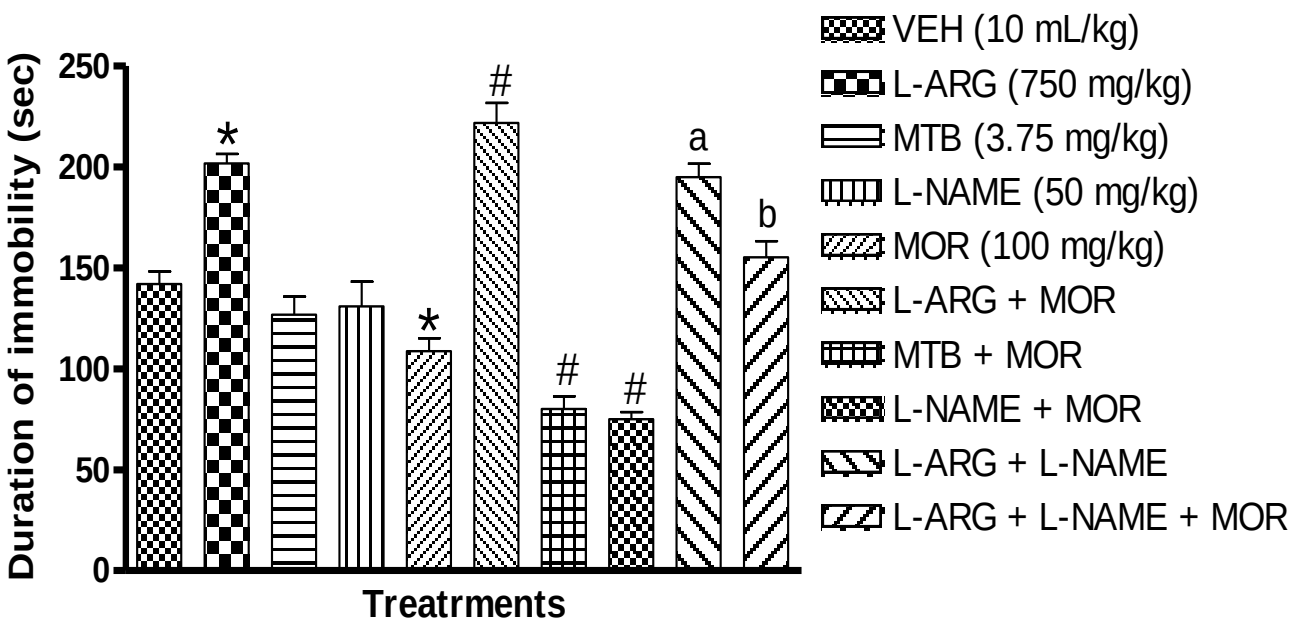


Figure 4.10: Effects of L-arginine, methylene blue and L-NAME on the activity of morin in forced swim test in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$P < 0.05$ compared to vehicle group. #$P < 0.05$ compared to MOR-treated group, $^{a}P < 0.05$ compared to L-NAME group, $^{b}P < 0.05$ compared to L-ARG plus L-NAME group (one-way or two-way ANOVA followed by bonferroni *post hoc* test).

VEH = Vehicle, **MOR** = Morin, **L-ARG** = L-Arginine, **MTB =** Methylene blue, **L-NAME =** L-Nitro arginine methyl ester

4.15 Effect of morin on ketamine-induced hyperlocomotion

The effect of MOR on KET-induced hyperlocomotion in the OFT in mice is shown in Table 4.12. Intraperitoneal administration of KET (20 mg/kg, i.p.) given once daily for 10 days, significantly ($p < 0.05$) increased the number of lines crossed in the OFT in comparison with vehicle (10 mL/kg, i.p.) treated group, suggesting hyperlocomotion. However, pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) once daily for 10 days prior to KET administration, significantly prevented increased hyperlocomotion caused by KET compared to KET-treated group ($p < 0.05$) (Table 4.12). HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented hyperlocomotion by KET (20 mg/kg, i.p.) in mice. The intraperitoneal administration of either HLP (1 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.) alone once daily for 10 days, demonstrated significant ($p < 0.05$) decrease in the number of lines crossed relative to vehicle group, whereas MOR (25, 50 and 100 mg/kg, i.p.) given alone once daily for 10 days, produced no significant alteration on these parameters (Table 4.12).

Table 4.12: Effect of morin on ketamine-induced hyperlocomotion in open field test in mice

Treatments	Numbers of line crossing(s)	Duration of ambulation (sec)
Vehicle (10 mL/kg)	71.60 ± 3.94	182.2 ± 12.66
MOR (25 mg/kg)	80.60 ± 5.00	192.2 ± 4.35
MOR (50 mg/kg)	69.60 ± 2.35	192.4 ± 4.56
MOR (100 mg/kg)	66.20 ± 3.20	205.8 ± 7.61
HLP (1 mg/kg)	14.20 ± 1.88*	250.2 ± 9.49*
RIS (0.5 mg/kg)	44.40 ± 5.70*	235.0 ± 8.82*
KET (20 mg/kg)	113.6 ± 3.93*	108.8 ± 7.91*
MOR (25 mg/kg) + KET	77.80 ± 7.26#	237.2 ± 9.49#
MOR (50 mg/kg) + KET	77.00 ± 4.56#	231.6 ± 10.97#
MOR (100 mg/kg) + KET	71.80 ± 3.52#	203.2 ± 9.52#
HLP (1 mg/kg) + KET	14.40 ± 0.50#	288.4 ± 2.87#
RIS (0.5 mg/kg) + KET	35.00 ± 4.20#	239.0 ± 6.62#

Values represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.16 Effect of morin on ketamine-induced alteration in spatial working memory

The effect of MOR on KET-induced deficit in spatial working memory based on the percentage (%) alternations in the YMT in mice is shown in Figure 4.11. Administration of KET (20 mg/kg, i.p.) once daily for 10 days produced a significant ($p < 0.05$) decrease in % alternation behaviour of the animals in the YMT relative to vehicle-treated group, which suggests impairment of spatial working memory navigation. Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) once daily for 10 days prior to KET administration, significantly ($p < 0.05$) increased the % level of cognitive alternation performance of the animals when compared with KET-treated group. In contrast, pretreatment with HLP (1 mg/kg, i.p.) once daily for 10 days, failed to prevent the spatial working memory impairment by KET when compared to KET-treated group. On the other hand, MOR (25, 50 and 100 mg/kg, i.p.) given alone once daily for 10 days, significantly ($p < 0.05$) increased spatial memory navigation in the Y-maze test in a dose-dependently manner relative to vehicle-treated group. Meanwhile, RIS (0.5 mg/kg, i.p.) alone did not show any changes in the spatial cognitive performance test, however, treatment with the typical antipsychotic reference drug, haloperidol (1 mg/kg, i.p.) alone once daily for 10, demonstrated significant ($p < 0.05$) decrease in the % alternations of the mice in the YMT compared to vehicle treated group (Figure 4.11).

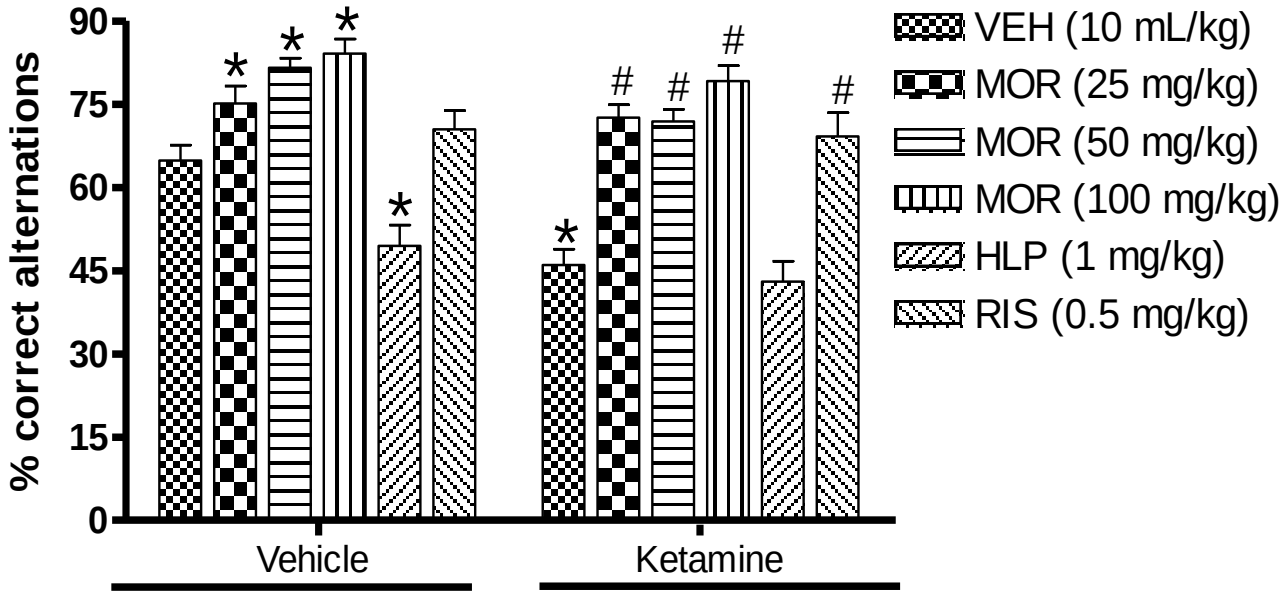


Figure 4.11: Effect of morin on ketamine-induced alteration in spatial working memory in mice

Bar represents the mean ± S.E.M of 5 animals / group. *$p < 0.05$ as compared to vehicle group; #$p < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.17 Effect of morin on ketamine-mediated alteration on recognition (non-spatial) memory

The effect of MOR on KET-induced non-spatial working memory impairment, as measured by the discrimination index, using the novel object recognition memory test (NORT) is shown in Figure 4.12. Intraperitoneal treatment with MOR (50 and 100 mg/kg, i.p.) alone given once daily for 10 days significantly ($p < 0.05$) increased the ratio of the amount of time spent exploring the novel object over the total time spent exploring both objects; as evidenced by the significant ($p < 0.05$) increase in the discrimination index (recognition memory) relative to vehicle-treated. Treatment with RIS (0.5 mg/kg, i.p.) alone did not demonstrate any significant change in the index of recognition memory compared to vehicle-treated group; whereas, treatment with HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) decreased the recognition memory of the novel object, as indexed by the decrease in the discrimination index compared to vehicle treated group. KET significantly ($p < 0.05$) decreased the index of recognition memory in the NORT relative to vehicle-treated group, suggesting impairment in non-spatial working memory performance. Interestingly, pretreatment with both MOR (25, 50 and 100 mg/kg, i.p.) and the reference atypical antipsychotic drug, RIS (0.5 mg/kg, i.p.) once daily for 10 days prior to KET treatment, significantly ($p < 0.05$) attenuated KET-induced non-spatial working memory deficit in mice (Figure 4.12). Pretreatment with the reference typical antipsychotic agent, HLP (1 mg/kg, i.p.) failed to prevent the defective non-spatial working memory by KET (20 mg/kg, i.p.) in mice hence, decreased discriminating capacity for the novel object (Figure 4.12).

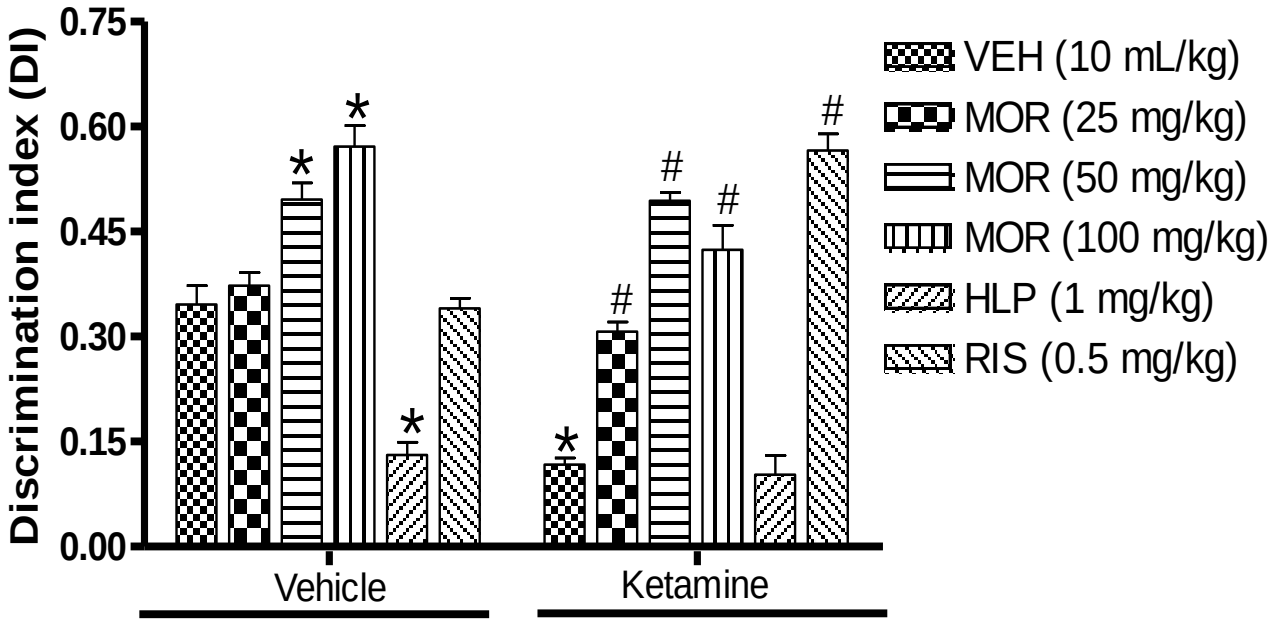


Figure 4.12: Effect of morin on ketamine-mediated alteration on recognition (non-spatial) memory in mice

Bar represents the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group; #$p < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.18 Effect of morin on ketamine-induced alteration on social preference

The effect of MOR on KET-induced deficit in social interaction, based on % social preference using the social interaction test is shown in Figure 4.13. Intraperitoneal administration of MOR (100 mg/kg), but not 25 and 50 mg/kg alone once daily for 10 days demonstrated significant ($p < 0.05$) increase in the % social preference relative to vehicle-treated. Treatment with RIS (0.5 mg/kg, i.p.) did not show any significant changes in the social behaviour when compared with vehicle-treated mice. However, treatment with HLP (1 mg/kg, i.p.) once daily for 10 days, produced a significant ($p < 0.05$) decrease in % social interaction preference compared with vehicle-treated group in mice. Repeated intraperitoneal injection of KET (20 mg/kg) significantly ($p < 0.05$) decreased the preference for social interaction in comparison with vehicle-treated group in mice, suggesting decrease in social activity. Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) once daily for 10 days showed a dose-dependent significant ($p < 0.05$) protection against KET-induced social withdrawal similar to the reference atypical antipsychotic drug, RIS (0.5 mg/kg, i.p.) compared with KET-treated group (Figure 4.12); although, MOR (100 mg/kg, i.p.) elicited more social activity than RIS. In contrast, pretreatment with HLP (1 mg/kg, i.p.) prior to KET administration failed to prevent the deficit in social interaction in KET-treated mice (Figure 4.13).

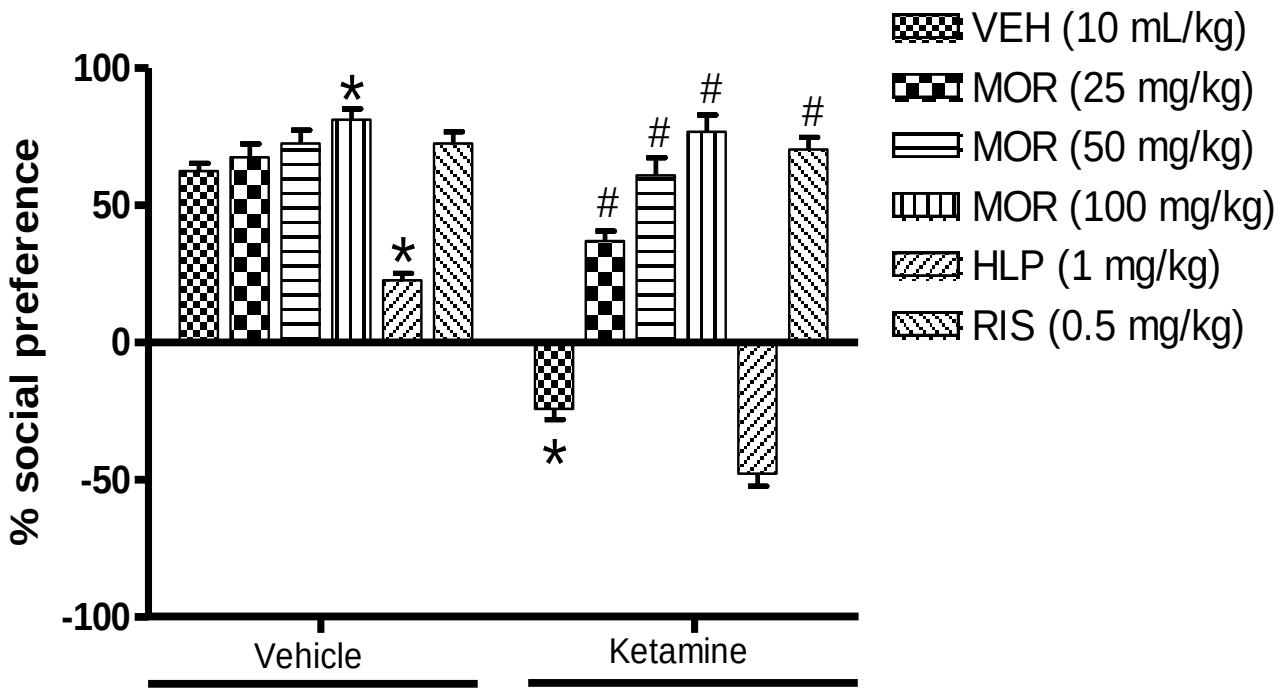


Figure 4.13: Effect of morin on ketamine-induced alteration on social preference in mice

Bar represents the mean ± S.E.M of 5 animals / group. *$p < 0.05$ as compared to vehicle group, #$p < 0.05$ as compared with KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.19 Effect of morin on ketamine-enhanced immobility in forced swim test

The effect of MOR on KET-enhanced immobility in the FST is shown in Figure 4.14. Intraperitneal injection of KET (20 mg/kg) significantly ($p < 0.05$) increased the duration of immobility in the FST in comparison with vehicle-treated group in mice, suggesting a behavioural despair which is indicative of negative symptom. However, pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) once daily for 10 days produced a dose-dependent reduction in duration of immobility in a significant ($p < 0.05$) manner when compared with KET-treated mice. Similarly, this increase in immobility time by KET was significant ($p < 0.05$) reduced by the atypical antipsychotic drug, RIS (0.5 mg/kg, i.p.). Pretreatment with typical antipsychotic reference agent, HLP (1 mg/kg, i.p.) failed to prevent the increase in duration of immobility in KET-treated mice. Administration of HLP (1 mg/kg, i.p.) alone for 10 days, significantly enhanced duration of immobility relative to vehicle-treated mice, whereas RIS (0.5 mg/kg, i.p.) alone for 10 days demonstrated significant ($p < 0.05$) decrease in immobility time similar to MOR (50 and 100 mg/kg, i.p.) in comparison with vehicle-treated group (Figure 4.14).

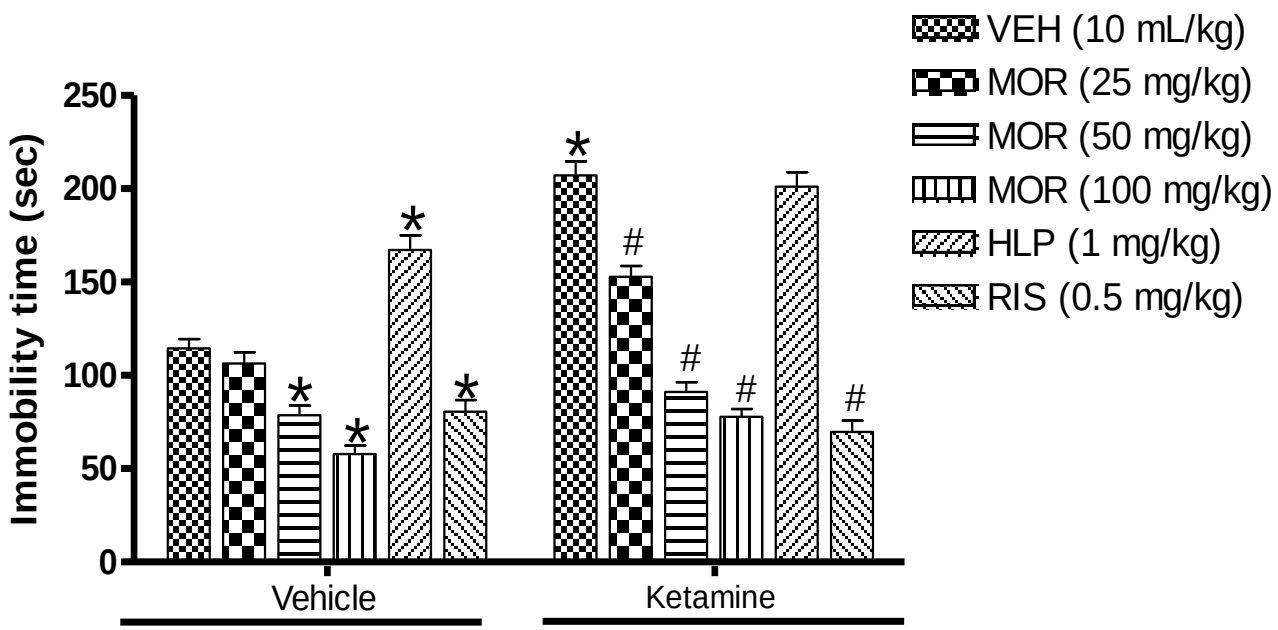


Figure 4.14: Effect of morin on ketamine-enhanced immobility in forced swim test in mice

Bar represents the mean ± S.E.M of 5 animals / group. *$p < 0.05$ as compared to vehicle group, #$p < 0.05$ as compared with KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.20 Effect of chronic administration of morin on catalepsy test

The effect of repeated administration of MOR on cataleptic (extrapyramidal) effects, as measured by descent latency using the wood block catalepsy test is shown in Figure 4.15. Intraperitoneal administration of MOR (25, 50 or 100 mg/kg, i.p.) once daily for 10 days did not cause any alteration in the descent latency in the wood block catalepsy test relative to vehicle-treated mice. Similarly, treatment with RIS (0.5 mg/kg, i.p.) did not affect the descent latency when compared with vehicle-treated group. However, HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) enhanced descent latency in the wood block test in comparison with vehicle-treated mice. Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.) prior to KET (20 mg/kg, i.p.) did not cause any significant alterations in the descent latency compared to KET-treated group. Moreover, the administration of KET (20 mg/kg, i.p.) once daily for 10 days did not demonstrate any alteration in the descent latency when compared with vehicle-treated group. Pretreatment with HLP (1 mg/kg, i.p.) significantly prolonged descent latency in KET-treated mice in comparison with vehicle- and KET-treated group respectively, suggesting evidence of extrapyramidal side effect (Figure 4.15).

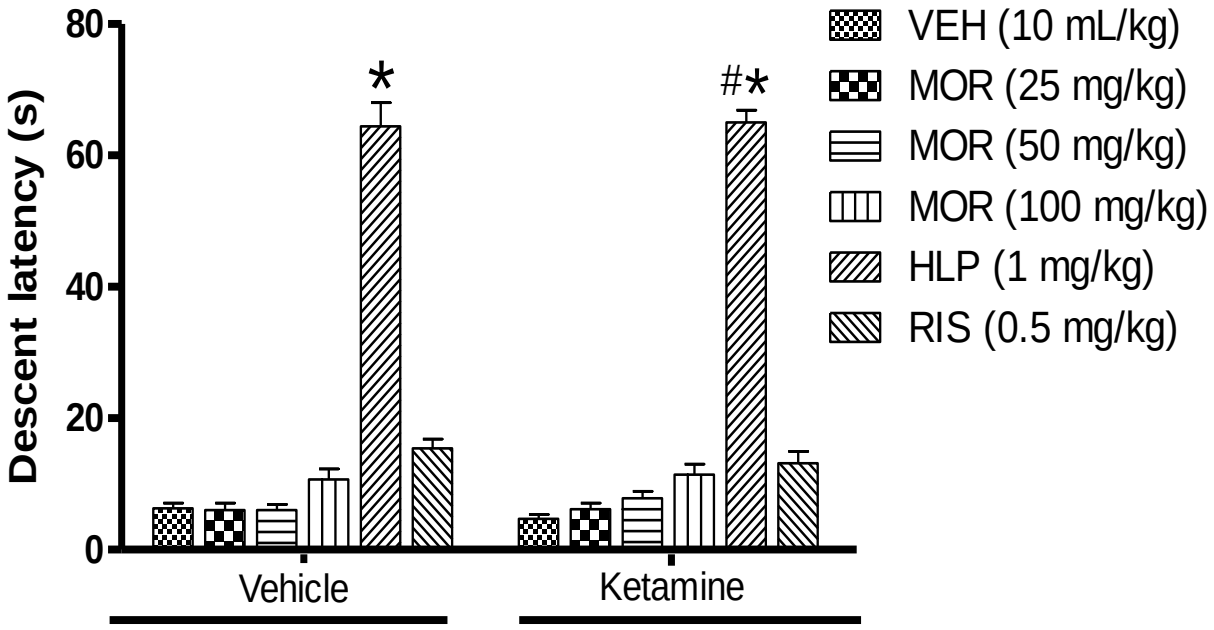


Figure 4.15: Effect of chronic administration of morin on catalepsy test in mice

Bar represents the mean ± S.E.M of 5 animals / group. *$p < 0.05$ as compared to vehicle group, #$p < 0.05$ as compared with KET group. Data was considered significant above 60 s (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.21 Effect of chronic administration of morin on rota rod test

The effect of MOR on motor coordination, as measured by the time of performance and number of falls, using the rota rod test is shown in Table 4.13. Intraperitoneal administration of MOR (25, 50 and 100 mg/kg) once daily for 10 days did not alter motoric activity, as evidenced by the increased time of performance and decreased number of falls from rota rod bar relative to vehicle-treated groups. Similarly, RIS (0.5 mg/kg, i.p.) did not produce any change in the time of performance and number of falls. However, treatment with HLP (1 mg/kg, i.p.) demonstrated significant ($p < 0.05$) decrease in the time of performance and increase number of falls in comparison with vehicle-treated mice. Intraperitoneal injection of KET (20 mg/kg) once daily for 10 days did not show alterations on the time of performance and number of falls of animals when compared with vehicle-treated groups (Table 4.13). However, the pretreatment with HLP (1 mg/kg, i.p.) prior to KET demonstrated significant ($p < 0.05$) increased of the number of falls and decreased time of performance from the rota rod bar, suggesting extrapyramidal side effect. In contrast, pretreatment with both MOR (25, 50 and 100 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.) did not alter the time of performance and number of falls in comparison with KET-treated mice, suggesting evidence of motor coordination and lack of extrapyramidal side effect (Table 4.13).

Table 4.13: Effect of chronic administration of morin on rota rod test in mice

Treatments	Time of performance (s)	Number of falls / 2 min
VEH (10 mL/kg)	117.6 ± 1.28	0.20 ± 0.20
MOR (25 mg/kg)	118.6 ± 0.98	0.20 ± 0.20
MOR (50 mg/kg)	111.4 ± 8.60	0.20 ± 0.20
MOR (100 mg/kg)	116.2 ± 2.37	0.20 ± 0.20
HLP (1 mg/kg)	59.40 ± 3.86*	1.20 ± 0.58*
RIS (0.5 mg/kg)	117.8 ± 1.74	0.20 ± 0.20
KET (20 mg/kg)	118.60 ± 8.01	0.20 ± 0.20
MOR (25 mg/kg) + KET	117.2 ± 3.12	0.20 ± 0.20
MOR (50 mg/kg) + KET	111.6 ± 8.40	0.40 ± 0.20
MOR (100 mg/kg) + KET	118.4 ± 1.60	0.20 ± 0.40
HLP (1 mg/kg) + KET	55.80 ± 5.11#	3.40 ± 0.50#
RIS (0.5 mg/kg) + KET	117.8 ± 1.35	0.20 ± 0.20

Values represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.22 Effect of morin on ketamine-induced alteration in superoxide dismutase (SOD) and catalase (CAT) activities in mice whole brains

The effect of MOR on KET-induced alterations on SOD and CAT activities in mice whole brains is shown in Table 4.14. MOR (25, 50 and 100 mg/kg, i.p.) administered once daily for 10 days significantly ($p < 0.05$) increased SOD and CAT activities when compared with vehicle-treated group. Treatment with atypical antipsychotic agent, RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) increased SOD activity but not CAT activity compared to vehicle-treated group. HLP (1 mg/kg, i.p.) failed to increase levels of SOD and CAT activities in comparison with vehicle-treated groups. Intraperitoneal injection of KET (20 mg/kg) once daily for 10 days was found to significantly ($p < 0.05$) decrease SOD and CAT activities when compared with vehicle-treated groups. Pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented the effect of KET on the activities of SOD and CAT. HLP (1 mg/kg, i.p.) failed to reverse the KET-induced suppressive effects on antioxidant enzymes activities of SOD and CAT relative to KET-treated groups (Table 4.14).

Table 4.14: Effect of morin on superoxide dismutase and catalase activities in mice whole brains treated with ketamine

Treatments	SOD (Unit/mg protein)	CAT (Unit/mg protein)
VEH (10 mL/kg)	5.88 ± 0.39	4.96 ± 0.24
MOR (25 mg/kg)	8.12 ± 0.14*	6.38 ± 0.36*
MOR (50 mg/kg)	8.26 ± 0.10*	6.20 ± 0.37*
MOR (100 mg/kg)	8.56 ± 0.30*	7.00 ± 0.38*
HLP (1 mg/kg)	4.36 ± 0.30	4.12 ± 0.28
RIS (0.5 mg/kg)	8.08 ± 0.33*	5.34 ± 0.22
KET (20 mg/kg)	3.38 ± 0.18*	3.20 ± 0.22*
MOR (25 mg/kg) + KET	5.60 ± 0.21#	4.52 ± 0.25#
MOR (50 mg/kg) + KET	6.16 ± 0.30#	5.48 ± 0.22#
MOR (100 mg/kg) + KET	7.34 ± 0.24#	5.56 ± 0.16#
HLP (1 mg/kg) + KET	3.70 ± 0.18	2.94 ± 0.43
RIS (0.5 mg/kg) + KET	6.88 ± 0.38#	5.78 ± 0.14#

Values represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.23 Effect of morin on reduced glutathione (GSH) concentration in mice whole brains treated with ketamine

The effect of MOR on KET-induced alteration on GSH concentrations in mice whole brain is shown in Table 4.15. Administration of MOR (25, 50 and 100 mg/kg, i.p.) alone once daily for 10 days significantly ($p < 0.05$) increased the concentration of GSH compared to vehicle-treated group. Treatment RIS (0.5 mg/kg, i.p.) alone for 10 days also significant ($p < 0.05$) increased GSH concentration when compared with vehicle-treated group. However, HLP (1 mg/kg, i.p.) failed to demonstrate significant ($p > 0.05$) increase in GSH concentration in comparison with vehicle-treated group. Moreover, KET (20 mg/kg, i.p.) alone once daily for 10 days caused a significant ($p < 0.05$) decrease in GSH concentration when compared with vehicle-treated group. However, pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented decreases in GSH concentrations in KET-treated group. In contrast, pretreatment with the reference typical antipsychotic, HLP (1 mg/kg, i.p.) failed to significantly prevent KET-induced decrease in GSH concentration in mice whole brains when compared with KET administration alone (Table 4.15).

4.24 Effect of morin on malondialdehyde (MDA) content in mice whole brains treated with ketamine

The effect of MOR on KET-induced alterations on MDA content in mice whole brain is shown in Table 4.15. Intraperitoneal administration of KET (20 mg/kg, i.p.) produced significant ($p < 0.05$) increase in brain MDA content relative to vehicle-treated group, which suggests increase in lipid peroxidation . However, pretreatment with both MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly reduced brains concentrations of MDA produced by KET (20 mg/kg, i.p.) in comparison with KET-treated group. In contrast, HLP (1 mg/kg, i.p.) pretreatment failed to attenuate lipid peroxidation by KET, evidenced by the increase in brain MDA concentrations. Intraperitoneal administration of HLP (1 mg/kg, i.p.) significantly increased brain MDA concentration relative to vehicle-treated group. However, MOR (25, 50 and 100 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.) alone significantly decreased MDA concentrations in comparison to vehicle-treated group (Table 4.15).

Table 4.15: Effect of morin on reduced glutathione and malondialdehyde concentrations in mice brains treated with Ketamine

Treatments	GSH (nmol/mg protein)	MDA (µmol/mg protein)
VEH (10 mL/kg)	168.4 ± 5.51	16.40 ± 0.92
MOR (25 mg/kg)	199.2 ± 9.09*	9.20 ± 0.80*
MOR (50 mg/kg)	192.2 ± 9.55*	9.40 ± 0.92*
MOR (100 mg/kg)	202.6 ± 4.35*	8.20 ± 0.96*
HLP (1 mg/kg)	125.4 ± 8.62	26.0 ± 1.70*
RIS (0.5 mg/kg)	205.6 ± 7.61*	9.00 ± 0.83*
KET (20 mg/kg)	95.20 ± 5.88*	30.20 ± 1.77*
MOR (25 mg/kg) + KET	151.0 ± 11.78#	17.80 ± 2.05#
MOR (50 mg/kg) + KET	159.8 ± 5.07#	13.80 ± 1.59#
MOR (100 mg/kg) + KET	182.8 ± 8.17#	12.60 ± 0.92#
HLP (1 mg/kg) + KET	79.60 ± 7.20	33.20 ± 1.24
RIS (0.5 mg/kg) + KET	187.4 ± 10.40#	13.20 ± 1.59#

Values represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.25 Effect of morin on ketamine-induced alteration in nitrite levels in mice whole brains

The effect of MOR on KET–induced alteration on nitrite content in mice whole brain is shown Figure 4.16. KET (20 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in the brain concentrations of nitrite relative to vehicle-treated control, which suggests increase in nitrergic stress. Pretreatment once daily for 10 days with MOR (25, 50 and 100 mg/kg, i.p.) before KET (20 mg/kg, i.p.) significantly ($p < 0.05$) reduced brain concentrations of nitrite caused by KET in a dose-dependent manner when compared with KET-treated group, suggesting the involvement of nitrergic pathway. Also, pretreatment with RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) decreased brain concentrations of nitrite, whereas treatment with HLP (1 mg/kg, i.p.) failed to prevent the increase in nitrite by KET in comparison with KET treatment alone. Treatment with HLP (1 mg/kg, i.p.) alone once daily for 10 days significantly increased brain concentration of nitrite compared with vehicle-treated control ($p < 0.05$). MOR (100 mg/kg, i.p.) or RIS (0.5 mg/kg, i.p.) significantly decreased concentration of nitrite in mice brains relative to vehicle-treated group (Figure 4.16).

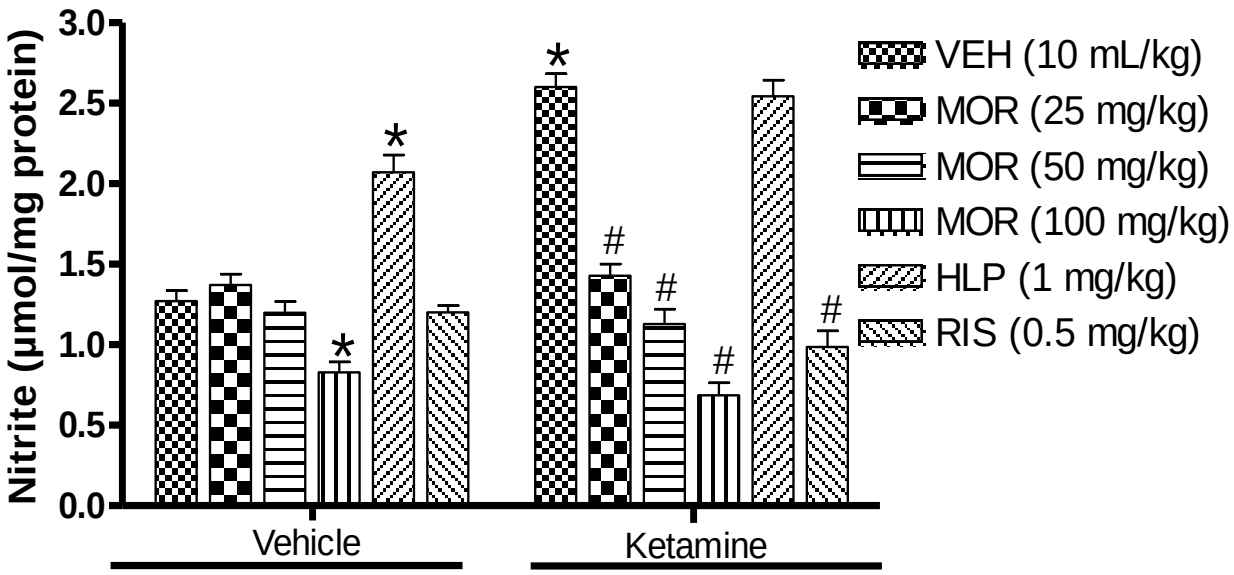


Figure 4.16: Effect of morin on ketamine-induced alteration in nitrite levels in mice brains

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.26 Effect of morin on ketamine-induced alteration in acetylcholinesterase (AChE) enzyme activity in mice whole brains

The effect of MOR on KET-induced alteration on AChE enzyme activity in mice whole brain is shown Figure 4.17. Administration of both MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) decreased brain AChE activity compared with vehicle-treated group in mice. In contrast, HLP (1 mg/kg, i.p.) given once daily for 10 days did not produce any significant change in brain activity of AChE in comparison with vehicle-treated mice. Intraperitoneal injection of KET (20 mg/kg, i.p.) significantly ($p < 0.05$) increased brain AChE enzyme activity in comparison with vehicle-treated mice, suggesting decrease in ACh concentration. Pretreatment with both MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) once daily prior to KET, demonstrated significant ($p < 0.05$) reduction in the brain AChE enzyme activity compared with KET-treated. Pretreatment with HLP (1 mg/kg, i.p.) failed to prevent the effect KET on the activity of brain AChE enzyme in mice relative to KET-treated group in mice (Figure 4.17).

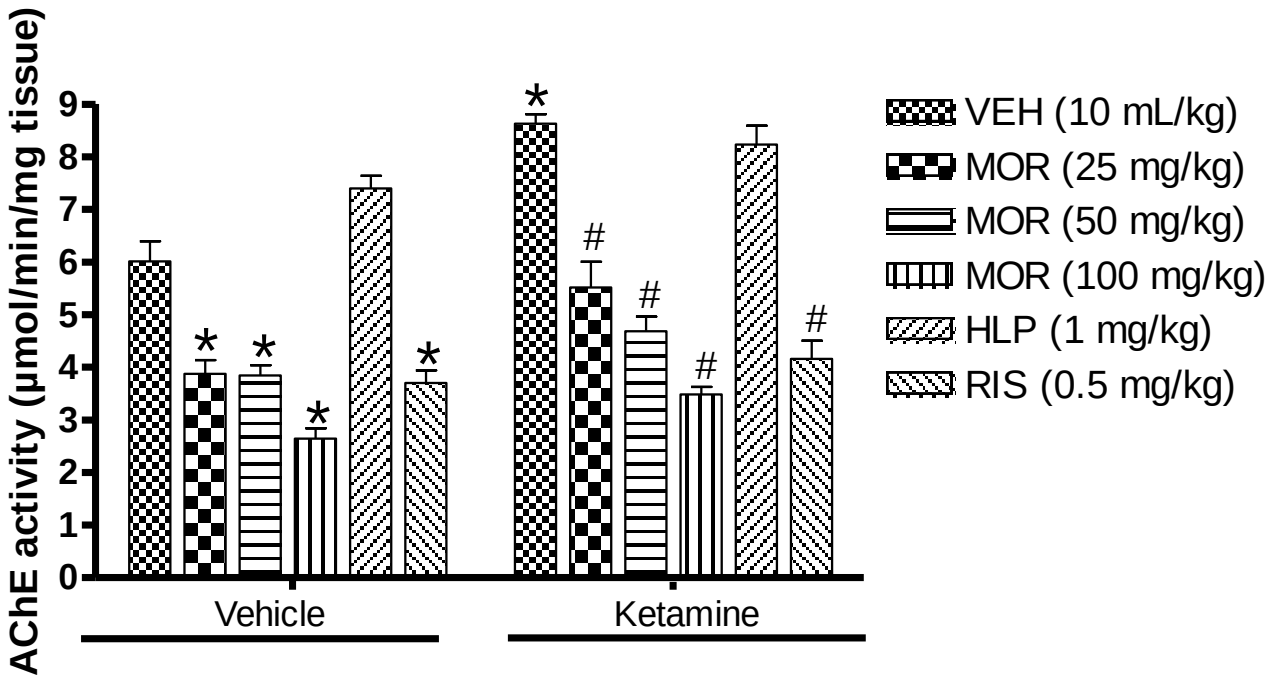


Figure 4.17: Effect of morin on brain acetylcholinesterase enzyme activity in ketamine-treated mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.27 Effect of morin on striatal neurons of mice brains

The photomicrographs of the effect of morin on histomorphological changes in the striatal neurons (caudate and putamen) of mice brains is shown in Plate 4.1 and Figure 4.18. Hematoxylin and Eosin (H&E) staining revealed that intraperitoneal injection of MOR (25 and 50 mg/kg, i.p.) once daily for 10 days did not demonstrate significant cytoarchitectural changes (Plate 4.1), as it did not affect viability of striatal neurons of caudate and putamen in comparison with vehicle-treated mice (Figure 4.18). Pretreatment with both MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p) daily for 10 days demonstrated significant ($p < 0.05$) increase in the population of viable neuronal cells and decreased pyknotic neurons of the striatum (caudate and putamen) (Figure 4.18); as evidenced by increased normal and open chromatin pattern (Plate 4.1) when compared with vehicle control. Pretreatment with HLP (1 mg/kg, i.p.) caused significant increase in the pyknotic populations of the striatal neurons with altered cytoarchiture, chromatin condensation and cytoplasmic shrinkage, with decreased population of viable striatal neurons relative to mice treated with vehicle, suggesting neuronal necrosis (Plate 4.1 and Figure 4.18).

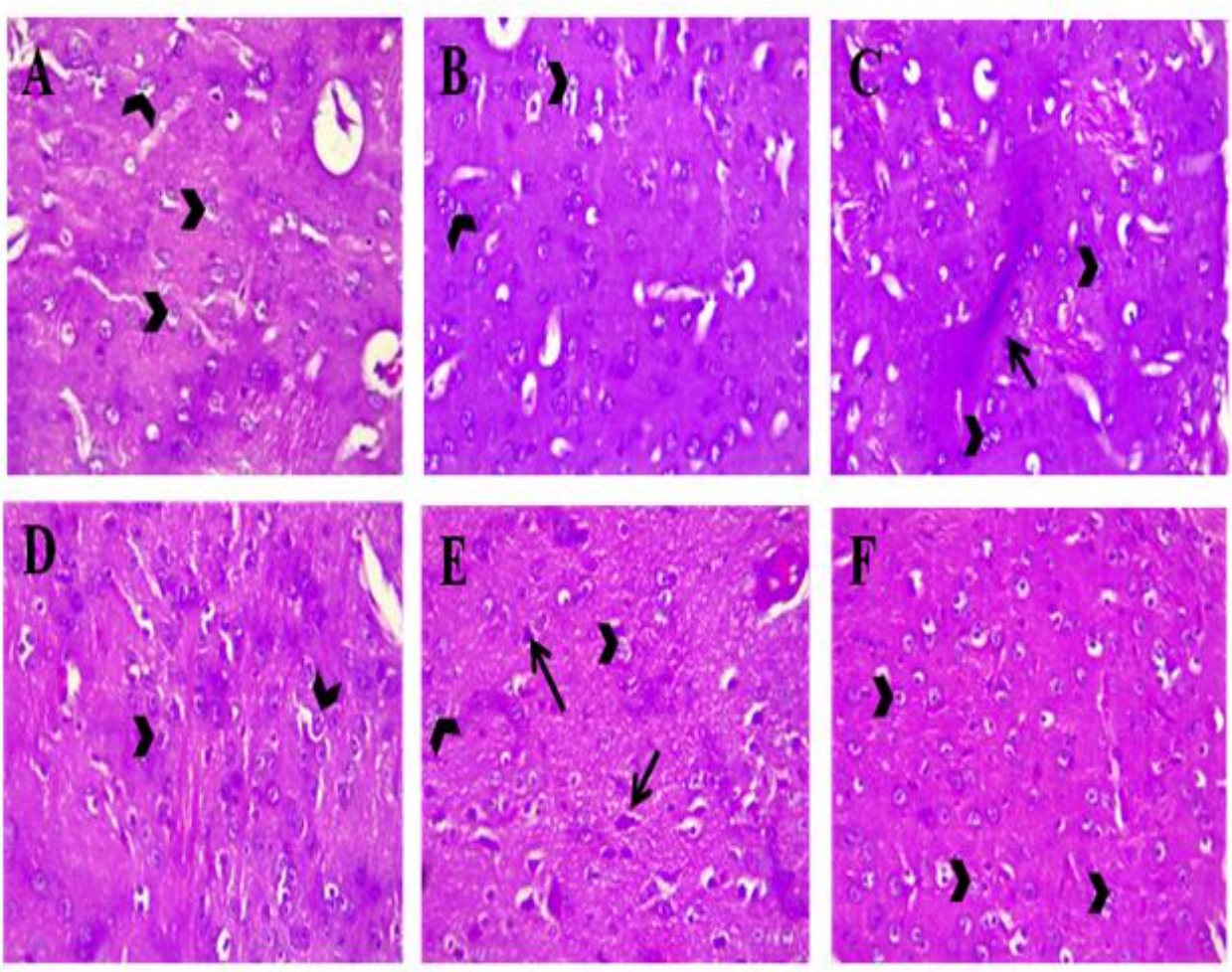


Plate 4.1: Representative photomicrographs **of *striatum* of mice brains treated with morin.** A: VEH; B: MOR (25 mg/kg); C: MOR (50 mg/kg); D: MOR (100 mg/kg); E: HLP (1 mg/kg); F: RIS (0.5 mg/kg). Slide A revealed normal histological open chromatin pattern and cytoarchiture of neurons. Slide B showed the presence of dark chromatin nuclei and decrease in the population of viable neuronal cell. Slide C shows normal neuronal cells with the presence of open chromatin nuclei. Slide D revealed increased population of open chromatin nuclei with few pyknotic striatal neurons. Slide E shows neurons with altered cytoarchiture and numerous dark chromatin nuclei. Slide F shows normal nuclei and decreased population of pyknotic neurons. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures.

VEH = Vehicle, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

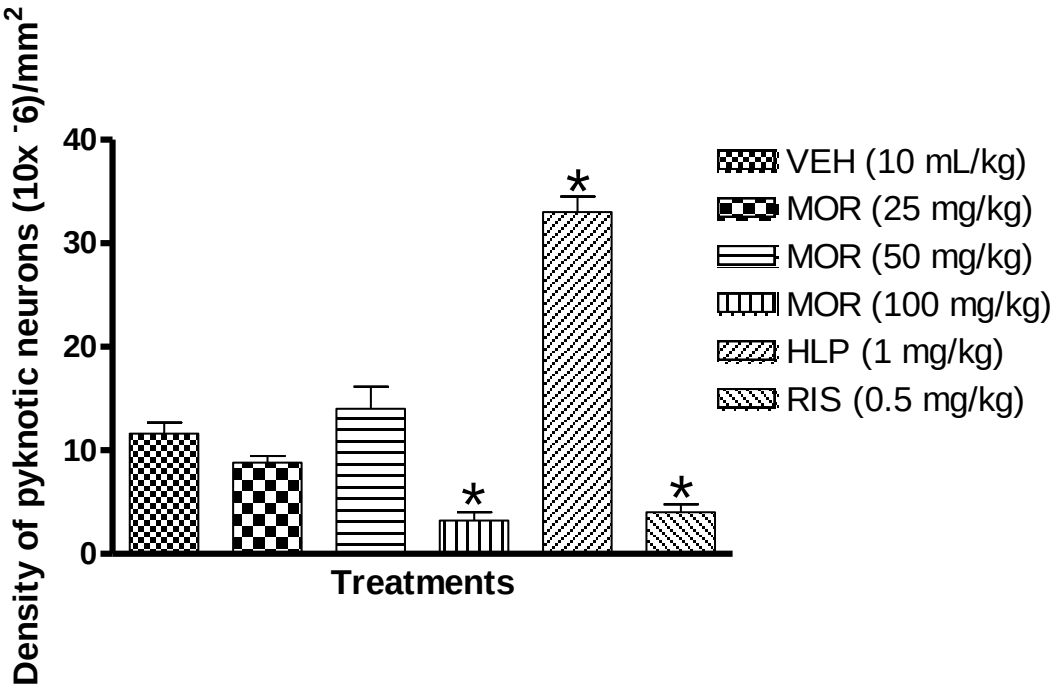


Figure 4.18: Effect of morin on neuronal cell density of the striatum of mice brains

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. #$p < 0.05$ compared to vehicle group and *$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.28 Neuroprotective effect of morin on the striatum of mice brains treated with ketamine

The photomicrographs of the effect of MOR on KET-induced histomorphological alterations and density of pyknotic neurons of the striatum of mice brains treated with KET are shown in Plate 4.2 and Figure 4.19. Intraperitoneal injection of KET (20 mg/kg) daily for 10 days demonstrated significant ($p < 0.05$) cytoarchitectural alterations, evidenced by numerous dark chromatin nuclei from chromatin condensation and shrinkage in comparison to vehicle-treated animals; showing open chromatin pattern (Plate 4.2). Furthermore, KET caused a significant ($p < 0.05$) decrease in the population of viable neuronal cells of the striatum when compared with vehicle control, suggesting neurodegeneration (Figure 4.19). Pretreatment with both MOR (100 mg/kg, i.p.), RIS (0.5 mg/kg, i.p) and HLP (1 mg/kg, i.p.) once daily for 10 days significantly ($p < 0.05$) protected the mice against neuropathological changes by KET (20 mg/kg, i.p.), as most of the striatal neuron of the caudate and putamen were repaired from cytoarchitectural alteration by KET relative to KET-treated mice alone. MOR (100 mg/kg, i.p.), RIS (0.5 mg/kg, i.p) and HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) decreased the loss of neuronal cells of the striatum both in the caudate and putamen of mice brains treated with KET in comparison with KET treatment alone, which is thus suggestive of neuroprotective effect. Meanwhile, pretreatment with lower doses of MOR (25 and 50 mg/kg, i.p.) failed to prevent neuropathological alterations and pyknotic index of cell survival of striatal neurons of mice brains treated with KET (Plate 4.2 and Figure 4.19).

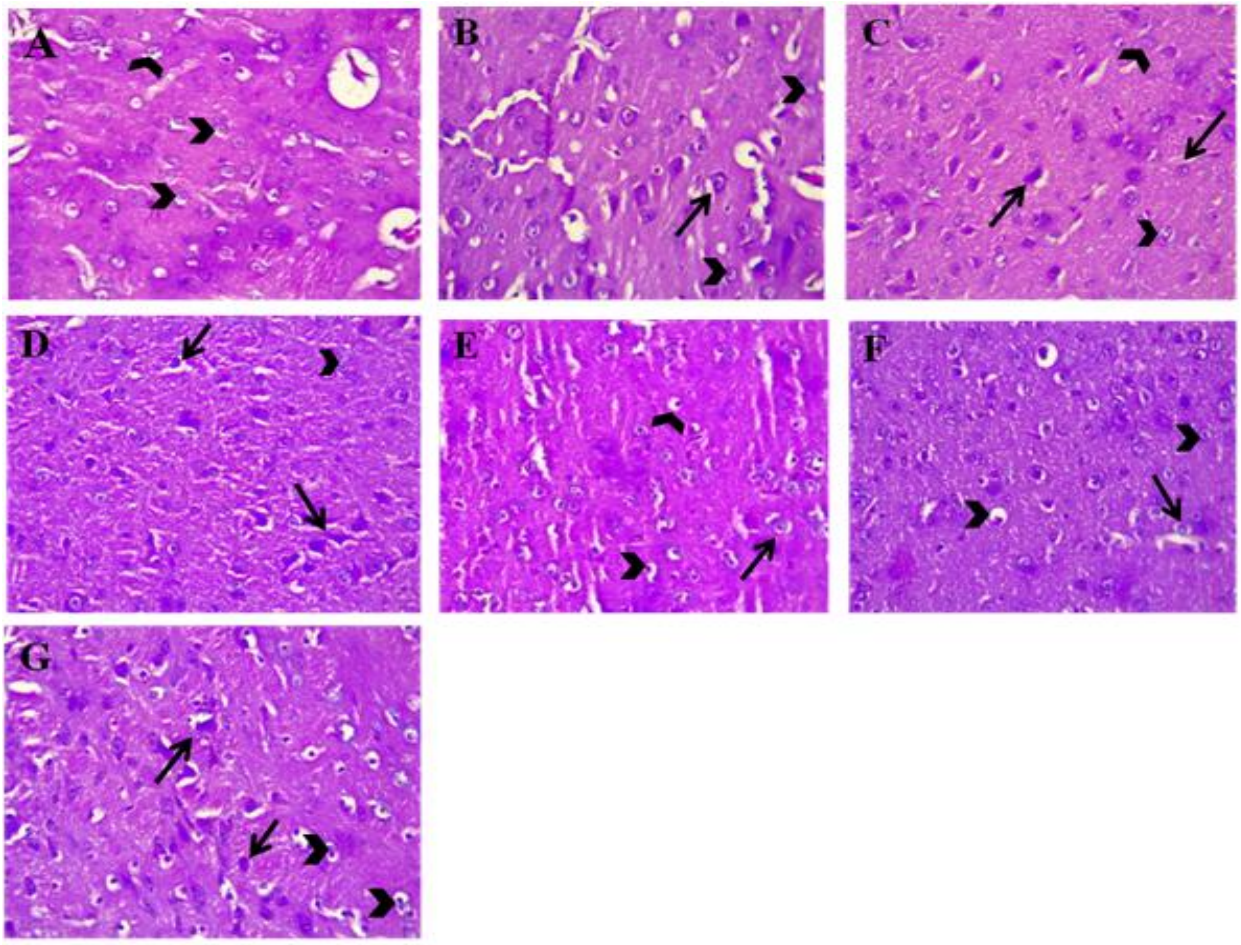


Plate 4.2: Representative photomicrographs showing the effects of morin treatments on the *striatum* of mice brains treated with ketamine. A: VEH; B: KET (20 mg/kg); C: MOR (25 mg/kg)+KET; D: MOR (50 mg/kg)+KET; E: MOR (100 mg/kg)+KET; F: HLP (1 mg/kg)+KET; G: RIS (0.5 mg/kg)+KET. Slide A revealed normal histological open chromatin pattern and cytoarchiture of neurons. Slide B showed the presence of degeneration, dark chromatin nuclei and decrease in the population of viable neuronal cell. Slide C shows dark neuronal layers and cellular outlines with evidence of some degenerating neurons. Slide D revealed neurons with the presence of condensed chromatin nuclei which are relatively not normal. Slide E revealed increased population of open chromatin nuclei and viable striatal neurons. Slide F shows neurons with altered cytoarchiture and numerous dark chromatin nuclei. Slide G shows normal nuclei and decreased population of pyknotic neurons. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures. **VEH** = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

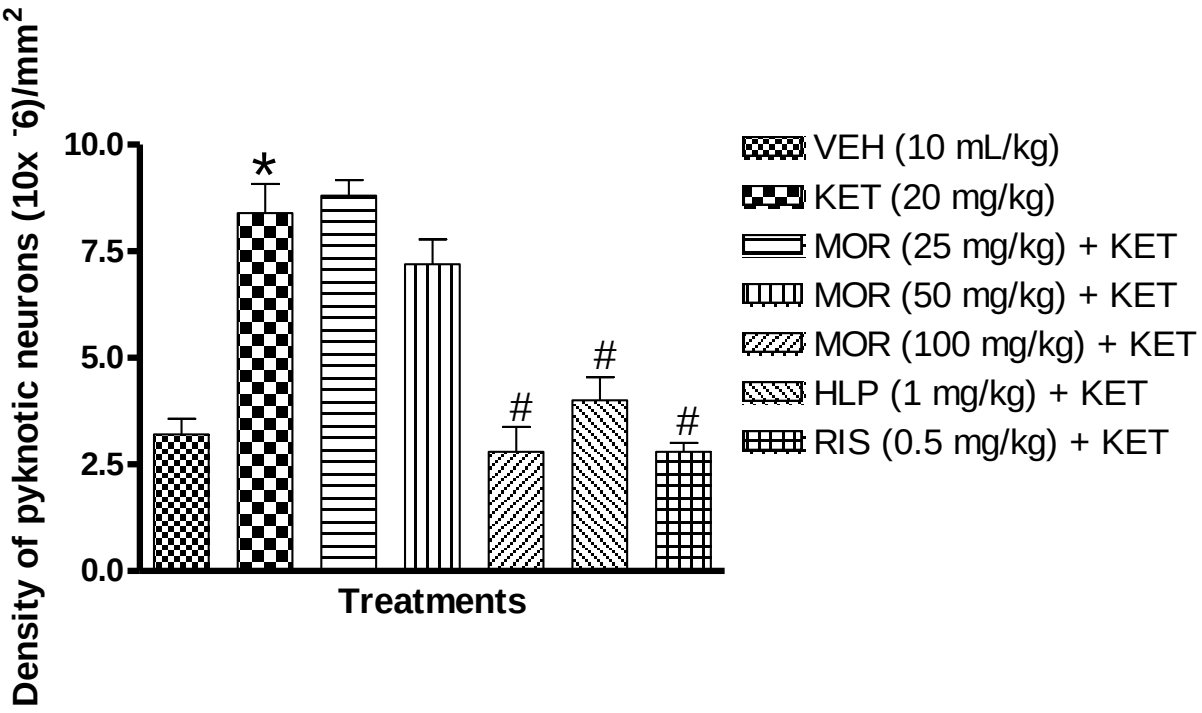


Figure 4.19: Effect of morin on neuronal cell density of the striatum of mice brains treated with ketamine

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. #$p < 0.05$ compared to vehicle group and *$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.29 Effect of morin on prefrontal cortical neurons of mice brains

Hematoxylin and Eosin photomicrographs revealed the histomorphological changes in the prefrontal cortex (PFC) of mice brains treated with MOR alone for 10 days (Plate 4.3 and Figure 4.20). Administration of MOR (25 mg/kg, i.p.) did not demonstrate significant ($p > 0.05$) cytoarchitectural changes (Plate 4.3), as did not affect the population of viable neuronal cells of the PFC (Figure 4.20) when compared with vehicle control. However, in comparison with vehicle control group; with normal round shape and open chromatin nuclei of neurons without condensation, decreased pyknotic cells with evidence of non-distorted cytoarchitecture (Plate 4.4) and significant ($p < 0.05$) increase in the population of viable prefrontal cortical neuronal cells (Figure 4.20) were observed in MOR (50 and 100 mg/kg, i.p.) treated mice brains. HLP (1 mg/kg, i.p.) caused significant ($p < 0.05$) distorted cytoarchitectural, dark chromatin patterns (Plate 4.3) and increased density of pyknotic neuronal cells (Figure 4.20) when compared with vehicle-treated mice. Meanwhile, treatment with RIS (0.5 mg/kg, i.p.) did not cause any significant cytoarchitectural and histomorphological changes in the PFC of the brains of mice relative to vehicle-treated mice alone (Plate 4.3 and Figure 4.20).

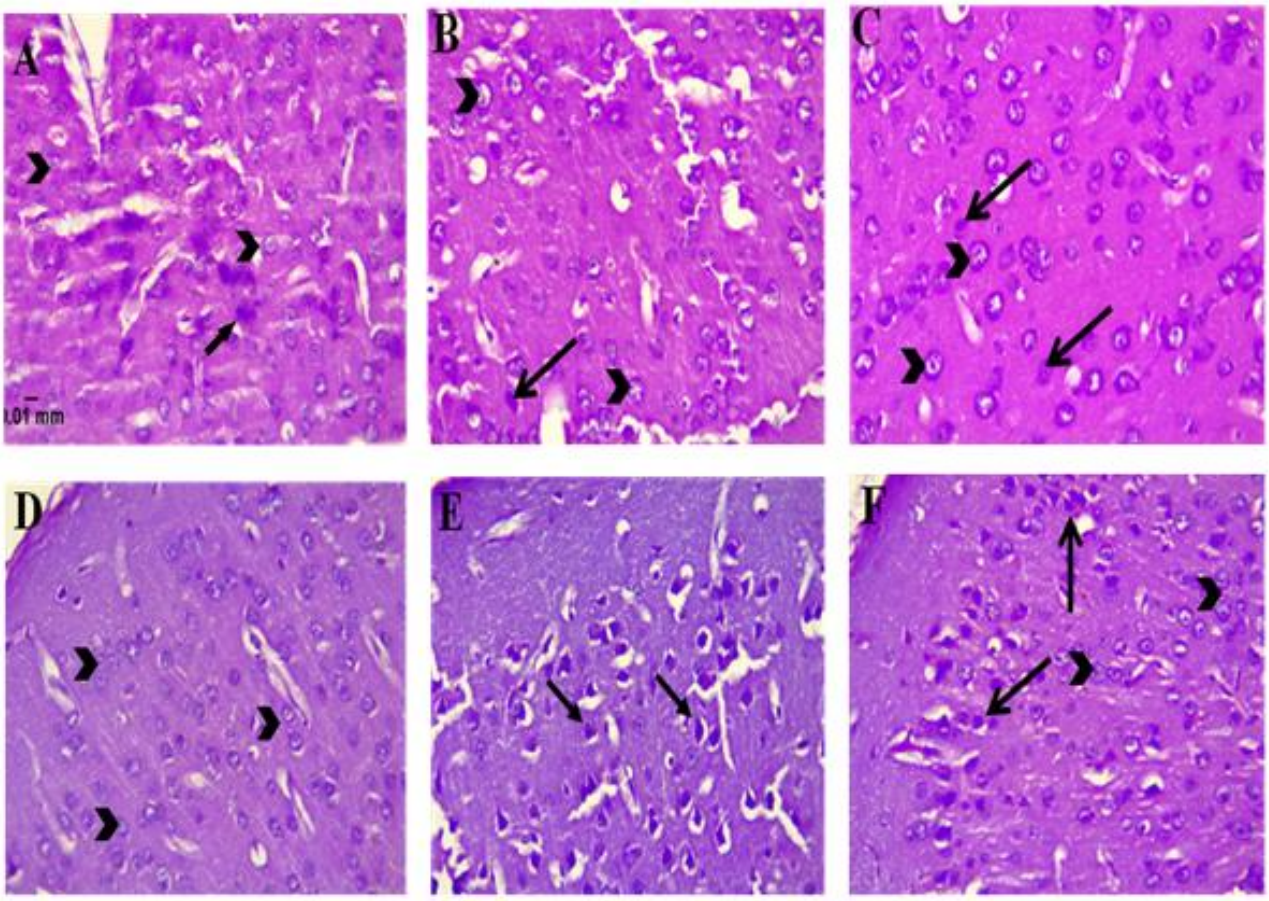


Plate 4.3: Representative photomicrographs **of *prefrontal cortex* (PFC) of mice brains treated with morin.** A: VEH; B: MOR (25 mg/kg); C: MOR (50 mg/kg); D: MOR (100 mg/kg); E: HLP (1 mg/kg); F: RIS (0.5 mg/kg). Slide A revealed normal histological open chromatin pattern and cytoarchiture of neurons. Slide B showed no changes in the chromatin nuclei and population of viable neuronal cell. Slide C revealed normal neuronal cells with the presence of open chromatin nuclei and increased population of viable neuronal cells. Slide D shows increased population of open chromatin nuclei with decreased density of pyknotic prefrontal cortical neuronal cells. Slide E shows neurons with distorted cytoarchiture and numerous dark chromatin condensation and shrinkages. Slide F shows fairly normal nuclei and unaffected population of viable neuronal cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures.

VEH = Vehicle, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

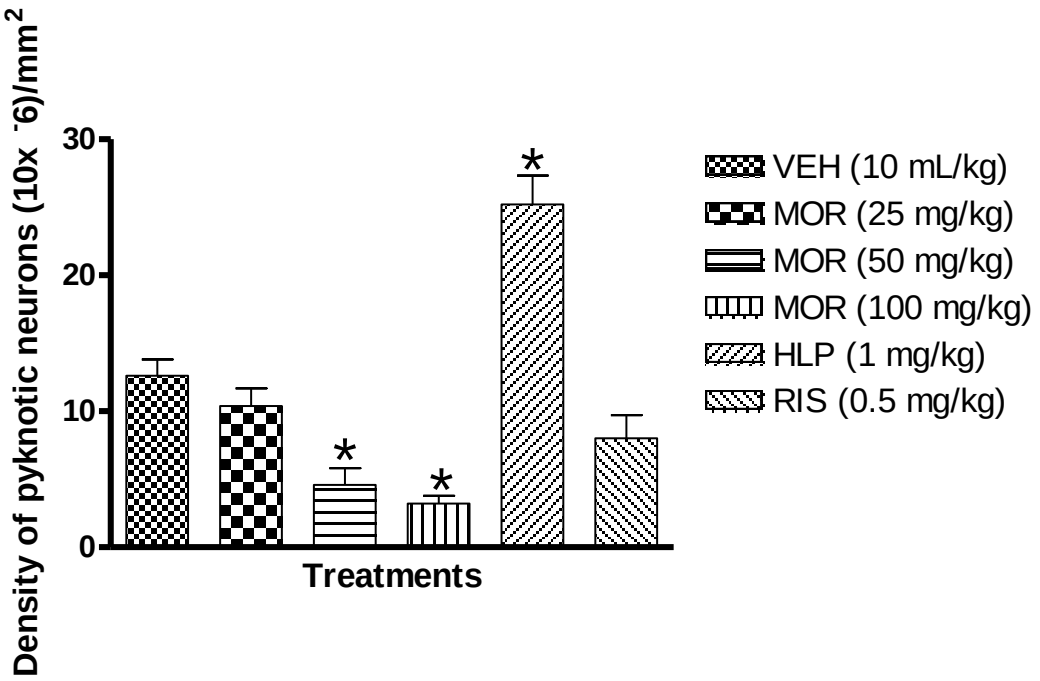


Figure 4.20: Effect of morin on neuronal cell density of the prefrontal cortex (PFC) of mice brains

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. #$p < 0.05$ compared to vehicle group and *$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

4.30 Neuroprotective effect of morin on prefrontal cortex of mice brains treated with ketamine

The photomicrographs and density of pyknotic neurons of the PFC of mice brains treated with MOR on KET-induced cytoarchitectural and histomorphological alterations respectively, is shown in Plate 4.4 and Figure 4.21. Treatment with KET (20 mg/kg, i.p.) once daily for 10 days produced a significant ($p < 0.05$) increases in chromatin condensation and shrinkages as indicated by cytoarchitectural alterations with dark chromatin nuclei when compared with vehicle-treated animals (Plate 4.4). KET increase the population of pyknotic neuronal cells of the PFC, as it demonstrated a significant ($p < 0.05$) increase in chromatin condensation when compared with vehicle control (Figure 4.21). However, pretreatment with MOR (25, 50 and 100 mg/kg, i.p.) once daily prior to KET treatment for 10 days demonstrated a dose-dependent prevention of the cytoarchitectural alterations in the PFC by KET (20 mg/kg, i.p.) in a significant ($p < 0.05$) manner (Plate 4.4). Furthermore, MOR (25, 50 and 100 mg/kg, i.p.) pretreatment interaction with KET significantly ($p < 0.05$) attenuated neuronal necrosis in the PFC in KET-treated mice in comparison with KET treatment alone, suggesting neuroprotective property (Figure 4.21). Similarly, pretreatment with the atypical antipsychotic standard drug, RIS (0.5 mg/kg, i.p.) caused a significant ($p < 0.05$) recovery and repair of cytoarchitectural alterations, as well as a decrease in the number of pyknotic neurons in the PFC of mice brains relative to KET-treated mice. However, pretreated animals with the typical antipsychotic drug, HLP (1 mg/kg, i.p.) showed no significant protection and repair against KET treatment, as most of the prefrontal cortical neuronal cells showed dark shrink chromatin nuclei, and increased pykontic index (dead neuronal cells) when compared with KET-treated mice alone (Plate 4.4 and Figure 4.21).

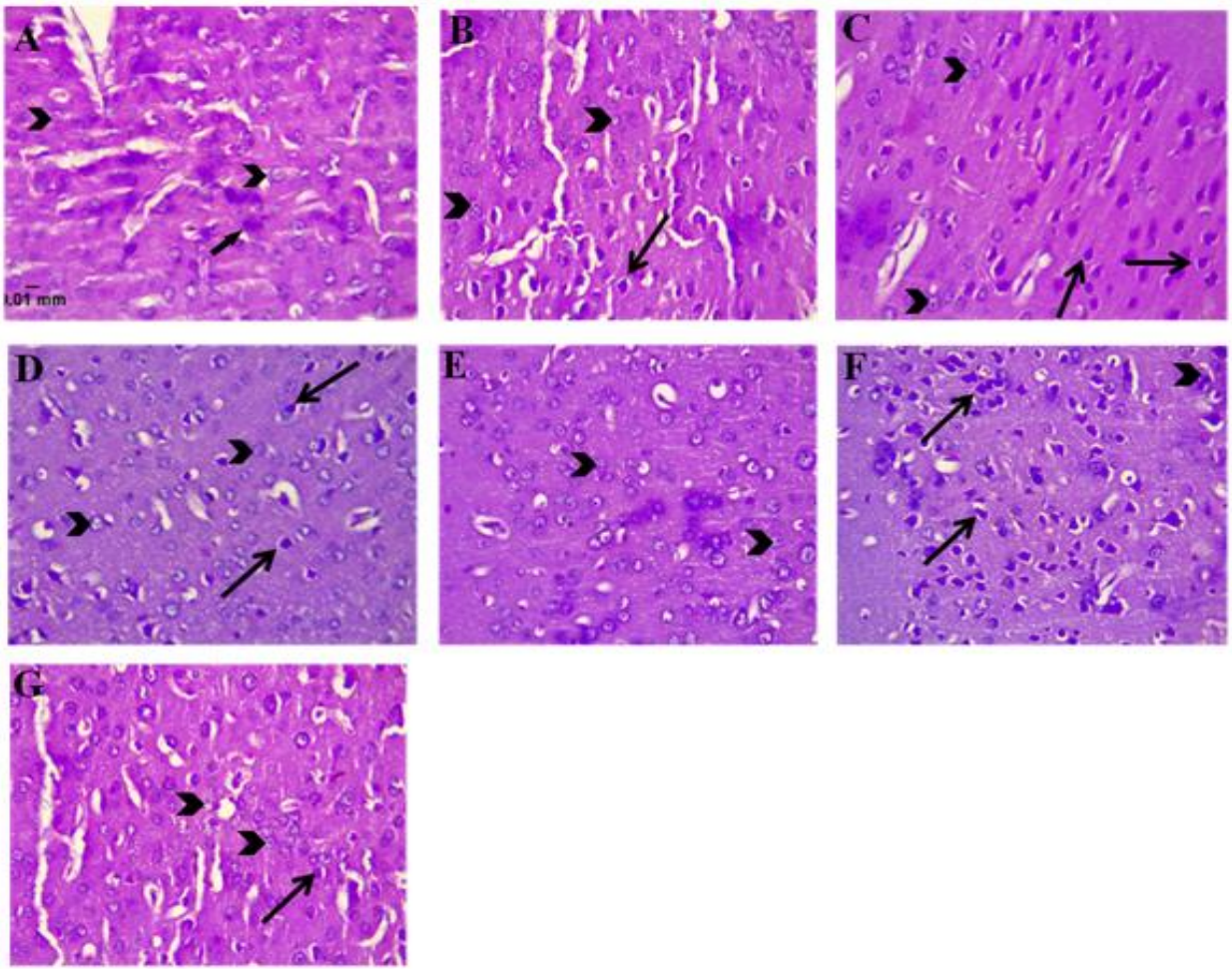


Plate 4.4: Representative photomicrographs showing the effects of morin treatments on the *prefrontal cortex* (PFC) of mice brains treated with ketamine. A: VEH; B: KET (20 mg/kg); C: MOR (25 mg/kg)+KET; D: MOR (50 mg/kg)+KET; E: MOR (100 mg/kg)+KET; F: HLP (1 mg/kg)+KET; G: RIS (0.5 mg/kg)+KET. Slide A revealed normal histological open chromatin pattern and cytoarchiture of neurons. Slide B showed the presence of dark chromatin nuclei and decrease in the population of pyknotic neuronal cell. Slide C shows normal neuronal cells with the presence of open chromatin nuclei. Slide D revealed neurons with the presence of open chromatin nuclei. Slide E revealed increased population of open chromatin nuclei and viable prefrontal neurons. Slide F shows neurons with altered cytoarchiture and numerous dark chromatin nuclei. Slide G shows normal nuclei and decreased population of pyknotic neurons. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures.

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

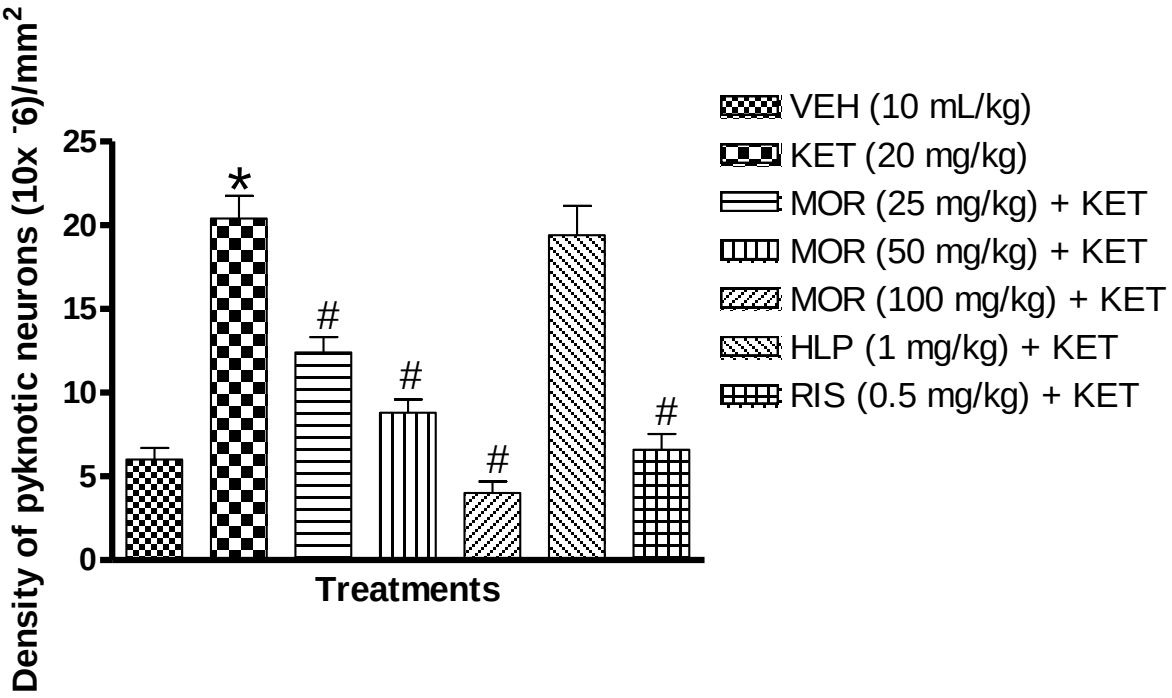


Figure 4.21: Effect of morin on neuronal cell density of the prefrontal cortex of mice treated with ketamine

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. #$p < 0.05$ compared to vehicle group and *$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.31 Effect of morin on cornus ammonis 1 region of the hippocampus of mice brains

The Hematoxylin and Eosin photomicrograph of the histomorphological changes and neuronal density in the cornus ammonis 1 (CA1) region of the hippocampus of mice treated with MOR alone for 10 days is shown in Plate 4.5 and Figure 4.22. Administration of both MOR (25, 50 and 100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) once daily for 10 days did not significantly ($p > 0.05$) affect cytoarchitectural changes (Plate 4.5) and population of viable neuronal cells of the CA1 region, including stratum oriens (SO), stratum pyramidalis (SP) and stratum radiatum (SR), of the hippocampus (Figure 4.22) when compared with vehicle control; with normal round shape and open chromatin nuclei of neurons, decreased pyknotic cells with evidence of non-distorted cytoarchitecture. However, HLP (1 mg/kg, i.p.) given once daily for 10 days showed neurons exhibiting distorted cytoarchitecture with dark chromatin nuclei (Plate 4.5) and increase density of pyknotic neurons of the CA1 region, particularly of the SP of the hippocampus in comparison with vehicle control group (Figure 4.22).

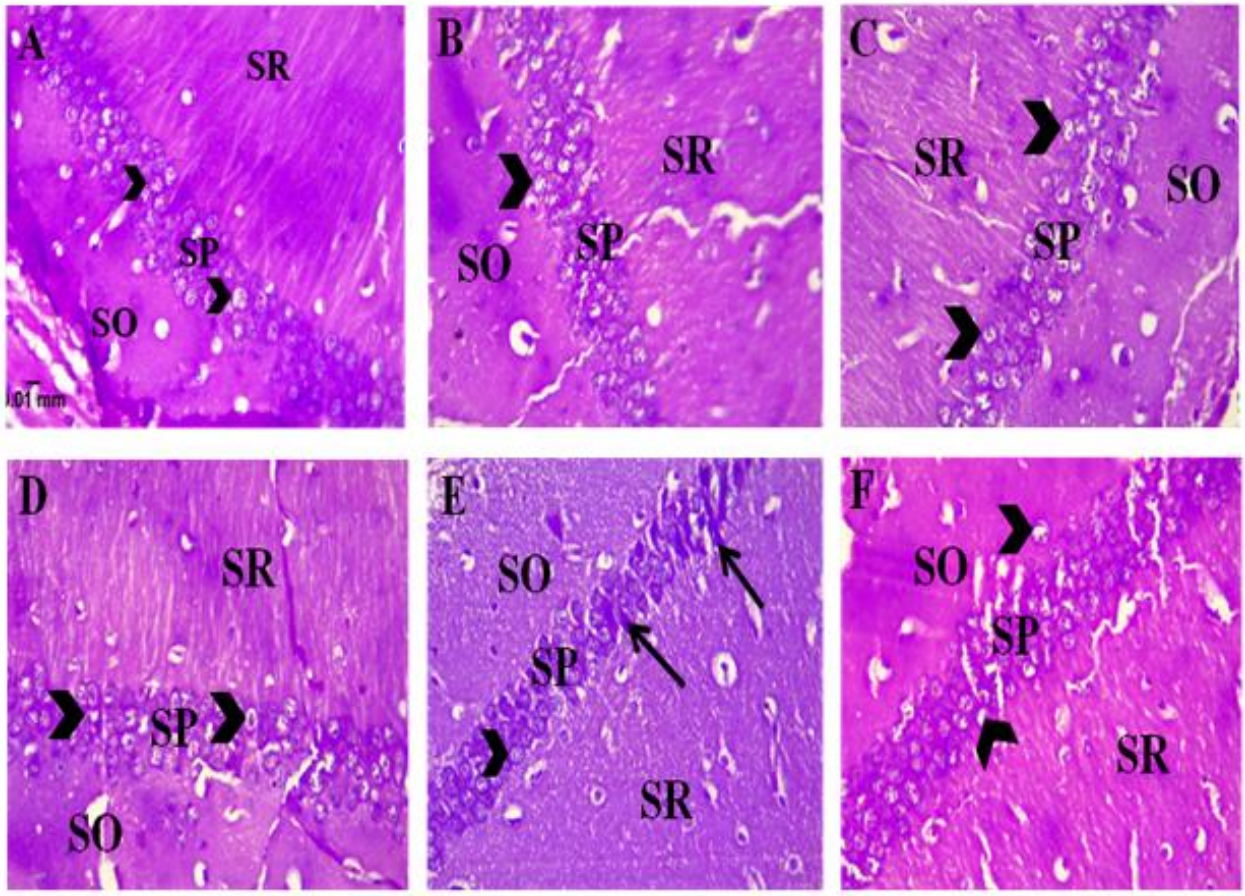


Plate 4.5: Representative photomicrographs **of cornus ammonis 1 region of the *hippocampus* of mice brains treated with morin.** A: VEH; B: MOR (25 mg/kg); C: MOR (50 mg/kg); D: MOR (100 mg/kg); E: HLP (1 mg/kg); F: RIS (0.5 mg/kg). Slide A revealed normal histological open chromatin pattern and cytoarchiture of neurons of stratum oriens (SO), stratum pyramidalis (SP) and stratum radiatum (SR). Slide B, C and D revealed normal neuronal cells and showed no changes in the chromatin nuclei and population of viable neuronal cell. Slide E shows neurons with distorted cytoarchiture and numerous dark chromatin condensations. Slide F shows fairly normal nuclei and unaffected population of viable neuronal cells. Arrow head – normal neuron; arrow - dark neurons. Heamotoxylin Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures.

VEH = Vehicle, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

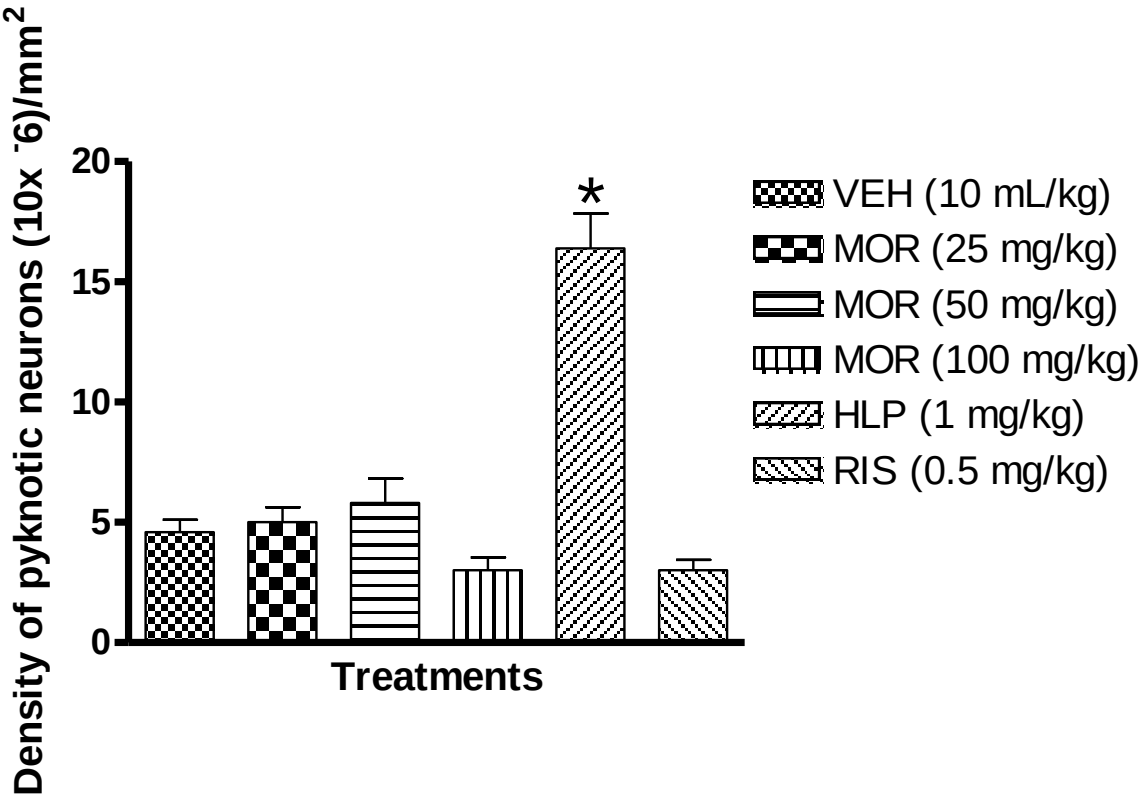


Figure 4.22: Effect of morin on neuronal cell density of the cornus ammonis 1 region of the hippocampus of mice brains

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. #$p < 0.05$ compared to vehicle group and *$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

4.32 Effect of morin on cornus ammonis 1 region of the hippocampus of mice brain treated with ketamine

The photomicrographs of the effect of MOR on histomorphological changes and neuronal density in the CA1 region of the hippocampus of mice treated with KET for 10 days is shown in Plate 4.6 and Figure 4.23. Intraperitoneal injection of KET (20 mg/kg, i.p.) once daily for 10 days did not produce significant ($p > 0.05$) cytoarchitecture changes, most of the neuronal cell of the hippocampal (CA1) areas remained intact when compared with vehicle-treated mice (Plate 4.6 and Figure 4.23). In the same pattern, pretreatment with MOR (25, 50 and 100 mg/kg, i.p.), RIS (0.5 mg/kg, i.p.) and HLP (1 mg/kg, i.p.) did not produced any significant changes in KET-treated mice, as the layers of the CA1, namely stratum oriens, stratum pyramidalis and stratum radiatum appears unperturbed in all the treatment groups (Plate 4.6 and Figure 4.23).

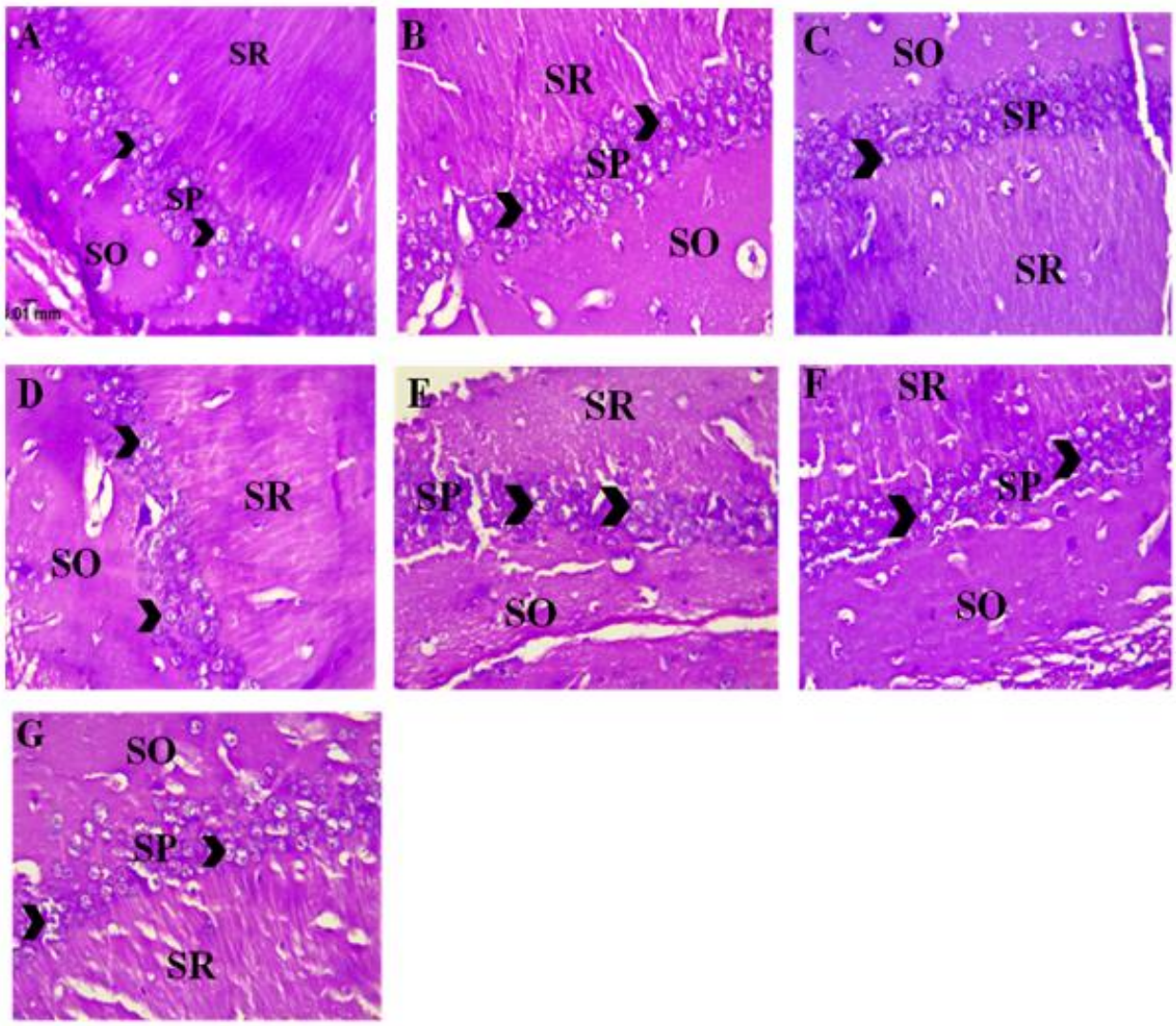


Plate 4.6: Representative photomicrographs showing the effects of morin on the **cornus ammonis 1 region of the *hippocampus*** of mice brains treated with ketamine. A: VEH; B: KET (20 mg/kg); C: MOR (25 mg/kg)+KET; D: MOR (50 mg/kg)+KET; E: MOR (100 mg/kg)+KET; F: HLP (1 mg/kg)+KET; G: RIS (0.5 mg/kg)+KET. Slide A, B, C, D, E and F revealed normal neuronal cells and showed no changes in the chromatin nuclei and population of viable neuronal cell of stratum oriens (SO), stratum pyramidalis (SP) and stratum radiatum (SR). Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all figures.

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP** = Haloperidol, **RIS** = Risperidone

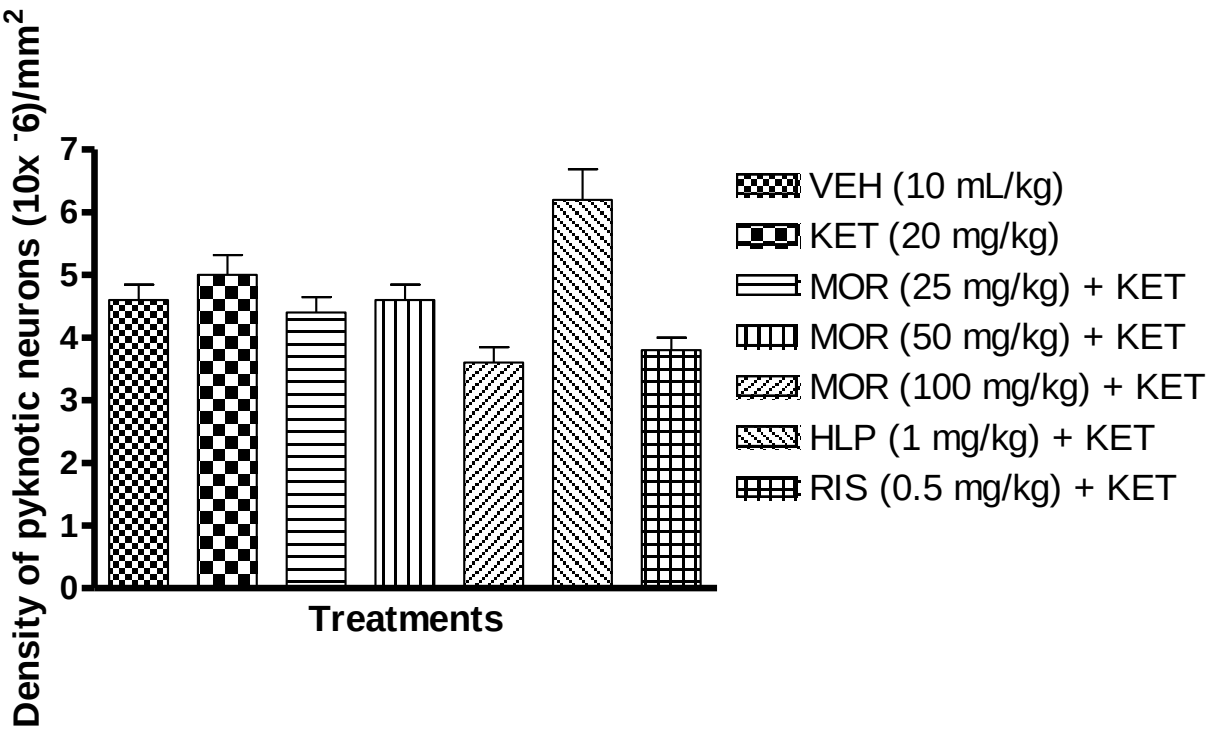


Figure 4.23: Effect of morin on neuronal cell density of the cornus ammonis 1 (CA1) region of the hippocampus of mice brains treated with ketamine

Bars represent densitometry analysis: Each column represents mean ± S.E.M for 3 animals / group. (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.33 Effect of morin on preventive and reversal treatments of ketamine-induced stereotypy

The effect of MOR on preventive and reversal treatment protocols of KET-induced stereotypy, as assessed by the stereotypy behaviours represented by repetitive head movements, intermittent sniffing, chewing and intense licking, using the stereotypy scale are shown in Figure 4.24 (A and B). Intraperitoneal administration of KET (20 m g/kg) significantly ($p < 0.05$) induced repetitive head movements, intermittent sniffing, chewing and intense licking, which suggests stereotypy behaviour in both preventive (Figure 4.24A) and reversal (Figure 4.24B) protocols. However, the marked stereotypic behaviours were significantly ($p < 0.05$) prevented and reversed by MOR (100 mg/kg, i.p.) when compared with KET-treated mice. Treatments with the standard drugs, HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated the stereotyped behaviour induced by KET in both treatment protocols in comparison with KET (20 mg/kg, i.p.) treatment alone (Figure 4.24A and B).

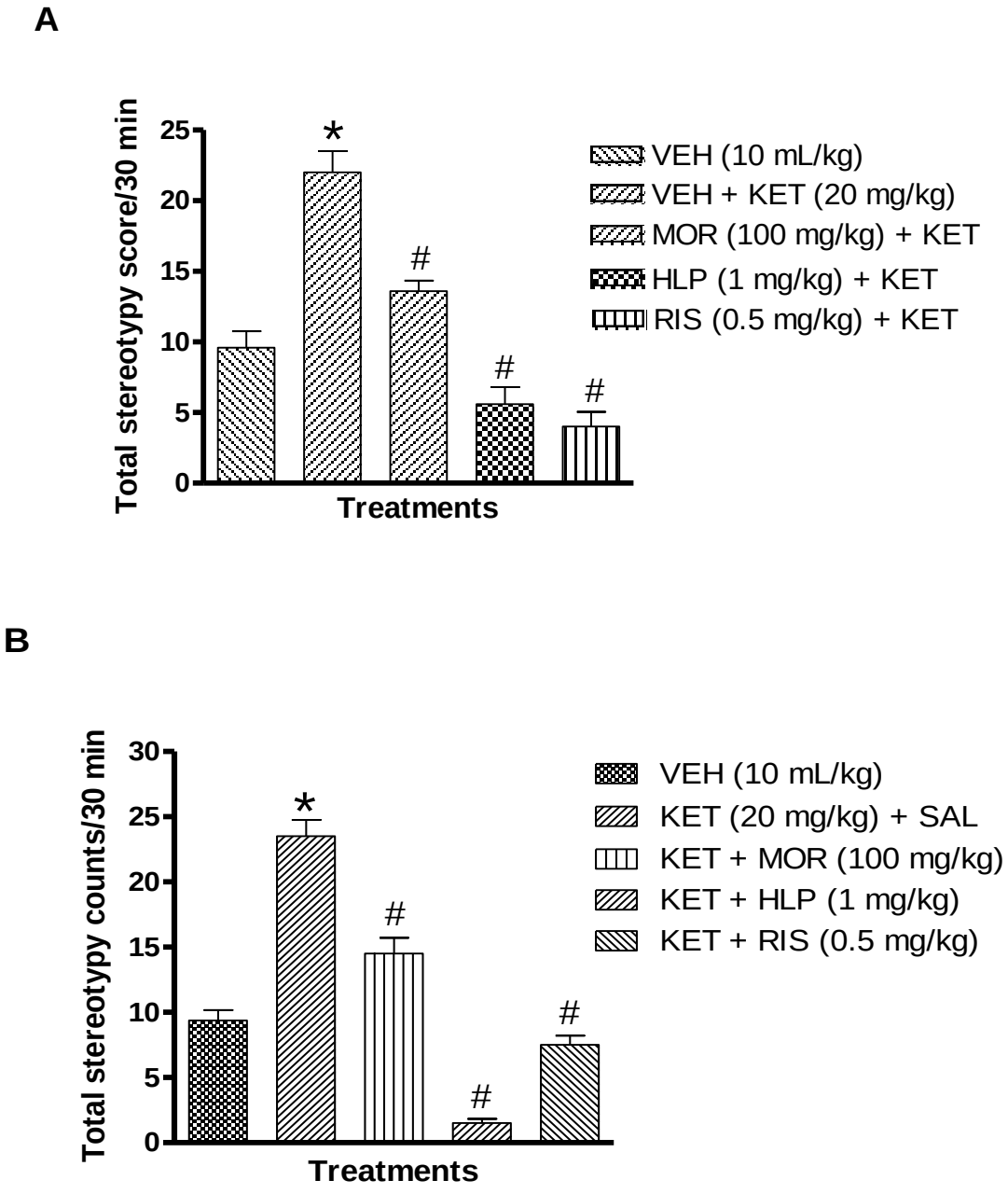


Figure 4.24: Effect of morin on ketamine-induced stereotypy in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.34 Effect of morin on ketamine-induced hyperlocomotion in the preventive and reversal treatment protocols

The effect of MOR on KET-induced hyperlocomotion as measured by the number of line crossings in the open field test (OFT) in the preventive and reversal treatment protocols in mice is shown in Figure 4.25 (A and B). Intraperitoneal injection of KET (20 mg/kg, i.p.) significantly ($p < 0.05$) increased the number of line crossings in the OFT in both protocols when compared with vehicle-treated group, which suggests hyperlocomotion. However, MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated KET-induced hyperlocomotion in the preventive (Figure 4.25A) and reversal (Figure 4.25B) treatment protocols relative to KET-treated mice. Similarly, both HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced the hyperlocomotion induced by KET (20 mg/kg, i.p.) in the preventive (Figure 4.25A) and reversal (Figure 4.25B) treatment protocols when compared with KET-treated mice.

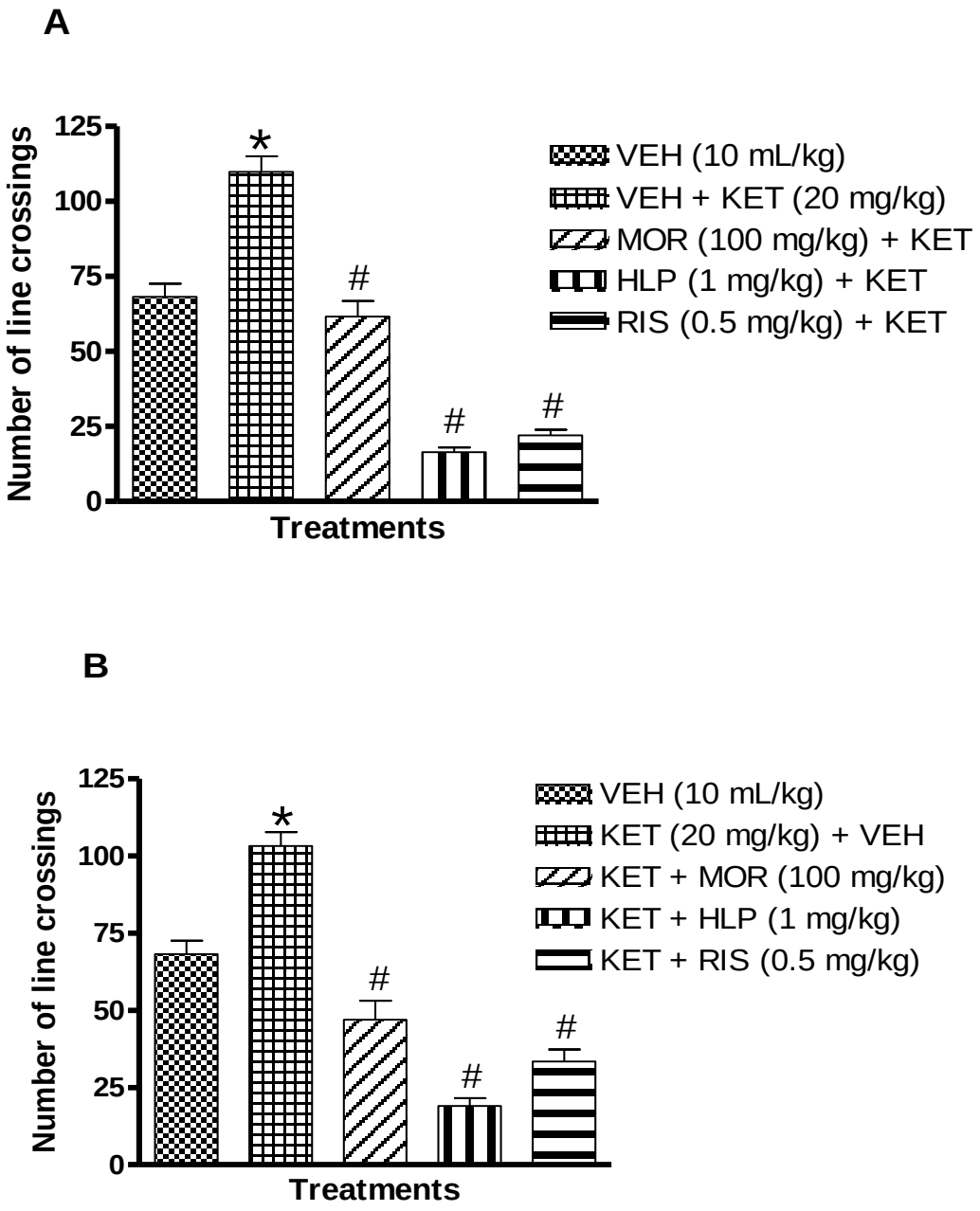


Figure 4.25: Effect of morin on ketamine-induced hyperlocomotion in the preventive (A) and reversal (B) treatment protocols in mice.

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.35 Effect of morin on ketamine-induced impairment of spatial working memory in the preventive and reversal treatment protocols

The effect of MOR on KET-induced deficit in spatial working memory based on alternation behaviour in the YMT in the preventive and reversal treatment protocols is shown in Figure 4.26 (A and B). KET (20 mg/kg, i.p.) in both chronic treatment protocols, significantly ($p < 0.05$) decreased percentage correct alternations in the YMT in comparison with vehicle-treated group, suggesting impairment in spatial working memory. However, both MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented (Figure 4.26A) and reversed (Figure 4.26B) the spatial memory impairment induced by KET when compared with KET treatment alone. Treatment with HLP (1 mg/kg, i.p.) failed to prevent and reverse the spatial working memory deficit by KET in both protocols in comparison with KET-treated mice (Figure 4.26A and B).

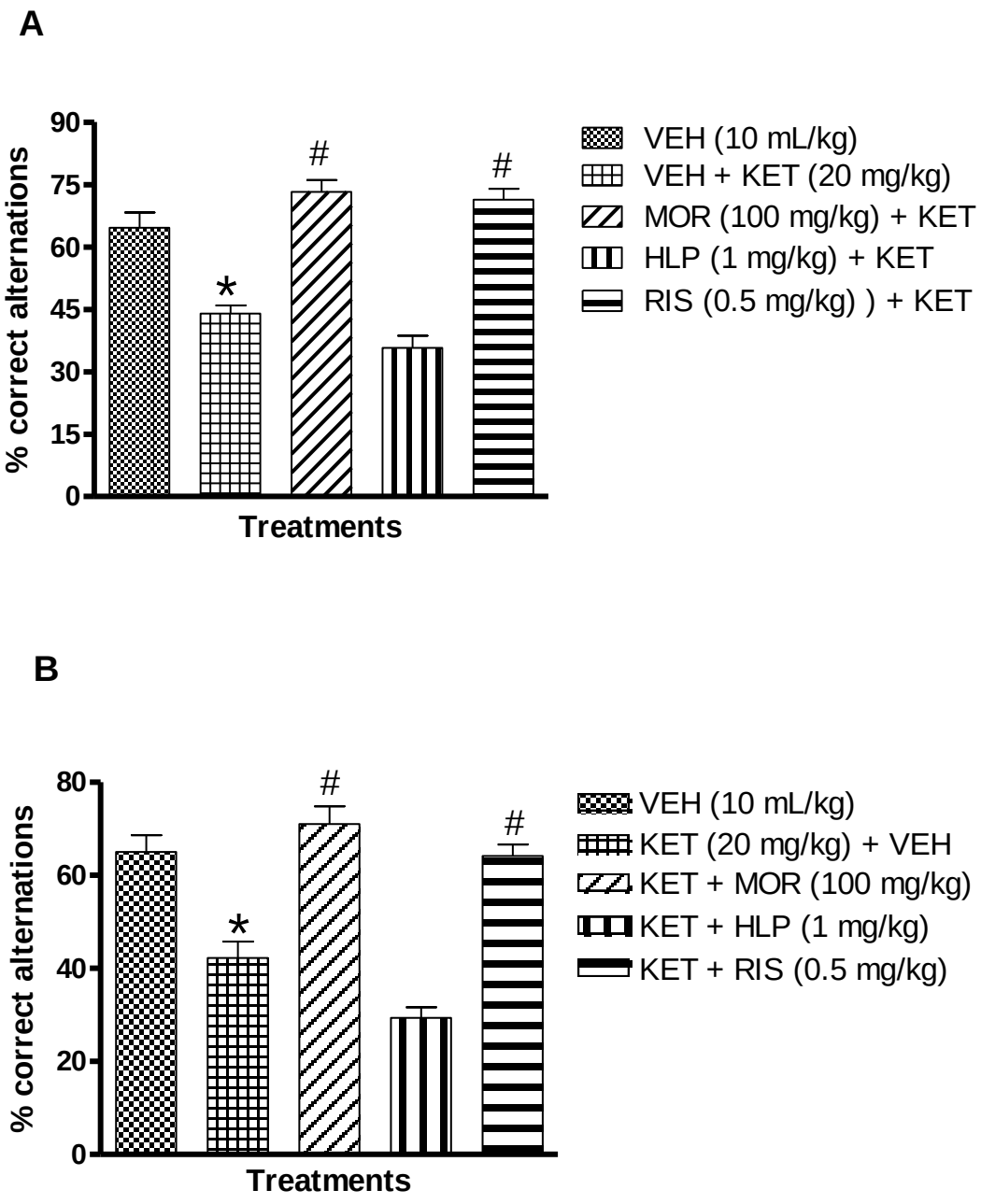


Figure 4.26: Effect of morin on ketamine-induced impairment in spatial working memory in the preventive (A) and reversal (B) treatment protocols in mice.

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.36 Effect of morin on ketamine-induced impairment of non-spatial recognition memory in the preventive and reversal treatment protocols

The effect of MOR on KET-induced impairment on recognition memory as measured by discrimination index in the novel object recognition test (NORT) in the preventive and reversal treatment protocols in mice is shown in Figure 4.27 (A and B). Chronic intraperitoneal injection of KET (20 mg/kg) demonstrated significant ($p < 0.05$) decreases in the percentage preferences for novel object, as evidenced by the decrease in the discrimination index of both treatment protocols relative to vehicle-treated; which suggests impairment of recognition memory function. Nevertheless, the interaction of MOR (100 mg/kg, i.p.) significantly prevented (Figure 4.27A) and reversed (Figure 4.27B) ($p < 0.05$) the recognition memory impairment induced by KET when compared with KET-treated group. Similarly, treatment with the reference drug, RIS (0.5 mg/kg, i.p.), but not HLP (1 mg/kg, i.p.), significantly ($p < 0.05$) attenuated KET-induced impairment on recognition memory when compared with KET-treated group in the preventive (Figure 4.27A) and reversal (Figure 4.27B) treatment protocols in mice.

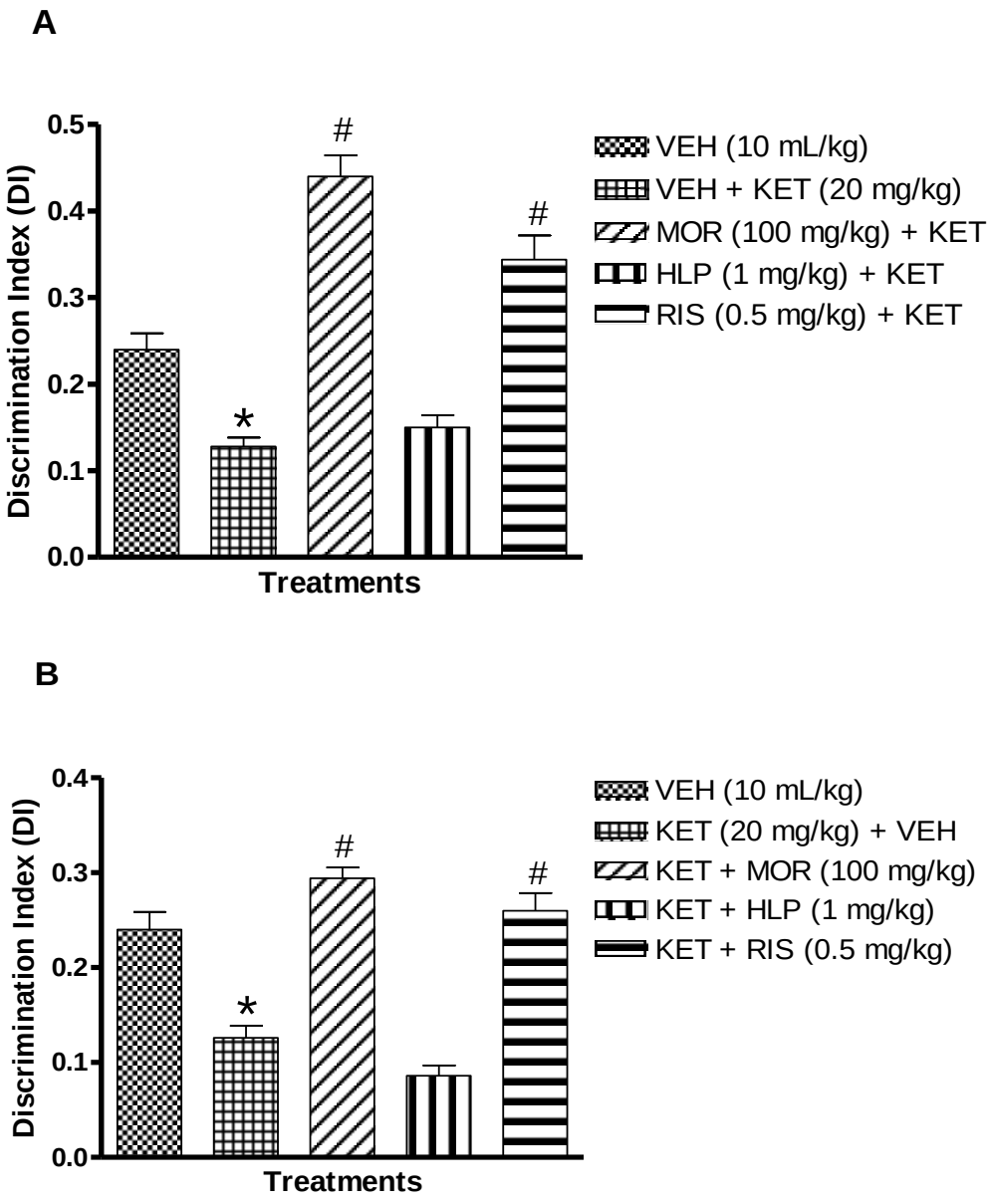


Figure 4.27: Effect of morin on ketamine-induced impairment in non-spatial recognition memory in the preventive (A) and reversal (B) treatment protocols in mice.

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.37 Effect of morin on ketamine-induced deficit in social interaction in the preventive and reversal treatment protocols

The effect of MOR on KET-induced deficit in social interaction based on percentage social preferences in the social interaction test (SIT) in the preventive and reversal treatment protocols in mice is illustrated in Figure 4.28 (A and B). Treatment with KET (20 mg/kg, i.p.) significantly ($p < 0.05$) caused a decrease in % social preference in the preventive (Figure 4.28A) and reversal (Figure 4.28B) treatments when compared with vehicle-treated group, which suggests social withdrawal. However, preventive and reversal treatments with MOR (100 mg/kg, i.p.) prevented and reversed KET-induced social withdrawal, by significantly ($p < 0.05$) elevating the level of % social interaction preferences in mice (Figure 4.28A and B). As shown in Figure 4.28 (A and B), RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented and reversed KET-induced social withdrawal in the SIT model in mice. Treatment with HLP (1 mg/kg, i.p.) did not show any significant prevention or reversal of KET-induced deficit in social interaction preference relative to KET-treated group; rather showed tendency of enhanced social withdrawal (Figure 4.28A and B).

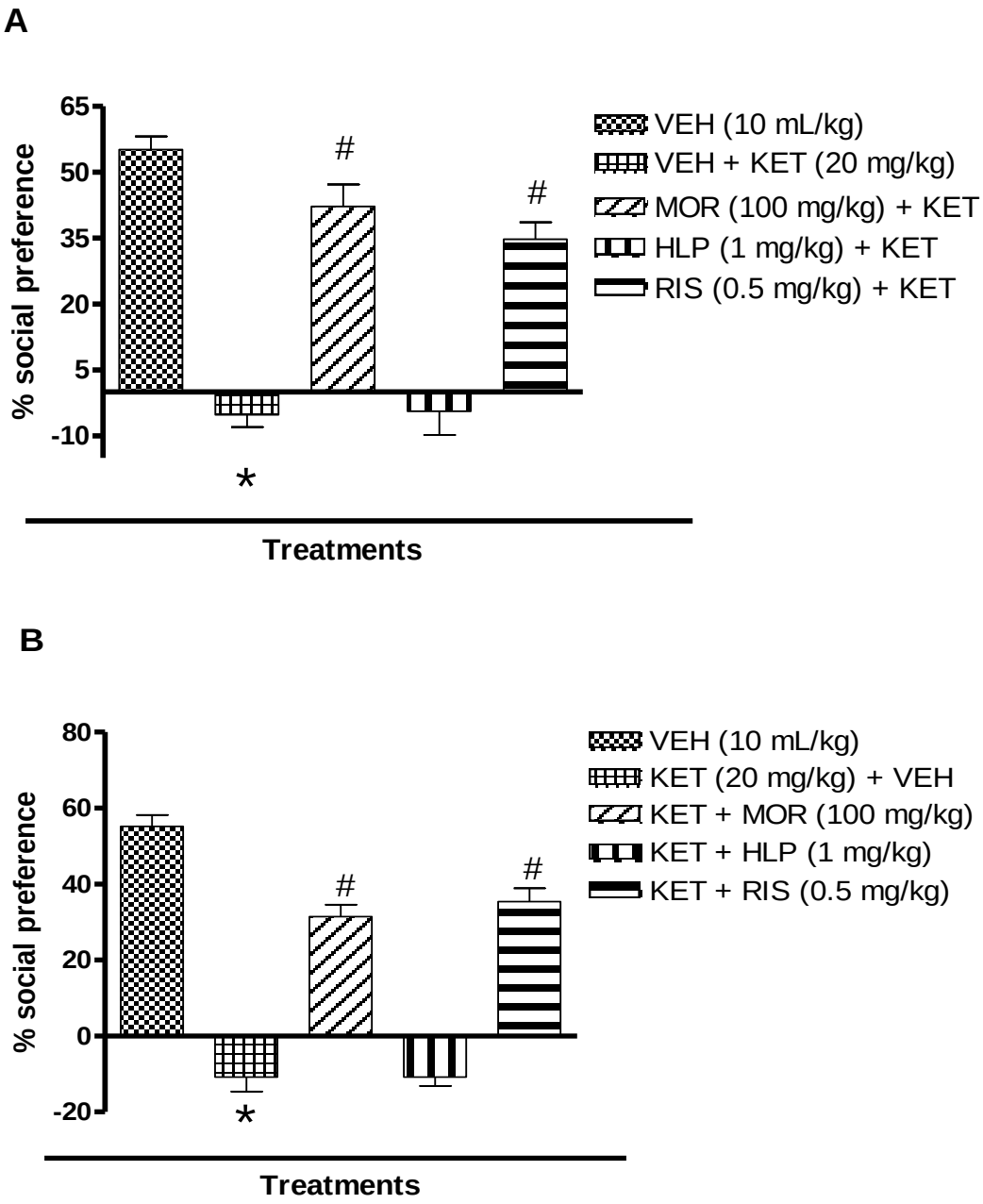


Figure 4.28: Effect of morin on ketamine-induced deficit in social interaction in the preventive (A) and reversal (B) treatment protocols in mice.

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.38 Effect of morin on ketamine-enhanced immobility in forced swim test in the preventive and reversal treatment protocols

Intraperitneal injection of KET (20 mg/kg) significantly ($p < 0.05$) increased the duration of immobility in the FST in the preventive and reversal treatment protocols in comparison with vehicle-treated group, which suggests behavioural despair indicative of negative symptom, as shown in Figure 29 (A and B). However, preventive (Figure 4.29A) and reversal (Figure 4.29B) study with MOR (100 mg/kg, i.p.) demonstrated a significant ($p < 0.05$) decrease in immobility time in both treatment plans when compared with KET-treated group. Treatment with the atypical antipsychotic standard drug, RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated KET-induced enhanced immobility time in the FST in both treatment protocols when compared with KET treatment alone. On the other hand, treatments with HLP (1 mg/kg, i.p.) in both protocols, failed to prevent or reverse the behavioural despair indicative of negative symptom by KET in FST model in mice relative to KET treatment alone (Figure 4.29A and B).

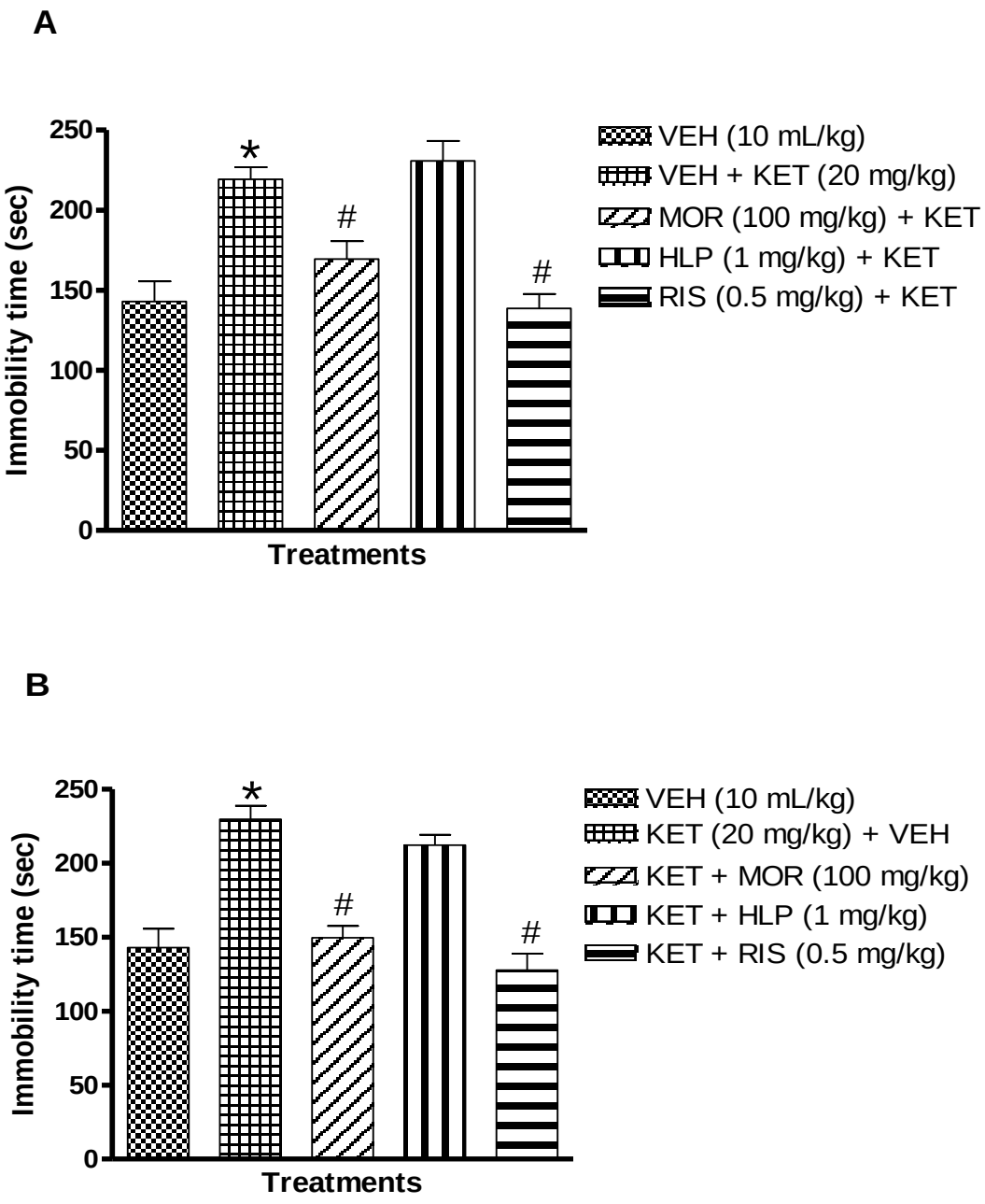


Figure 4.29: Effect of morin on ketamine-enhanced immobility in forced swim test in the preventive (A) and reversal (B) treatment protocols in mice.

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.39 Effect of morin on glutathione concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-induced changes of GSH concentration in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is illustrated in Figure 4.30 (A and B). In the preventive treatment, there was no significant ($p > 0.05$) decrease in GSH concentration in the hippocampus by KET (20 mg/kg, i.p.), however KET significantly ($p < 0.05$) decreased brain concentration of GSH in the striatum and prefrontal cortex when compared with vehicle group (Figure 4.30A). Preventive treatment with both MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) increased GSH concentrations in the striatum and prefrontal cortex, but not in the hippocampus, when compared with KET (20 mg/kg, i.p.) treatment respectively. Pretreatment with HLP (1 mg/kg, i.p.) failed to prevent the decrease in GSH levels in the striatum and prefrontal cortex by KET when compared with KET-treated mice. Pretreatment with HLP (1 mg/kg, i.p.) significantly decreased hippocampal brain GSH concentration in comparison with KET treatment alone in the preventive treatment protocol (Figure 4.30A).

Furthermore, intraperitoneal injection of KET (20 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in GSH concentrations in the striatum, prefrontal cortex and hippocampus in the reversal treatment relative to vehicle-treated mice (Figure 4.30B). However, the decrease was reversed by MOR (100 mg/kg, i.p.); as it significantly ($p < 0.05$) elevated the levels of GSH in the striatum, prefrontal cortex and hippocampus when compared with KET (20 mg/kg, i.p.) treatment. Similarly, treatment with RIS (0.5 mL/kg, i.p.) significantly ($p < 0.05$) increased GSH concentrations in all three regions of mice brain in comparison with KET-treated mice. Treatment with HLP (1 mg/kg, i.p.) failed to reverse the decrease in GSH concentration in the striatum, prefrontal cortex and hippocampus by KET (20 mg/kg, i.p.) when compared with KET-treated group (Figure 4.30B).

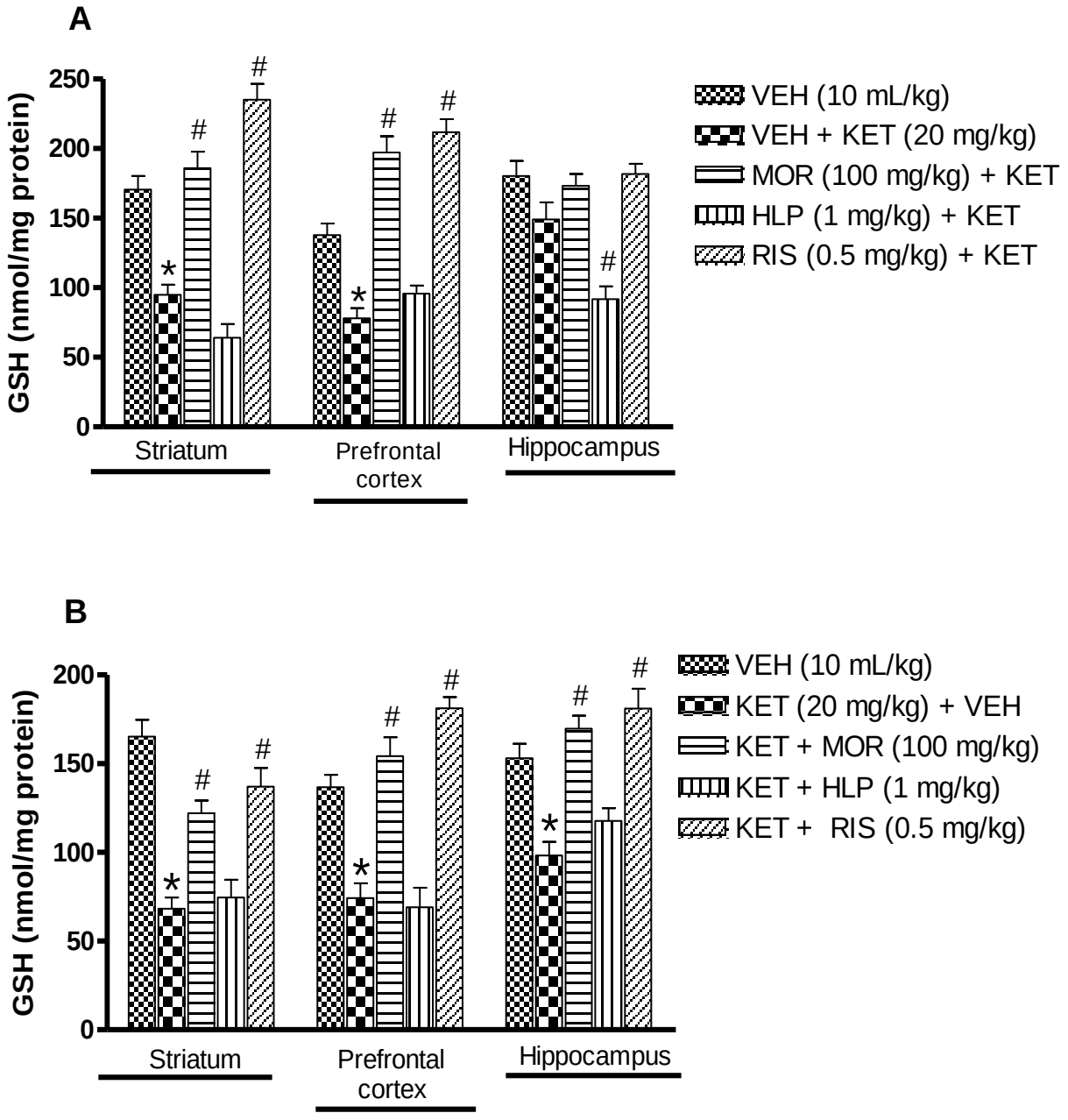


Figure 4.30: Effect of morin on ketamine-induced alteration on glutathione levels in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.40 Effect of morin on superoxide dismutase activity in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-induced changes of SOD activity in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is shown in Figure 4.31 (A and B). Administration of KET (20 mg/kg, i.p.) in the preventive protocol significantly ($p < 0.05$) decreased the antioxidant enzyme activity of SOD in the prefrontal cortex and hippocampus, without significant change in the striatum, when compared with vehicle group. However, pretreatment with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) attenuated the effect of KET on SOD activity in the prefrontal cortex and hippocampus (but not in the striatum) in comparison with KET-treated mice in the preventive protocol, which suggests increase in antioxidant activity. On the other hand, preventive treatment with HLP (1 mg/kg, i.p.) did not demonstrate any significant protection against KET-induced alteration in SOD activity in the prefrontal cortex and hippocampus relative to KET treatment. Pretreatment with HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) decreased antioxidant enzyme activity of SOD in the striatum when compared with KET-treated mice (Figure 4.31A).

In the reversal treatment, chronic treatment with KET (20 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in all brain regions (striatum, prefrontal cortex and hippocampus) in comparison with vehicle treatment, suggesting decrease in brain regional antioxidant activity. Treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) reversed KET-induced decrease in SOD activity in the striatum, prefrontal cortex and hippocampus when compared with KET-treated group. RIS (0.5 mg/kg, i.p.) reversed the alteration in SOD activity in all three regions by KET in a significant manner ($p < 0.05$) in comparison with KET-treatment alone. HLP (1 mg/kg, i.p.) did not reverse the effect of KET on SOD activity in the striatum, prefrontal cortex and hippocampus in comparison with KET-treatment (Figure 4.31B).

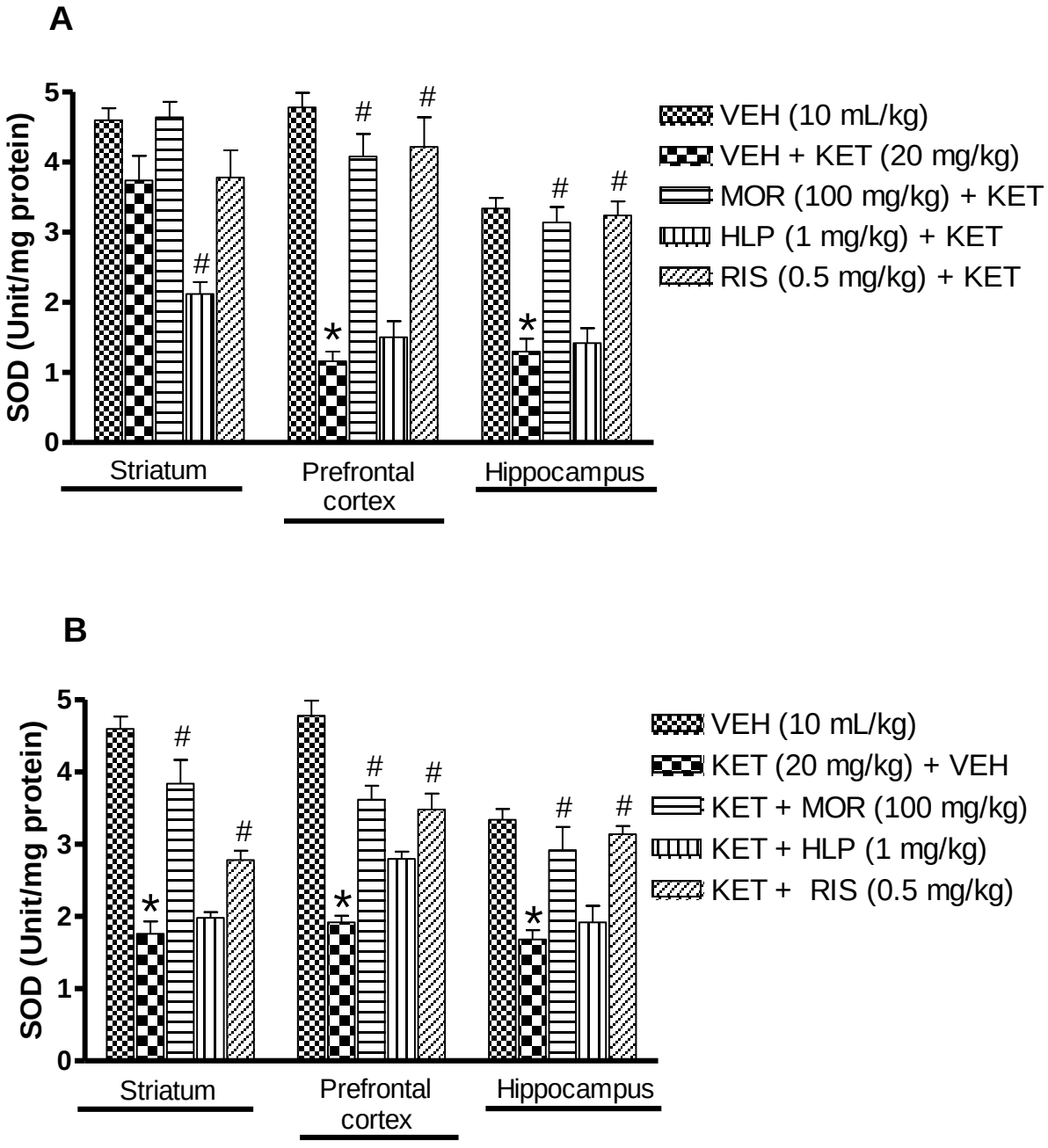


Figure 4.31: Effect of morin on ketamine-induced alteration on superoxide dismutase activity in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.41 Effect of morin on catalase activity in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-induced alterations of CAT activity in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is illustrated in Figure 4.32 (A and B). In the preventive treatment, there was no significant ($p > 0.05$) decrease in CAT activity in the hippocampus by KET (20 mg/kg, i.p.) relative to vehicle-treated mice, but KET significantly ($p < 0.05$) decreased brain activity of CAT in the striatum and prefrontal cortex when compared with vehicle group (Figure 4.32A). However, pretreatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) increased CAT activity in the striatum when compared with KET (20 mg/kg, i.p.) treatment, with no significant difference ($p > 0.05$) observed in the prefrontal cortex relative to KET-control. RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented KET-induced decreases in the prefrontal cortical and striatal CAT levels. Pretreatment with HLP (1 mg/kg, i.p.) did not prevent the decrease in CAT activity in the striatum and prefrontal cortex by KET when compared with KET-treated mice. HLP (1 mg/kg, i.p.) significantly attenuated hippocampal CAT activity of mice brain in comparison with KET-treated mice in the preventive treatment protocol (Figure 4.32A).

In the reversal treatment, chronic treatment with KET (20 mg/kg, i.p.) caused a decrease in CAT activity in the striatum, prefrontal cortex and hippocampus manner in comparison with vehicle treatment in a significant ($p < 0.05$) manner, which suggests region-specific decrease in CAT activity (Figure 4.32B). Treatment with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reversed the decrease in CAT activity by KET in the striatum, prefrontal cortex and hippocampus when compared with KET-treated group. HLP (1 mg/kg, i.p.) did not reverse the effect of KET on CAT activity in the striatum, prefrontal cortex and hippocampus relative to KET-treatment (Figure 4.32B).

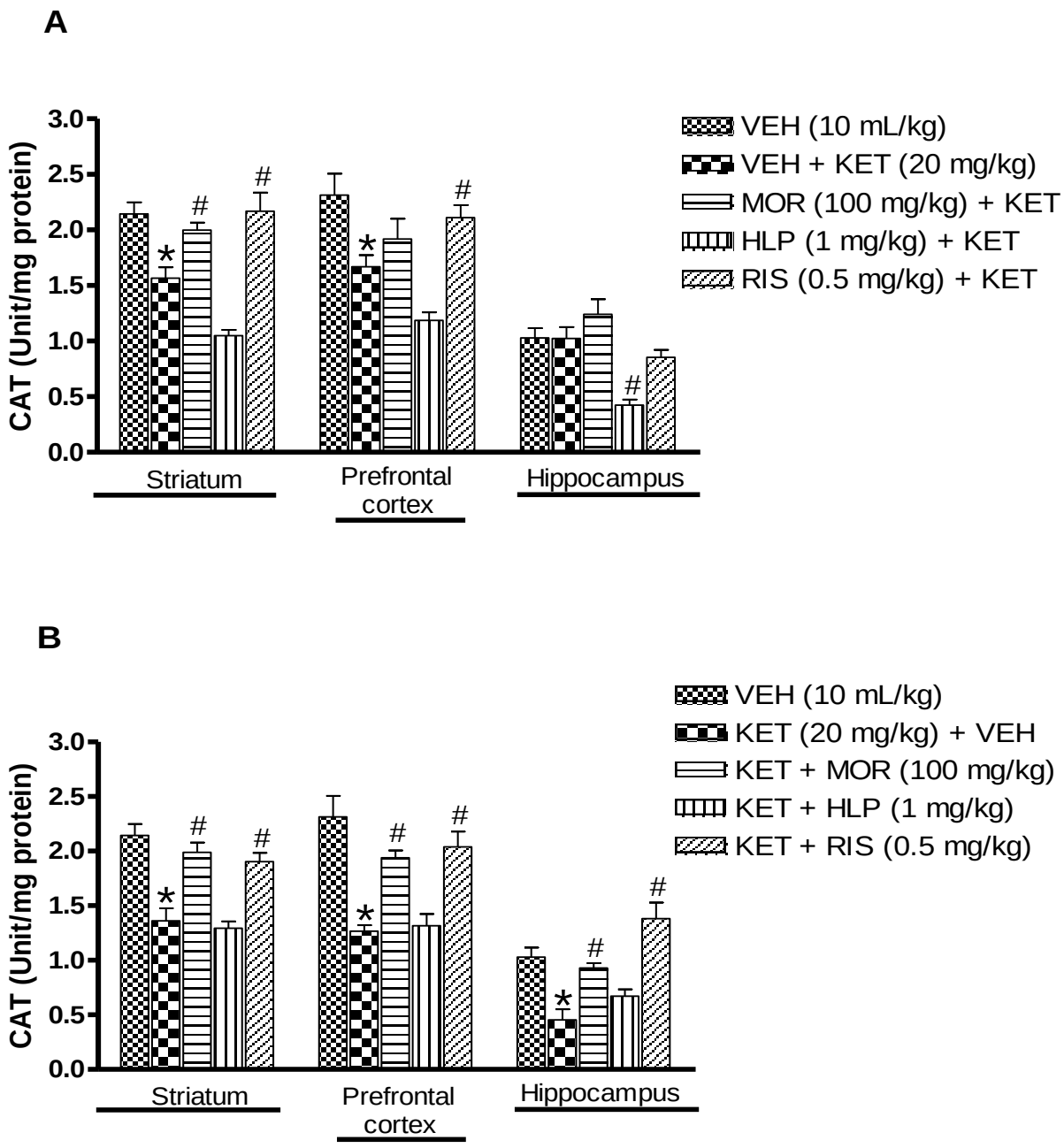


Figure 4.32: Effect of morin on ketamine-induced alteration on catalase activity in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.42 Effect of morin on malondialdehyde concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

Intraperitoneal injection of KET (20 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in regional brain (striatum, prefrontal cortex and hippocampus) MDA concentrations of mice brains when compared with vehicle-treated animals, which suggests regional brain lipid peroxidation, as shown in Figure 4.33 (A and B). Treatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) prevented (Figure 4.33A) and reversed (Figure 4.33B) KET-induced increase in MDA concentrations in the striatum, prefrontal cortex and hippocampus when compared with KET-treated animals, suggesting decrease in lipid peroxidation. Similarly, RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced brain concentrations of MDA in the striatum, prefrontal cortex and hippocampus relative to KET treatment alone. However, HLP (1 mg/kg, i.p.) failed to prevent and reverse the increase in MDA concentration by KET in the striatum, prefrontal cortex and hippocampus (Figure 4.33A and B).

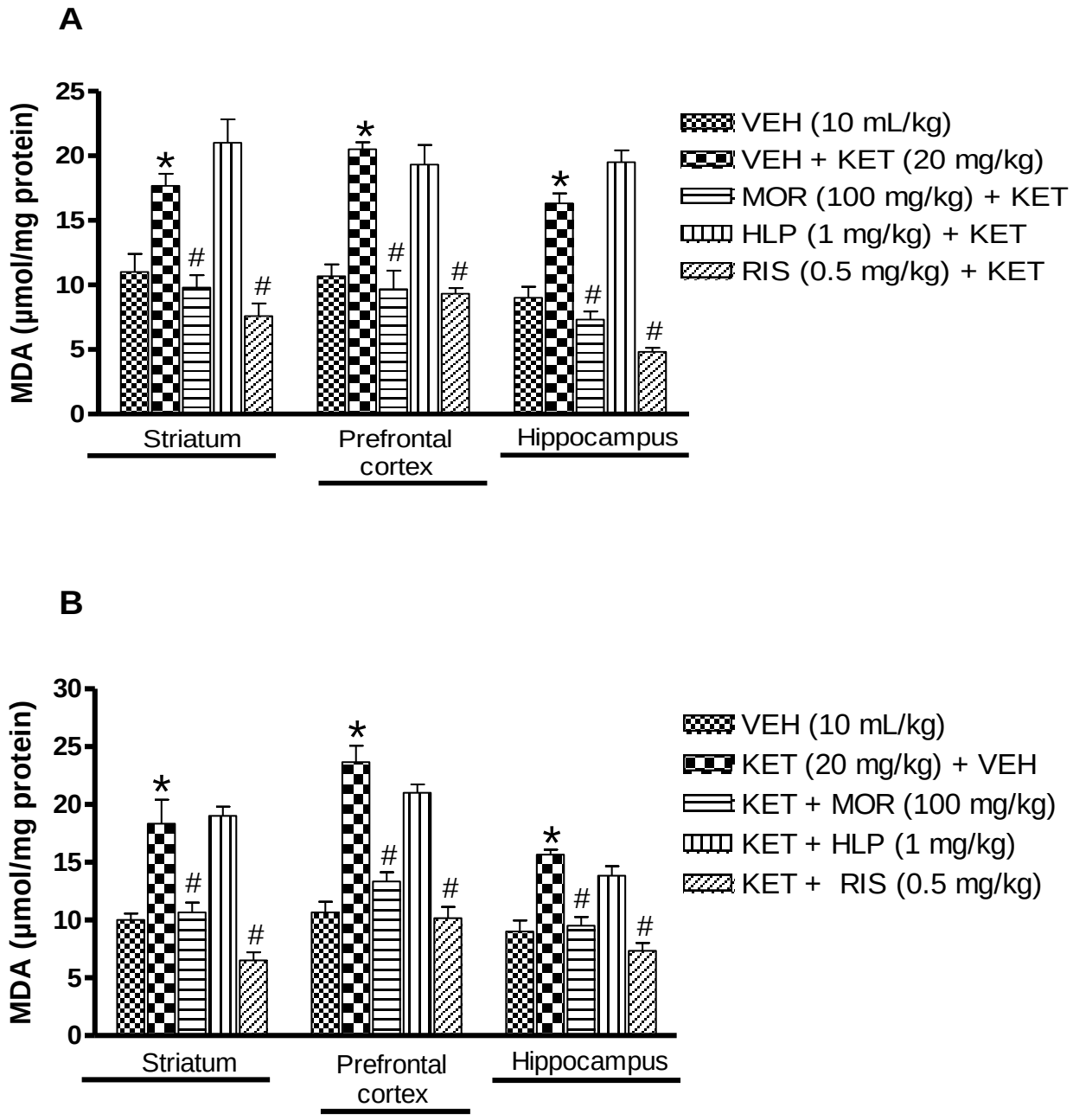


Figure 4.33: Effect of morin on ketamine-induced alteration on malondialdehyde concentrations in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.43 Effect of morin on nitrite concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-induced alteration of nitrite concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is shown in Figure 4.34 (A and B). In both protocols, administration of KET (20 mg/kg, i.p.) caused a significant ($p < 0.05$) increase in brain level of nitrite concentration in the striatum when compared with vehicle-treated animals, which suggests nitrergic stress in the striatum; however, administration of KET did not cause any significant changes in the nitrite levels of the prefrontal cortex and hippocampus relative to vehicle-treated animals. As presented in Figure 4.34 (A and B), MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) reduced brain concentration of nitrite in the striatum relative to KET-treated mice in the preventive and reversal protocols, although did not produce any significant change in the prefrontal cortex and hippocampus when compared with KET-treated animals. Also, preventive (Figure 4.34A) and reversal (Figure 4.34B) treatments with RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated brain levels of nitrite in the striatum in comparison with KET-treated mice in both treatments plans. Meanwhile, pretreatment with RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced nitrite concentrations in the prefrontal cortex and hippocampus when compared with KET-treated animals in the preventive treatment. Nevertheless, pretreatment with HLP (1 mg/kg, i.p.) failed to prevent the increase in brain level of nitrite in the striatum by KET relative to KET-treated animals (Figure 4.34A). In the reversal, treatment with HLP (1 mg/kg, i.p.) decreased nitrite level in the striatum when compared with KET treatment alone (Figure 4.34B).

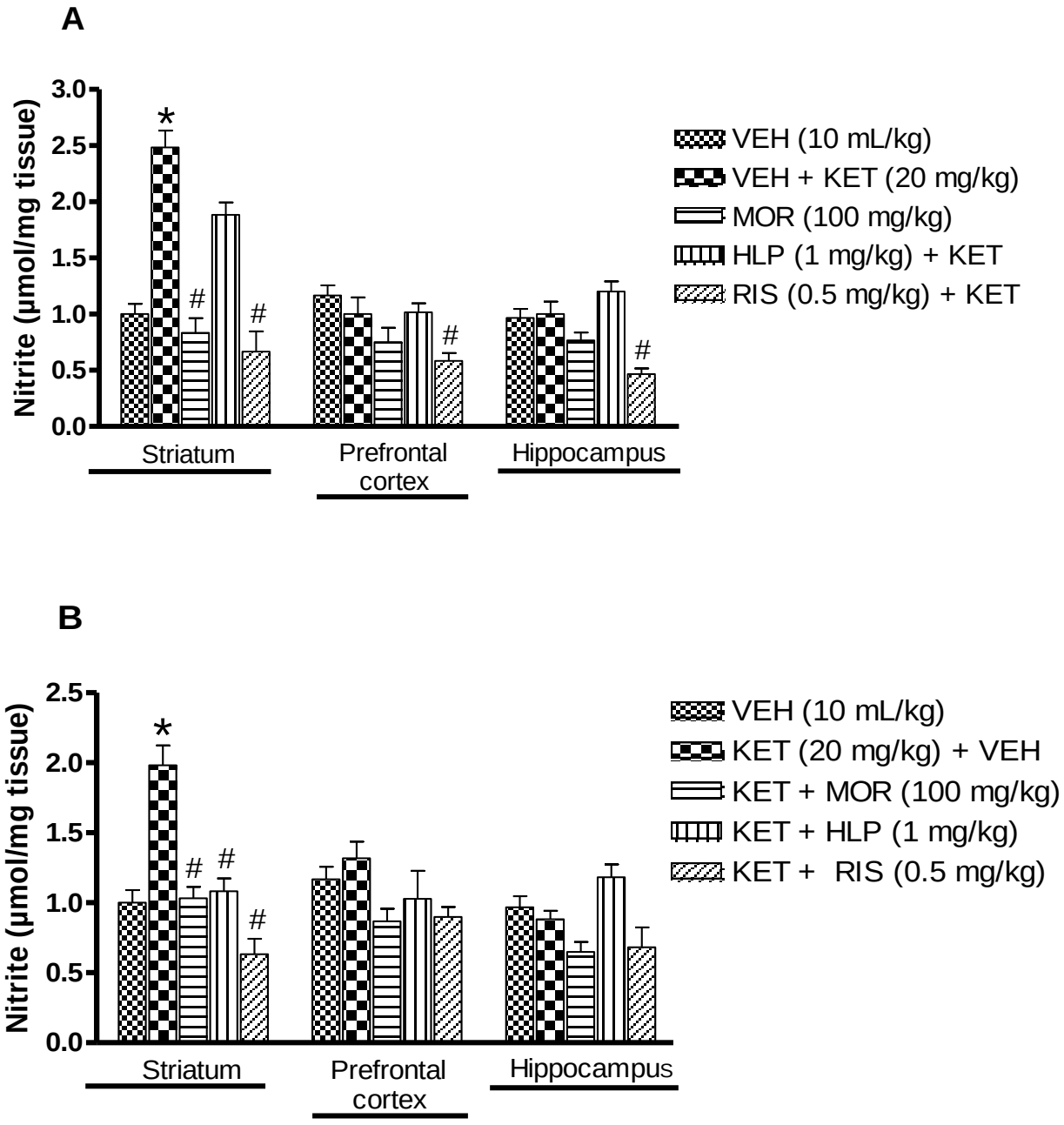


Figure 4.34: Effect of morin on ketamine-induced alteration on nitrite concentrations in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.44 Effect of morin on acetylcholinesterase activity in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-induced alteration of AChE enzyme activity in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is shown in Figure 4.35 (A and B). Intraperitoneal injection of KET (20 mg/kg) produced a significant ($p < 0.05$) increase in AChE activity in the prefrontal cortex and hippocampus, but not in the striatum, when compared with vehicle-treated group in the preventive (Figure 4.35A) and reversal (Figure 4.35B) treatment protocols, which suggests memory impairment. Preventive (Figure 4.35A) and reversal (Figure 4.35B) treatments with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated the increased activity of AChE enzyme by KET in the prefrontal cortex and hippocampus when compared with KET-treated group. Similarly, treatment with RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced KET-induced alterations in AChE activity in the prefrontal cortex and hippocampus when compared to KET treated group. HLP (1 mg/kg, i.p.) failed to prevent and reverse the increase in AChE enzyme activity relative to KET treatment alone (Figure 4.35A and B).

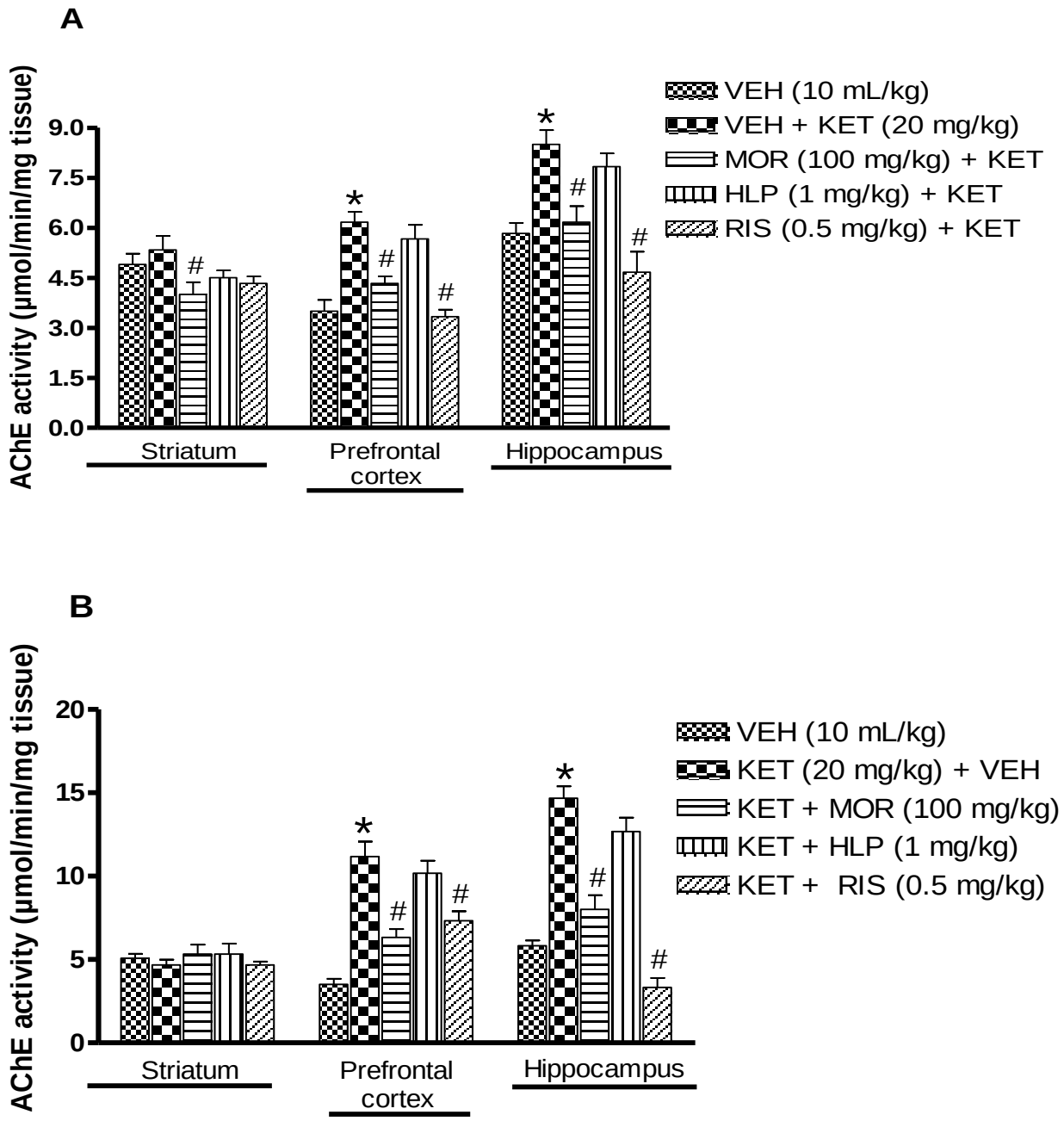


Figure 4.35: Effect of morin on ketamine-induced alteration on acetylcholinesterase activity in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.45 Effect of morin on dopamine concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effects of MOR on KET-mediated alterations of dopamine concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is illustrated in Figure 4.36 (A and B). In the preventive treatment, administration of KET (20 mg/kg, i.p.) significantly ($p < 0.05$) increased dopamine concentrations in the striatum and prefrontal cortex, but not in the hippocampus when compared with vehicle-treated mice. Pretreatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) prevented the increase in dopamine concentrations in the striatum and prefrontal cortex by KET. MOR significantly ($p < 0.05$) reduced hippocampal dopamine concentration of KET-treated mice (Figure 4.36A). HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) decreased KET-mediated increase in dopamine concentrations in the striatal, cortical (prefrontal) and hippocampal regions of the mice brain (Figure 4.36A).

In the reversal treatment protocol, chronic intraperitoneal injection of KET (20 mg/kg, i.p.) caused a significant ($p < 0.05$) increase in striatal and hippocampal dopamine concentrations relative to vehicle-treated mice, suggesting sub-cortical hyperdopaminergic activity (Figure 4.36B). Furthermore, KET significantly ($p < 0.05$) decreased dopamine concentration in the prefrontal cortex when compared with vehicle-treated mice, suggesting cortical hypodopaminergic activity (Figure 4.36B). MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) reverse KET-induced hyperdopaminergic activities in the striatum and hippocampus when compared with KET treatment alone. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) restored prefrontal cortical dopamine transmission relative to KET-treated mice. RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated striatal and hippocampal hyperdopaminergic activities induced by KET (20 mg/kg, i.p.), and increased dopamine concentration in the prefrontal cortex in mice. HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) reduced dopamine concentrations in the striatum and hippocampus, and did not increase prefrontal cortical dopamine concentration when compared with KET treatment alone (Figure 4.36B).

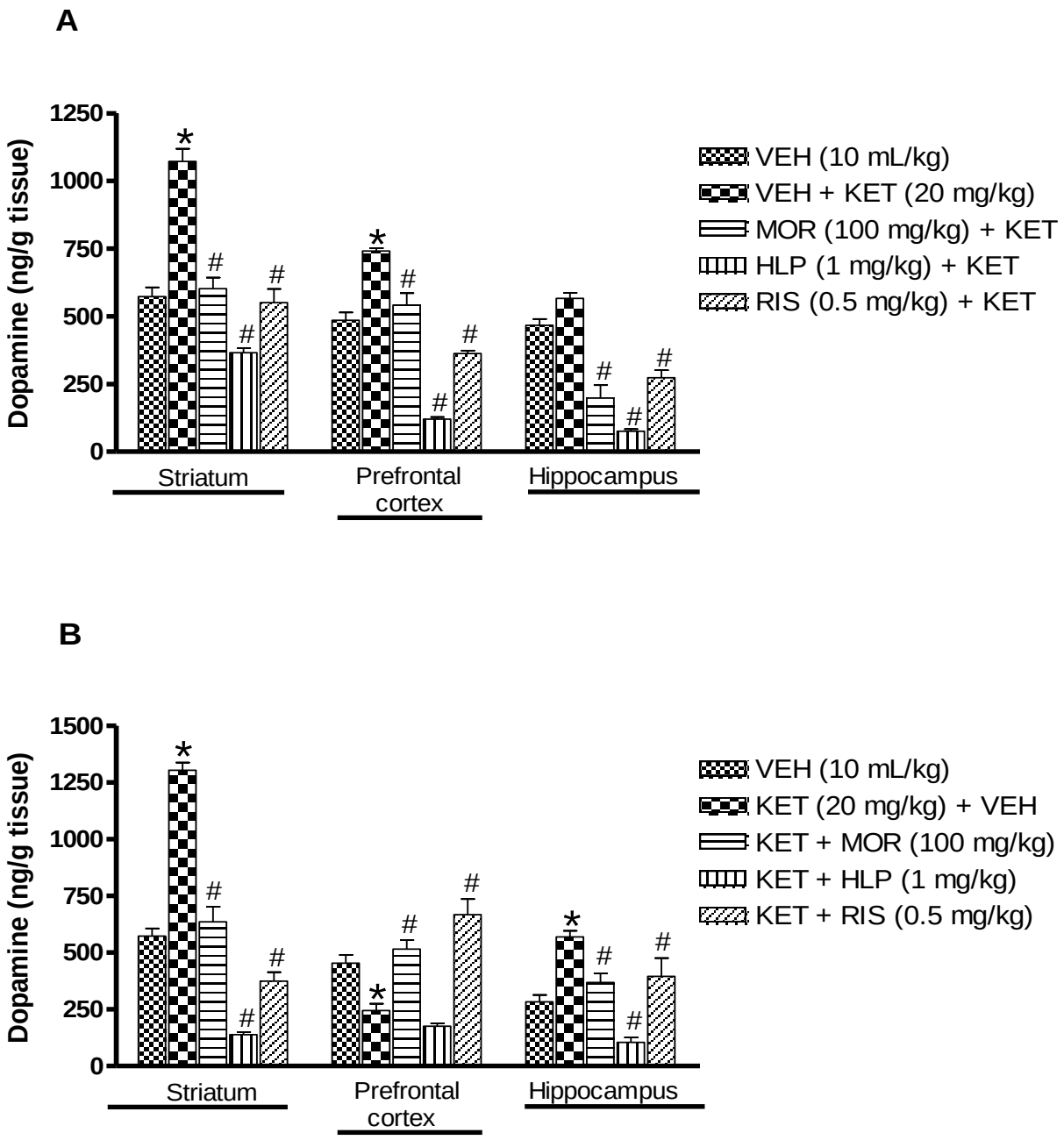


Figure 4.36: Effect of morin on ketamine-induced alteration on dopamine concentration in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.46 Effect of morin on 5-hydroxytryptaminergic concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effect of MOR on KET-induced changes on 5-HT concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatment protocols is shown in Figure 4.37 (A and B). Intraperitoneal injection of KET (20 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in 5-HT concentrations in the striatum and prefrontal cortex, but not in the hippocampus when compared with vehicle-treated group in the preventive treatment (Figure 4.37A). MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated KET-induced increase in 5-HT transmissions in the striatum and prefrontal cortex similar to RIS (0.5 mg/kg, i.p.) when compared with KET-treated mice (Figure 4.37A). HLP (1 mg/kg, i.p.) failed to prevent KET-mediated increase in 5-HT concentrations in the striatum and prefrontal cortex in comparison with KET-treated mice (Figure 4.37A).

In the reversal treatment protocol, KET (20 mg/kg, i.p.) significantly ($p < 0.05$) increased 5-HT concentrations in the striatum, prefrontal cortex and hippocampus in comparison with vehicle-treated mice (Figure 4.37B). MOR (100 mg/kg, i.p.) reversed the effect of KET on 5-HT concentrations in the striatum and prefrontal cortex, but not in the hippocampus (Figure 4.37B). RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated KET-evoked increase in 5-HT concentrations in the striatum, prefrontal cortex and hippocampus when compared with KET-treated mice. HLP (1 mg/kg, i.p.) did not inhibit KET-mediated increase in 5-HT transmissions in the striatum, prefrontal cortex and hippocampus in comparison with KET treatment alone in the reversal treatment protocol (Figure 4.37B).

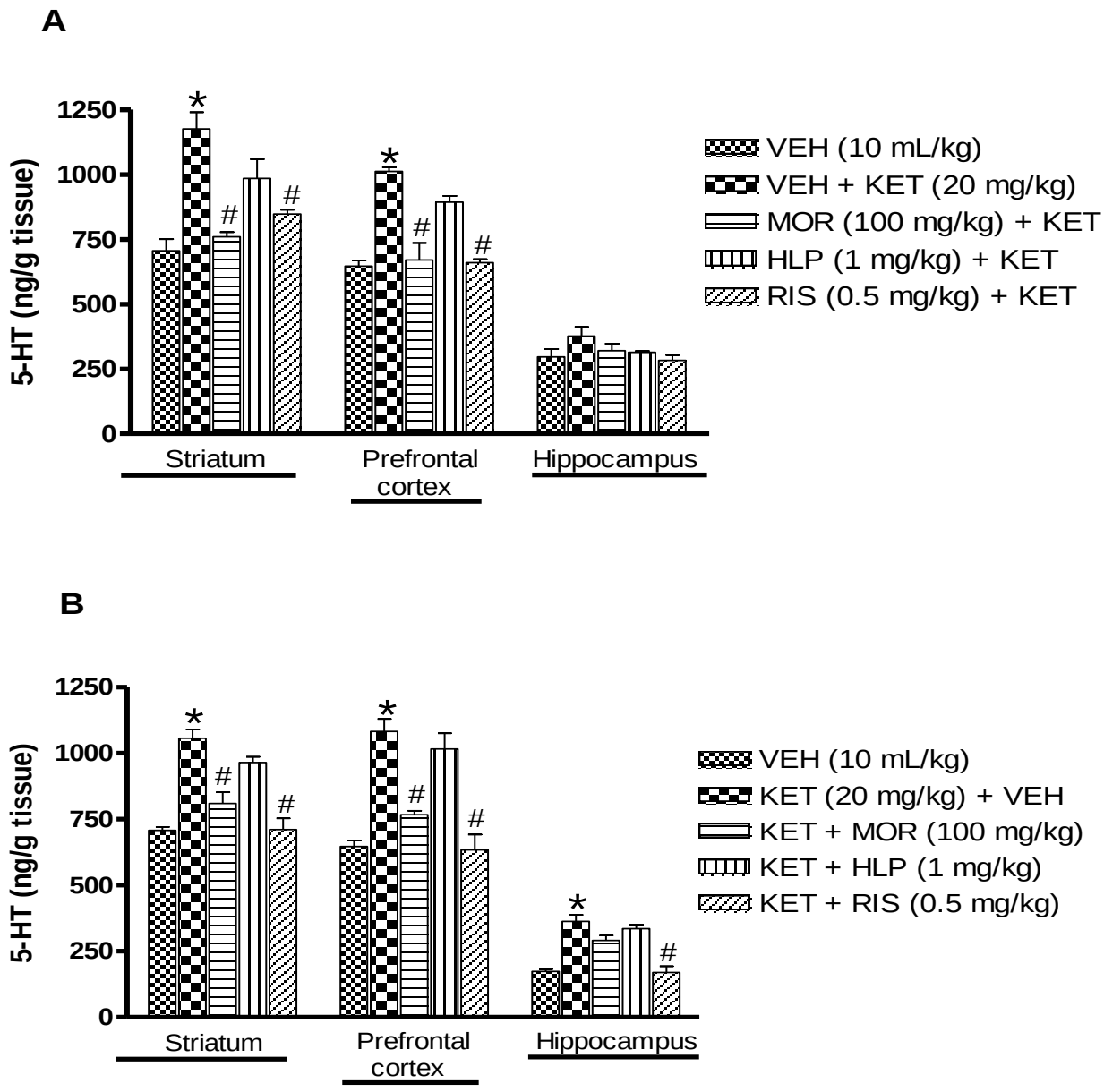


Figure 4.37: Effect of morin on ketamine-induced alteration on 5-hydroxytryptaminergic concentration in the preventive (A) and reversal (B) treatment protocols in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.47 Effect of morin on glutamate concentrations in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The effect of MOR on KET-induced alteration on glutamate concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatments is shown in Figure 4.38 (A and B). Treatment with KET (20 mg/kg, i.p.) significantly ($p < 0.05$) decreased glutamate concentrations in the striatum and hippocampus, whereas significantly ($p < 0.05$) increased glutamate concentration in the prefrontal cortex when compared with vehicle-treated group in the preventive (Figure 4.38A) and reversal (Figure 4.38B) treatment protocols. MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented the decrease in glutamate concentration by KET in the striatum and hippocampus in the preventive (Figure 4.38A) and reversal (Figure 4.38B) treatment protocols respectively. Also, MOR (0.5 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly decreased glutamate concentration in the prefrontal cortex relative to KET-treated mice in both protocols (Figure 4.38A and B). HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) increased striatal glutamate concentration in both treatment protocols compared with KET treatment alone, but did not reverse the effect of KET on the prefrontal cortex and hippocampus, as it failed to attenuate KET-mediated glutamatergic increase in the prefrontal cortex, or increase KET-induced decrease in hippocampal glutamate levels when compared with KET-treated mice in both protocols (Figure 4.38A and B).

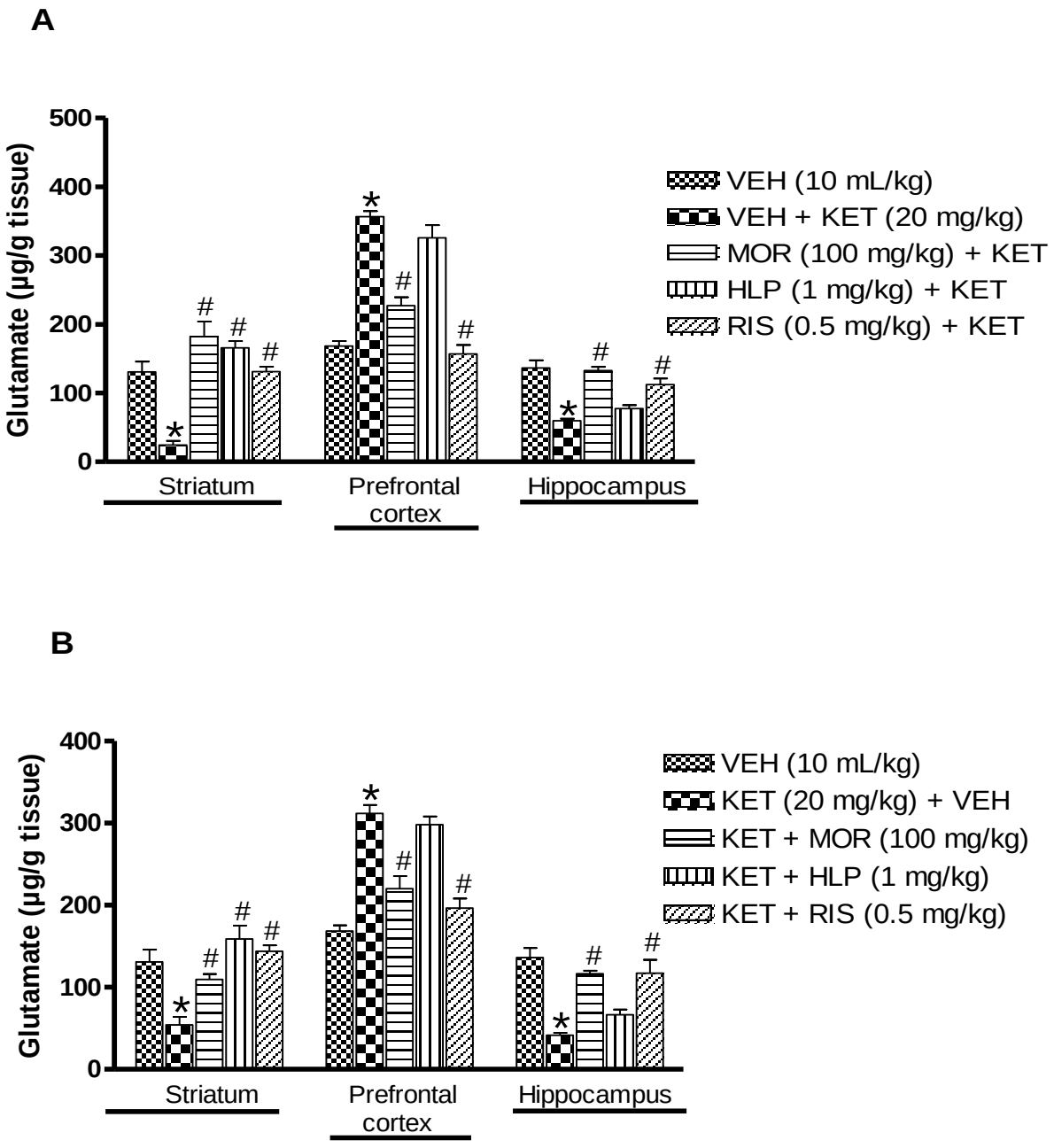


Figure 4.38: Effect of morin on ketamine-induced alteration on glutamate concentration in the preventive (A) and reversal (B) treatment protocols in mice

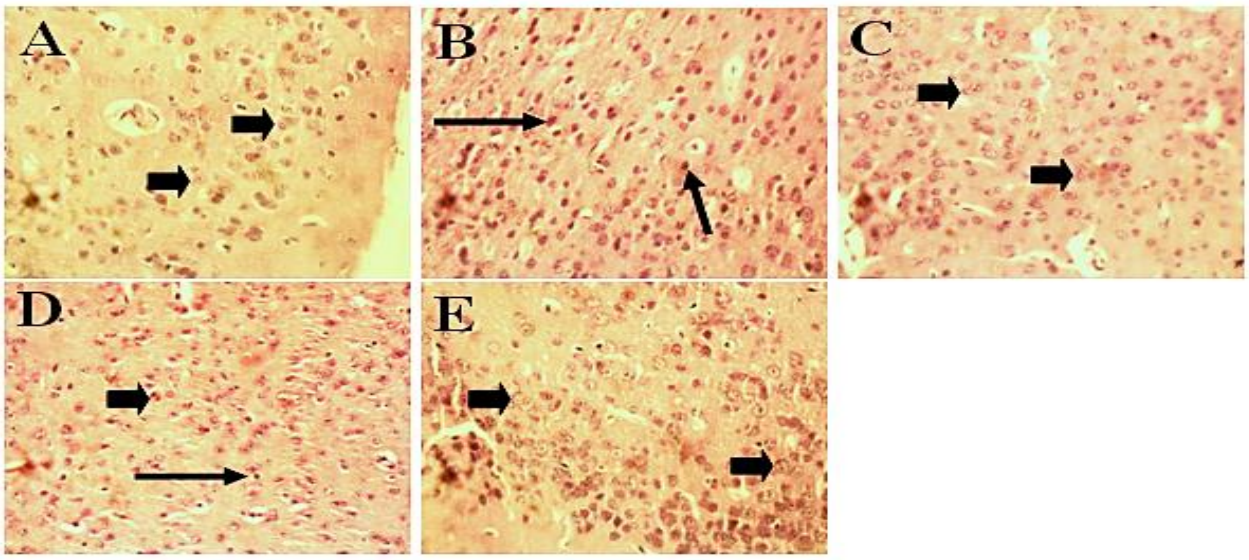
Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.48 Neuroprotective and neurorestorative effects of morin in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The photomicrographs of the effect of morin on KET-induced histological and histomorphological (density of viable cells) changes in mice brains in the preventive and reversal treatments are shown in Plates 4.7 (A and B), 4.8 (A and B), 4.9 (A and B) and Figure 4.39 (A and B). Hematoxylin and Eosin staining revealed that intraperitoneal injection of KET (20 mg/kg, i.p.) produced a significant cytoarchitectural changes in the striatum (Plate 4.7A and B) and prefrontal cortex (Plate 4.8A and B), but not in the CA1 of the hippocampal region (Plate 4.9A and B); as evidenced increased population of highly condensed (pyknotic) and angulated neuronal cells (Figure 4.39A and B) relative to vehicle-treated mice in the preventive and reversal treatments. KET (20 mg/kg, i.p.) significantly ($p < 0.05$) decreased the population of viable neuronal cells of the striatum and prefrontal cortex in comparison with vehicle control group in both treatment protocols (Figure 4.39A and B). Treatments with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly reduced these histomorphological alterations induced by KET in the striatum (Plate 4.7A and B) and prefrontal cortex (Plate 4.8A and B) in both treatments plans. MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) decreased the loss of viable neuronal cells of the striatum and cortical regions when compared with KET treatment alone (Figure 4.39A and B). HLP (1 mg/kg, i.p.) did not prevent or reverse KET-induced neuropathological changes and loss of viable neuronal cells in the striatum (Figure 4.39A and B) and prefrontal cortex (Figure 4.39A and B) in the preventive and reversal protocols compared with KET alone.

A



B

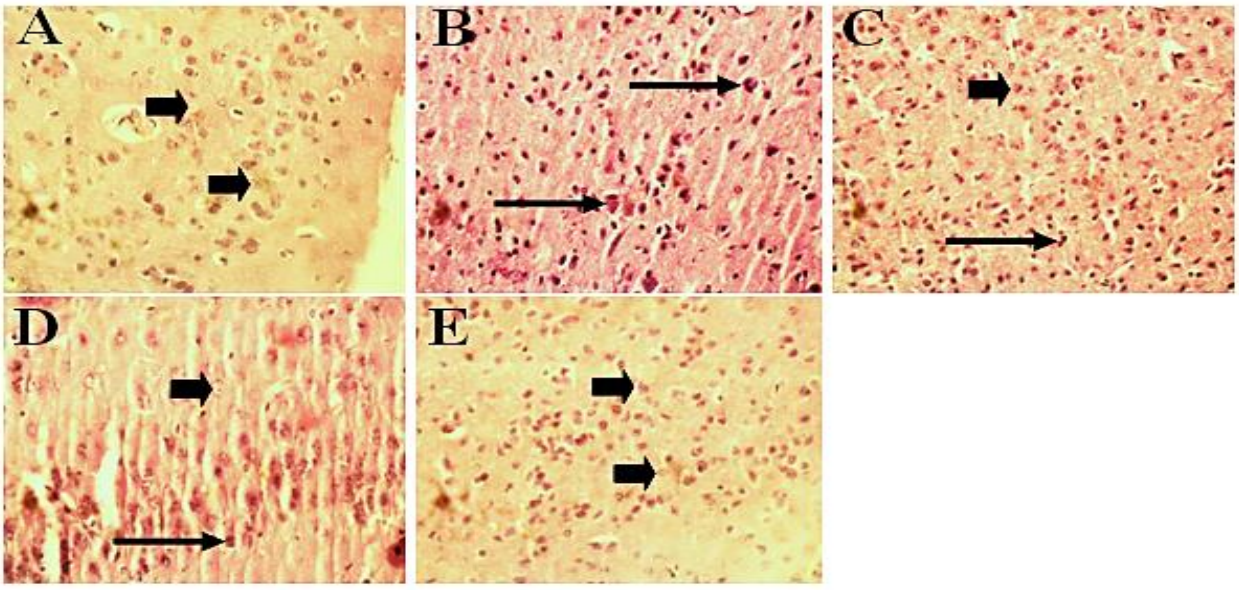
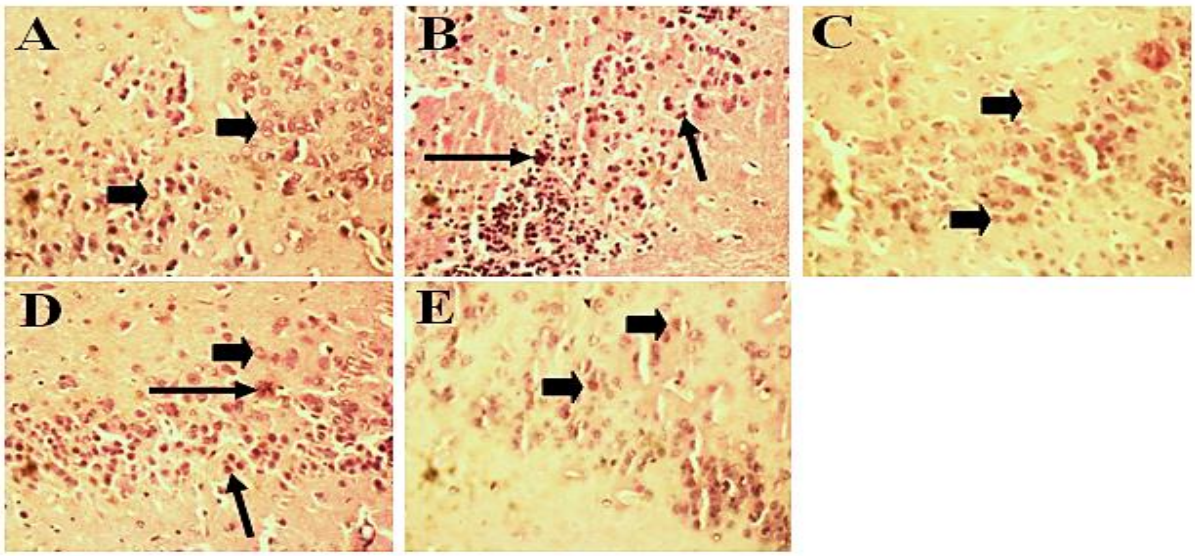


Plate 4.7: Representative stained sections of the effect of morin on the *striatum* of mice brains treated with ketamine in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg + Ketamine, D = Haloperidol 1 mg/kg + Ketamine, and E = Risperidone 0.5 mg/kg + Ketamine. Slide A shows normal multiple neuronal cells with hyperchromatic nuclei. Slide B revealed neurons with highly condensed, pyknotic and angulated cells. Slide C shows a few ghost neuronal cells and normal cells. Slide D reveals few normal neurons with angulated cells. Slide E showed a few ghost neuronal cells and a few normal cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

A



B

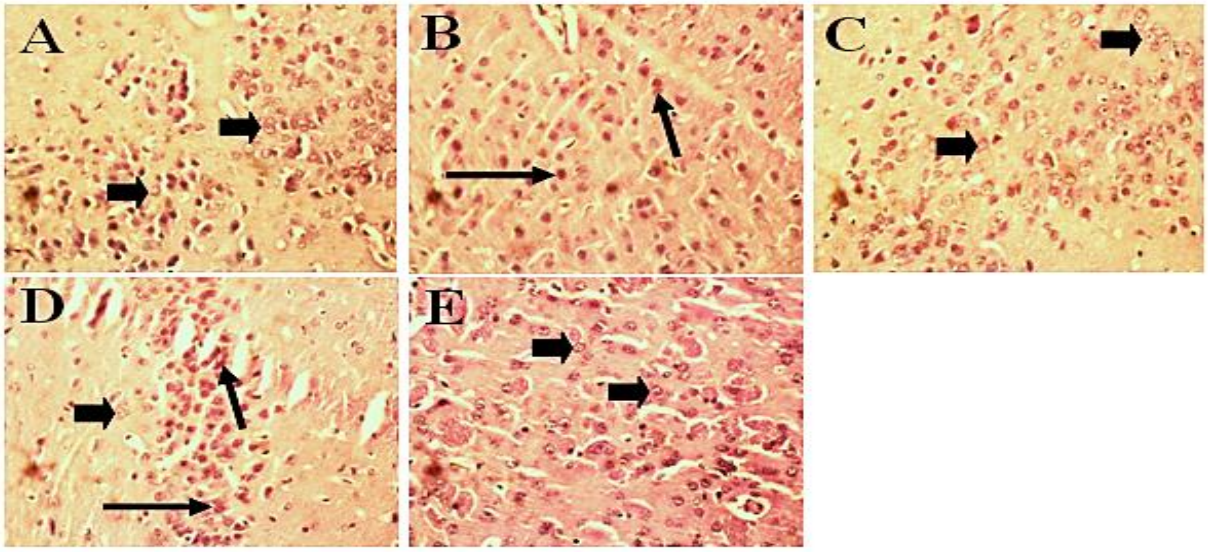
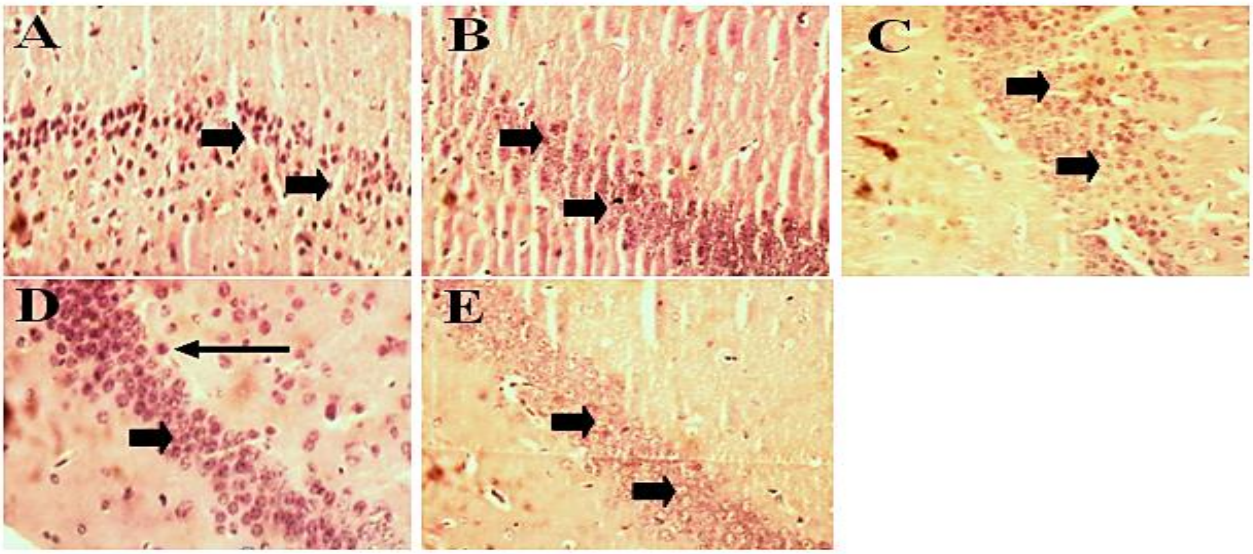


Plate 4.8: Representative photomicrographs of the effect of morin on the *prefrontal cortex* of mice brains treated with ketamine in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg + Ketamine, D = Haloperidol 1 mg/kg + Ketamine, and E = Risperidone 0.5 mg/kg + Ketamine. Slide A revealed normal multiple neuronal cells with open chromatin pattern. Slide B showed cells with hypochromatic nuclei and increased population of pyknotic cells. Slide C shows normal neuronal cells and a few ghost neuronal cells. Slide D shows neurons with numerous dark chromatin nuclei with pyknotic cells. Slide E revealed normal neuronal cells and a few pyknotic cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

A



B

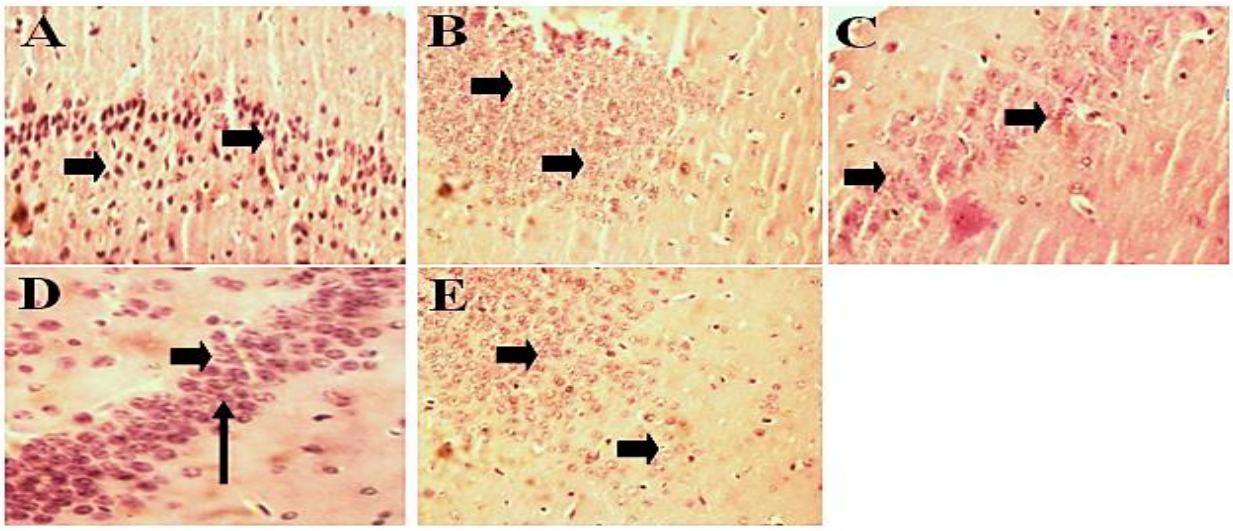


Plate 4.9: Representative photomicrographs of the effect of morin on the *hippocampus* (CA1) of mice brains treated with ketamine in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg + Ketamine, D = Haloperidol 1 mg/kg + Ketamine, and E = Risperidone 0.5 mg/kg + Ketamine. Slide A revealed normal multiple neuronal cells with open chromatin pattern. Slide B showed cells with hyperchromatic nuclei and decreased population of pyknotic cells. Slide C shows normal neuronal cells and a few pyknotic neurons. Slide D shows neurons with dark chromatin nuclei with pyknotic cells. Slide E showed normal neuronal cells with hyperchromatic nuclei cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

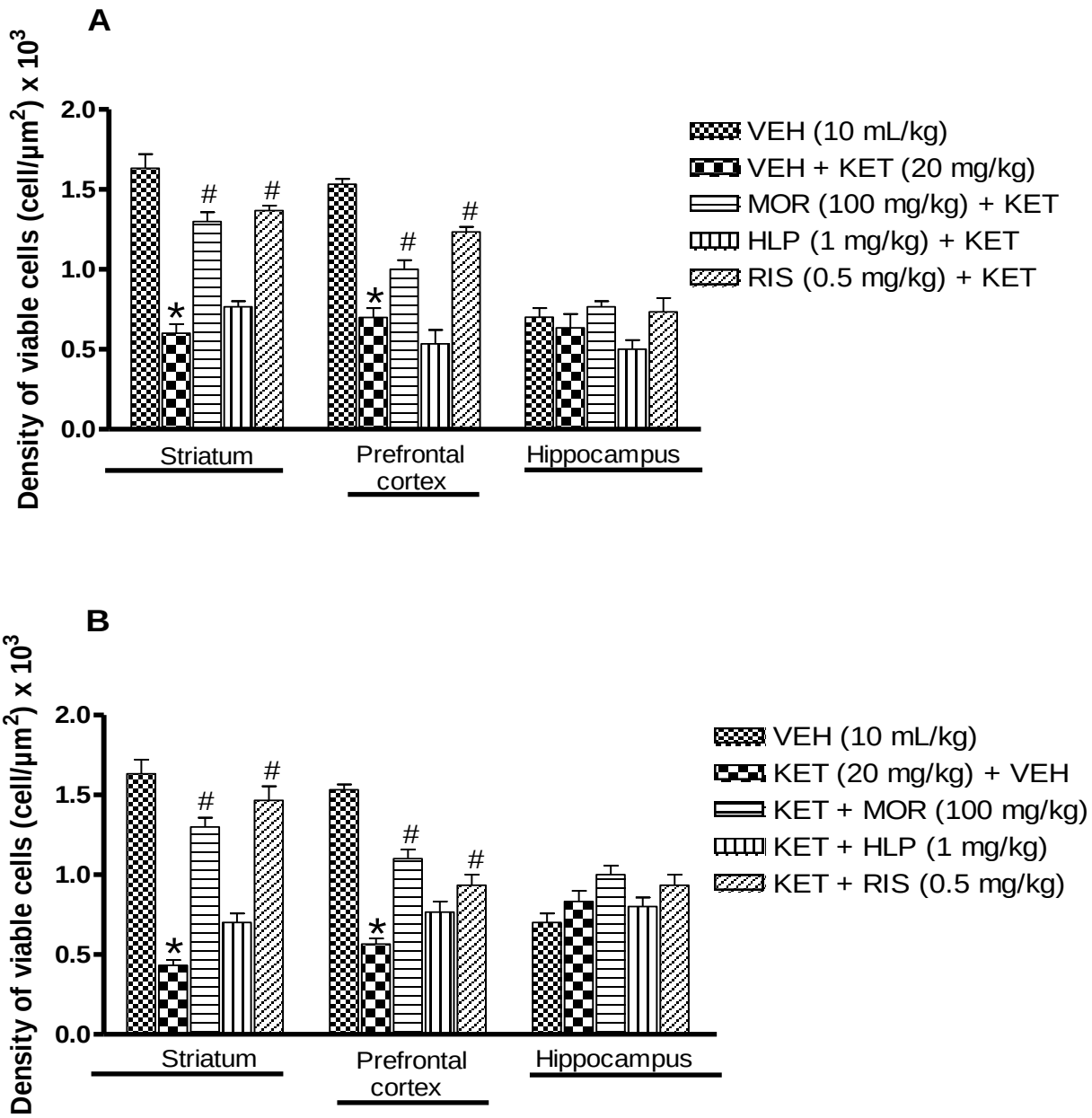


Figure 4.39: Effect of morin on the density of viable neuronal cells of the striatum, prefrontal cortex and hippocampus of mice brains in the preventive (A) and reversal (B) treatments with ketamine

Bars represent the mean ± S.E.M of 3 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.49 Effect of morin on GAD_{67} expressions in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The photomicrographs of the effect of MOR on KET-induced immunohistochemical changes and expressions of GAD_{67} (glutamic acid decarboxylase-67) immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatments are illustrated in Plates 4.10 (A and B), 4.11 (A and B), 4.12 (A and B) and Figure 4.40 (A and B). Chronic injections of KET (20 mg/kg, i.p.) produced significant ($p < 0.05$) immunohistochemical alterations of GAD_{67} in the striatum (Plate 4.10A and B), prefrontal cortex (Plate 4.11A and B) and hippocampus (Plate 4.12A and B), there was a relative decreases in the expressions of GAD_{67} iummunopositive cells (percentage optical density, % O.D.) in the preventive (Figure 4.40A) and reversal (Figure 4.40B) treatment protocols when compared with vehicle-treated group, suggesting decreased GABAergic neurotransmissions. Pretreatment with MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) prevented immunohistochemical changes and expressions of GAD_{67} positive cells induced by KET in the striatum (Plate 4.10A and Figure 4.40A), prefrontal cortex (Plate 4.11A and Figure 4.40A) and hippocampus (Plate 4.12A and Figure 4.40A) in the preventive treatment relative to KET-treated mice, which indicates increased GABAergic neurotransmissions. RIS (0.5 mg/kg, i.p.) did not prevent decreased GAD_{67} expression by KET in the striatum. It significantly ($p < 0.05$) increased GAD_{67} in the prefrontal cortex and hippocampus relative to KET-treated mice. HLP (1 mg/kg, i.p.) failed to prevent KET-induced decreased expressions of GAD_{67} immunopositive cells in the striatum, prefrontal cortex and hippocampus when compared to KET-treated mice brains (Figure 4.40A).

In the reversal treatment, MOR (100 mg/kg, i.p.) reversed immunohistochemical alterations and significantly ($p < 0.05$) increased the expressions of GAD_{67} positive cells in the striatum (Plate 4.10B and Figure 4.40B), prefrontal cortex (Plate 4.11B and Figure 4.40B) and CA1 region of the hippocampus (Plate 4.12B and Figure 4.40B) in comparison with KET-treated mice. Also, reversal treatment with the reference antipsychotic drug, RIS (0.5 mg/kg, i.p.), but not, HLP (1 mg/kg, i.p.), significantly ($p < 0.05$) reversed the effect of KET on the expressions of GAD_{67} immunopositive cells in the three brain region examined when compared with KET (20 mg/kg, i.p.) treatment alone (Figure 4.40B).

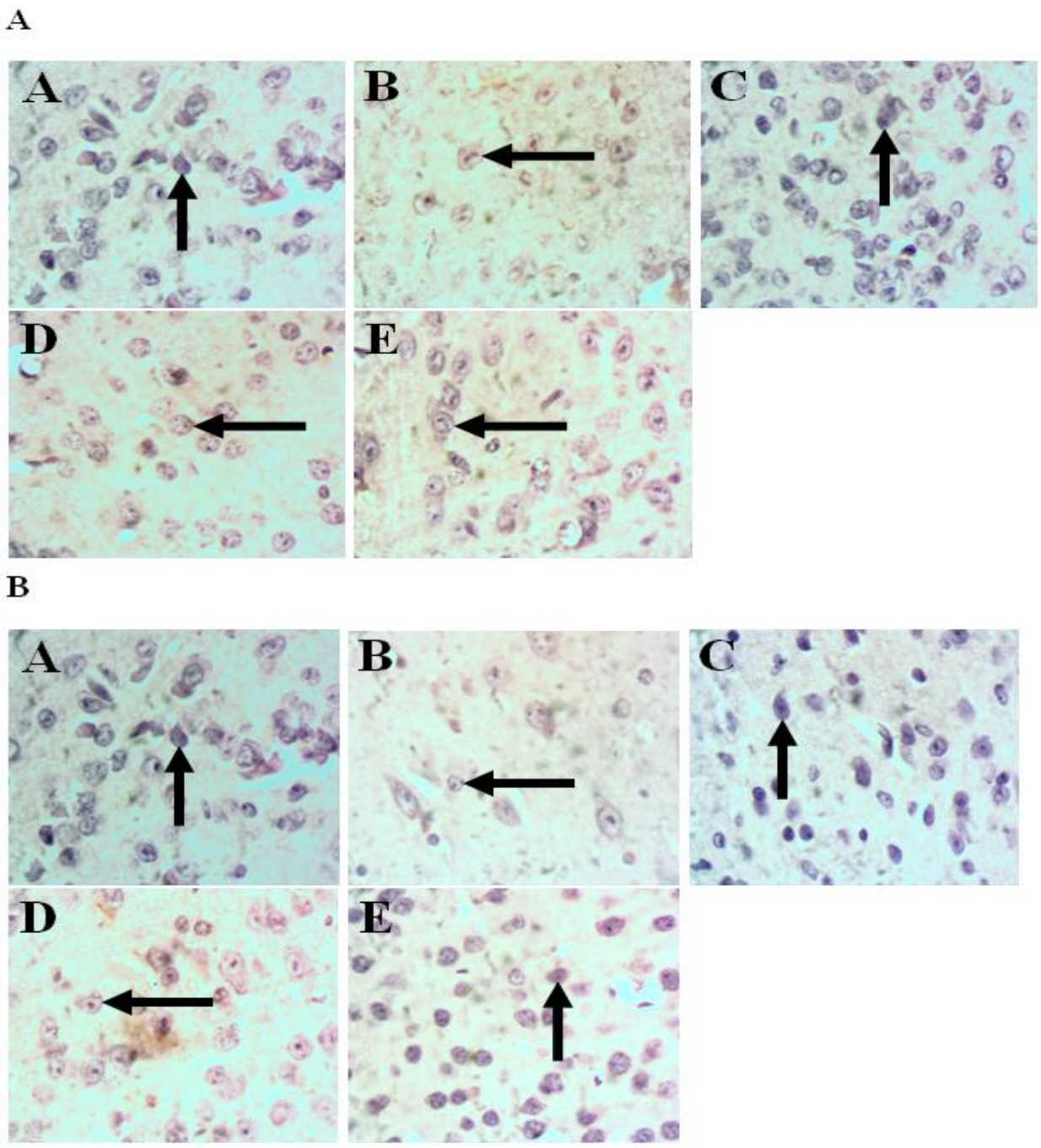


Plate 4.10: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of GAD_{67} immunopositive cells in the *striatum* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

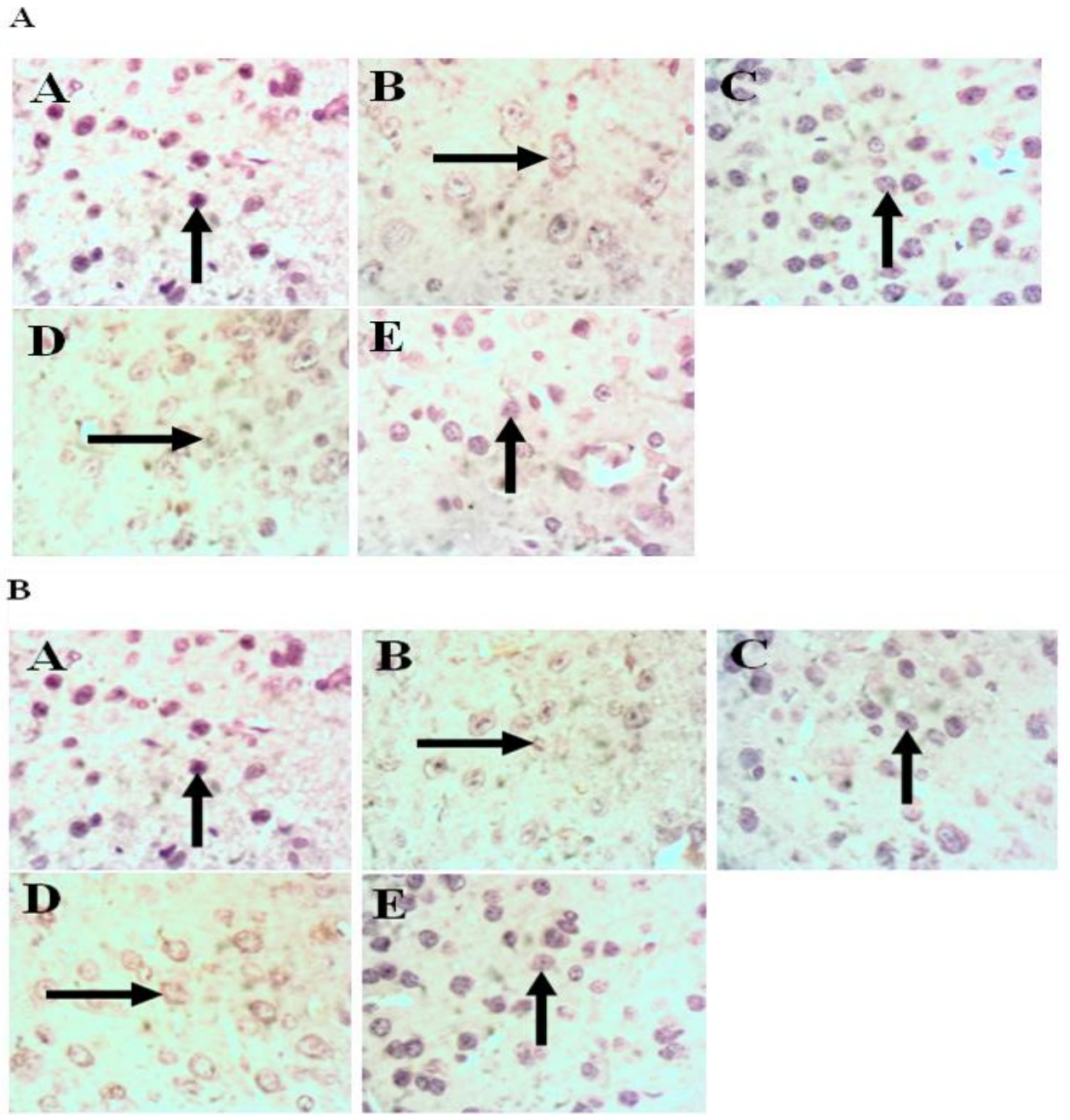
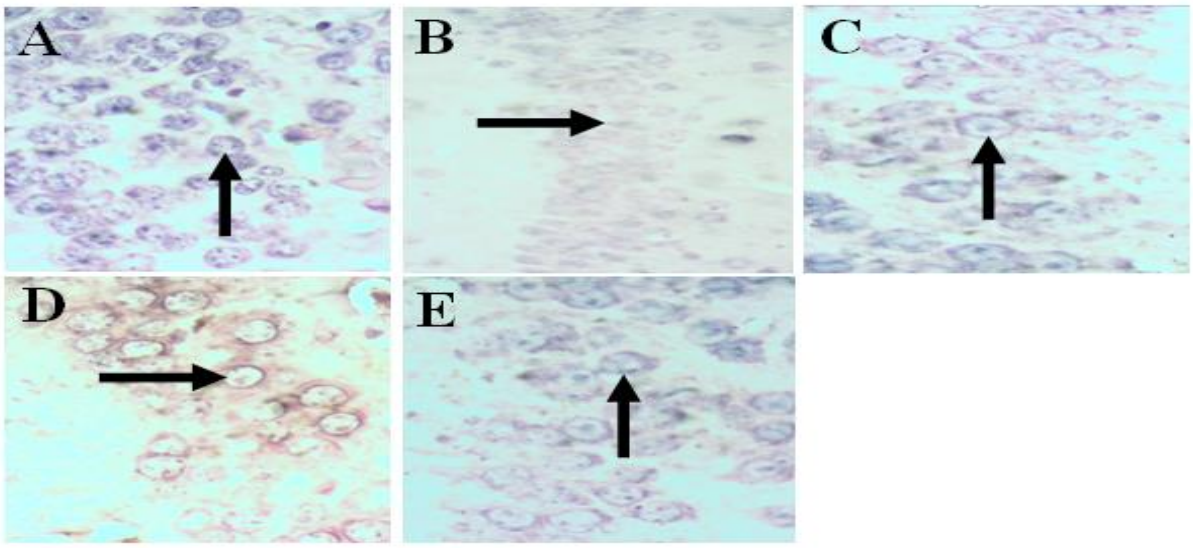


Plate 4.11: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of GAD_{67} immunopositive cells in the *prefrontal cortex* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

A



B

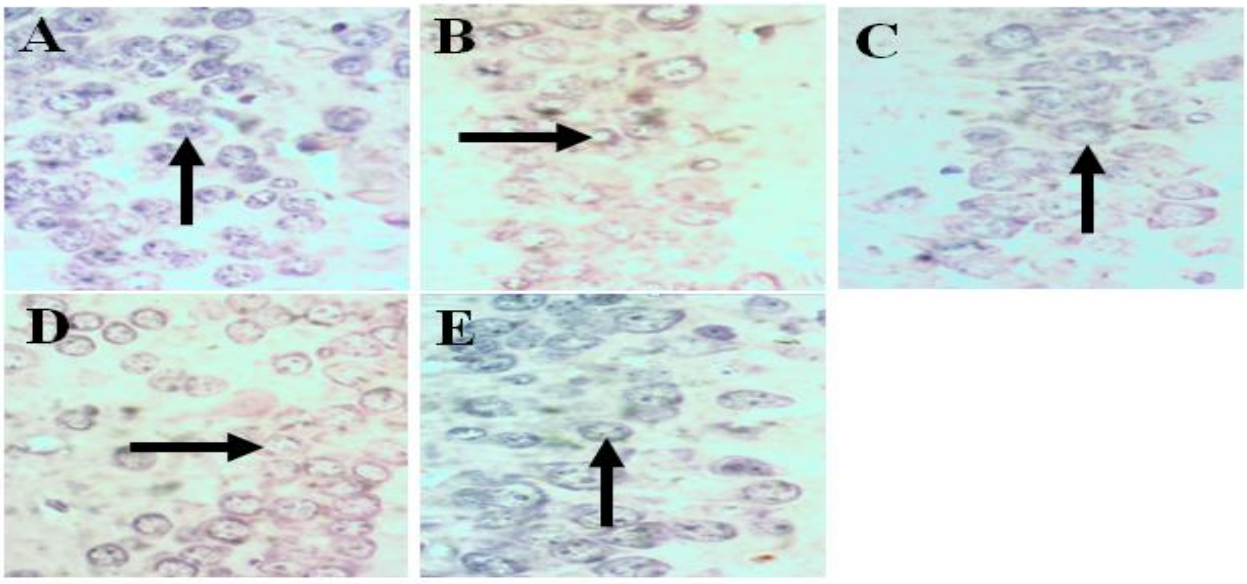


Plate 4.12 Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of GAD_{67} immunopositive cells in the CA1 region of the *hippocampus* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

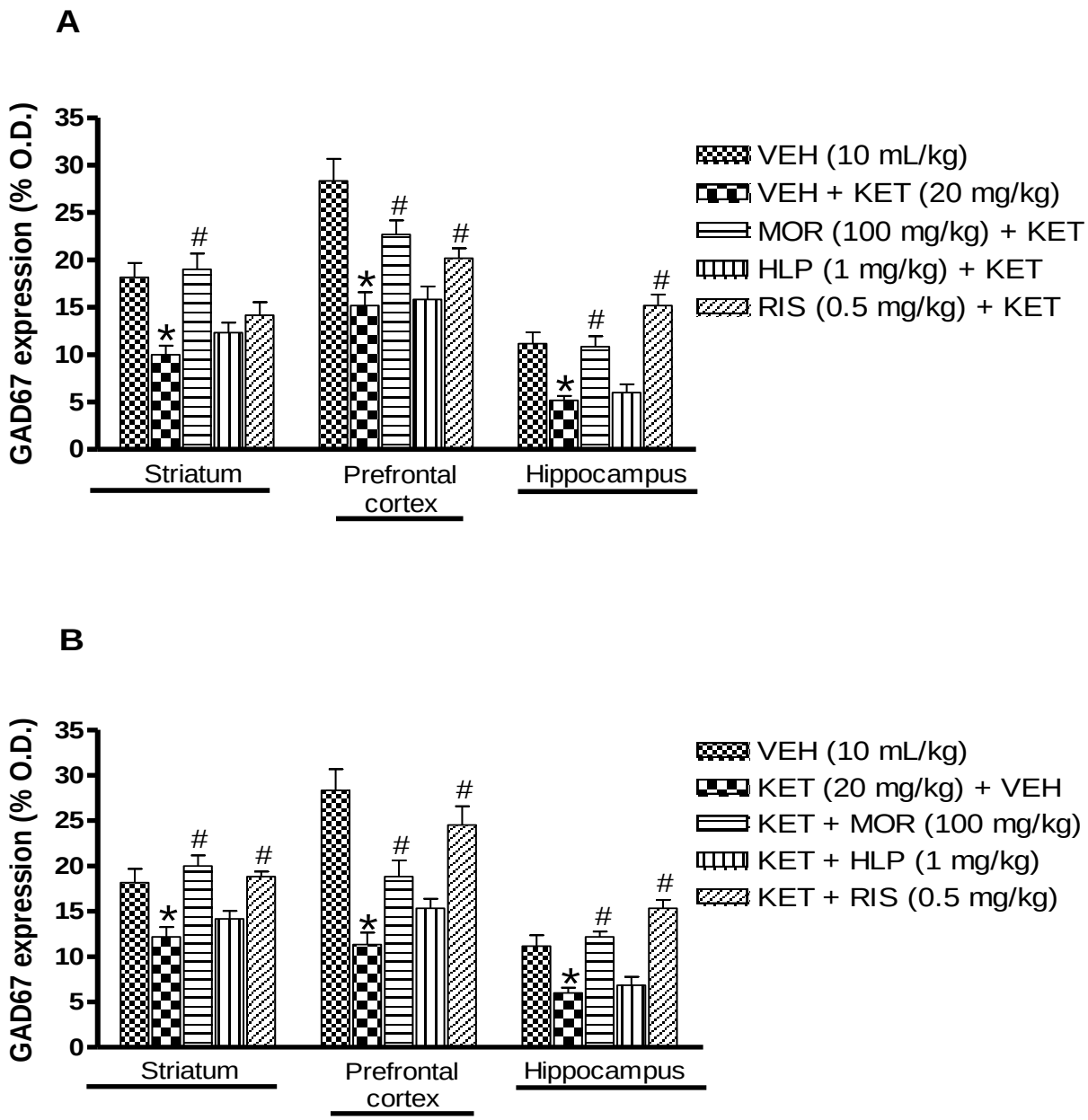


Figure 4.40: Effect of morin on the expression of GAD_{67} immunopositive cells of the striatum, prefrontal cortex and hippocampus of mice brains in the preventive (A) and reversal (B) treatments with ketamine.

Bars represent the mean ± S.E.M of 3 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

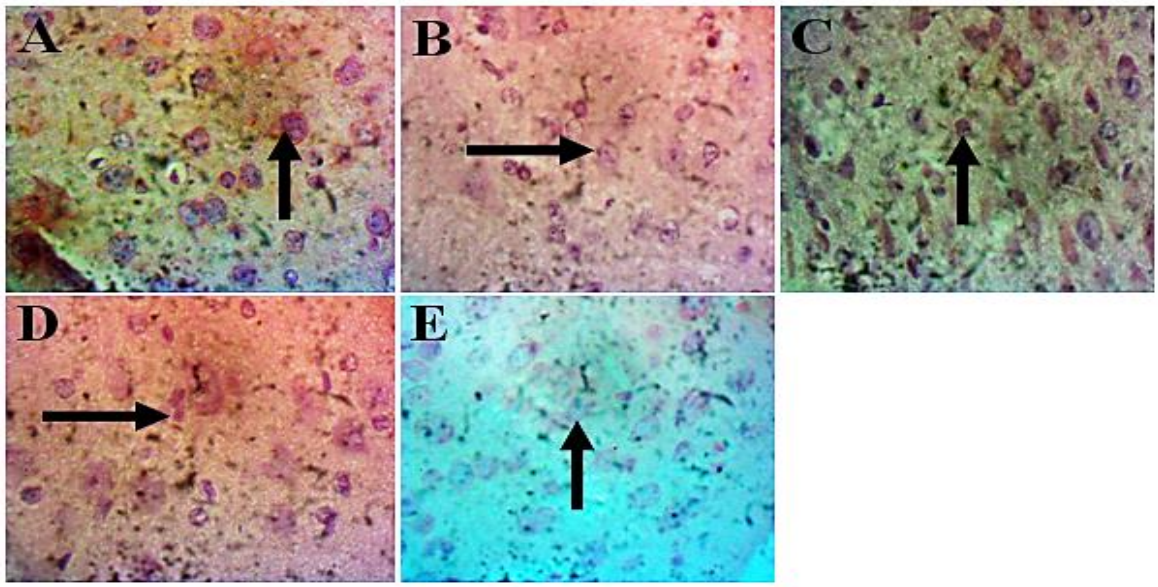
VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.50 Effect of morin on BDNF expressions in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The photomicrographs of the effect of MOR on KET-induced immunohistochemical changes and expressions of BDNF immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatments are shown in Plates 4.13 (A and B), 4.14 (A and B), 4.15 (A and B) and Figure 4.41 (A and B). Treatment with KET (20 mg/kg, i.p.) produced significant ($p < 0.05$) immunohistochemical alterations of BDNF in the striatum (Plate 4.13A and B), prefrontal cortex (Plate 4.14A and B) and CA1 region of the hippocampus (Plate 4.15A and B), with a corresponding decreases in the expressions of BDNF positive cells (percentage optical density, % O.D.) in the preventive (Figure 4.41A) and reversal (Figure 4.41B) treatment protocols when compared with vehicle control. MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented the decreased expressions of BDNF immunopositive cells induced by KET in the striatum (Plate 4.13A and Figure 4.41A), prefrontal cortex (Plate 4.14A and Figure 4.41A) and hippocampus (Plate 4.15A and Figure 4.41A) in the preventive treatment relative to KET-treated mice, suggesting evidence of neurotrophic property. HLP (1 mg/kg, i.p.) did not increase the expressions of BDNF positive cells decreased by KET in the striatum, prefrontal cortex and hippocampus when compared to KET-treated mice brains (Figure 4.41A).

In the reversal treatment, treatment with MOR (100 mg/kg, i.p.) increased the expressions of BDNF positive cells in the striatum (Plate 4.13B and Figure 4.41B) and prefrontal cortex (Plate 4.14B and Figure 4.41B) in comparison to KET-treated mice significantly ($p < 0.05$), but did not reverse decreased expressions of BDNF positive cells induced by KET (20 mg/kg, i.p.) in the hippocampal (CA1) region (Plate 4.15B and Figure 4.41B) when compared with KET treatment alone. Atypical antipsychotic drug, RIS (0.5 mg/kg, i.p.), but not typical antipsychotic drug, HLP (1 mg/kg, i.p.), significantly ($p < 0.05$) reversed the effect of KET on the expressions of BDNF positive cells in the striatum, prefrontal cortex and hippocampus when compared with KET (20 mg/kg, i.p.) treatment alone (Figure 4.41B).

A



B

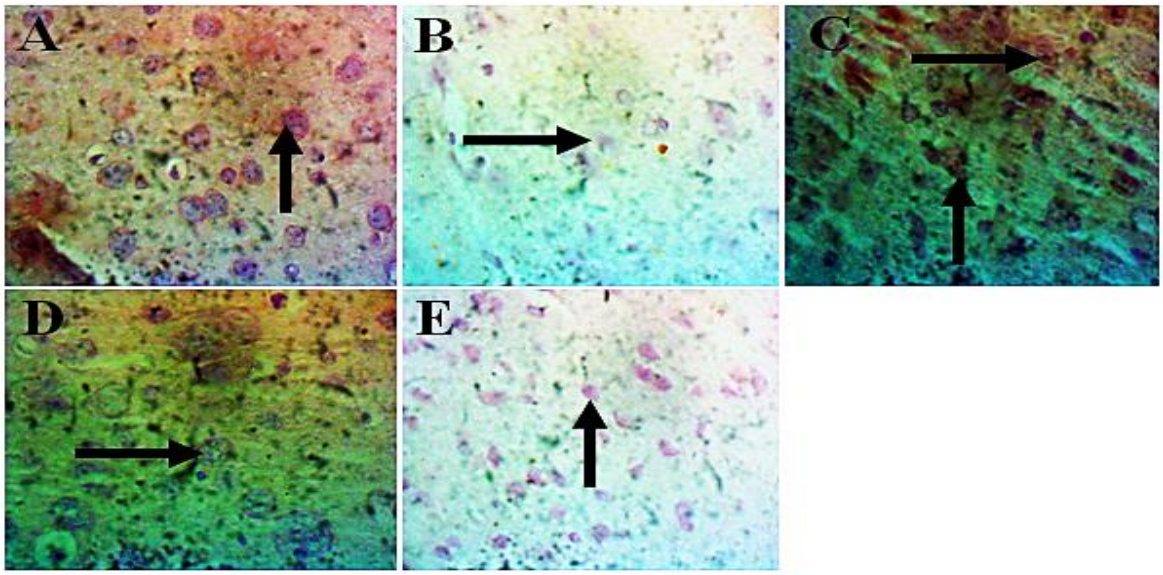
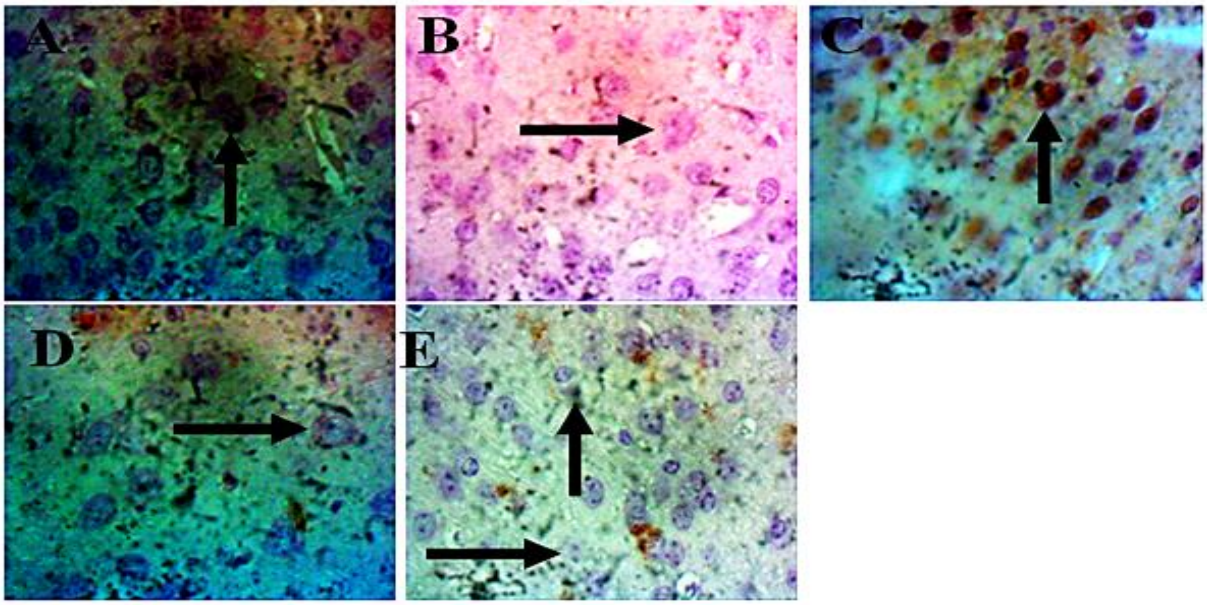


Plate 4.13: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of BDNF immunopositive cells in the *striatum* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

A



B

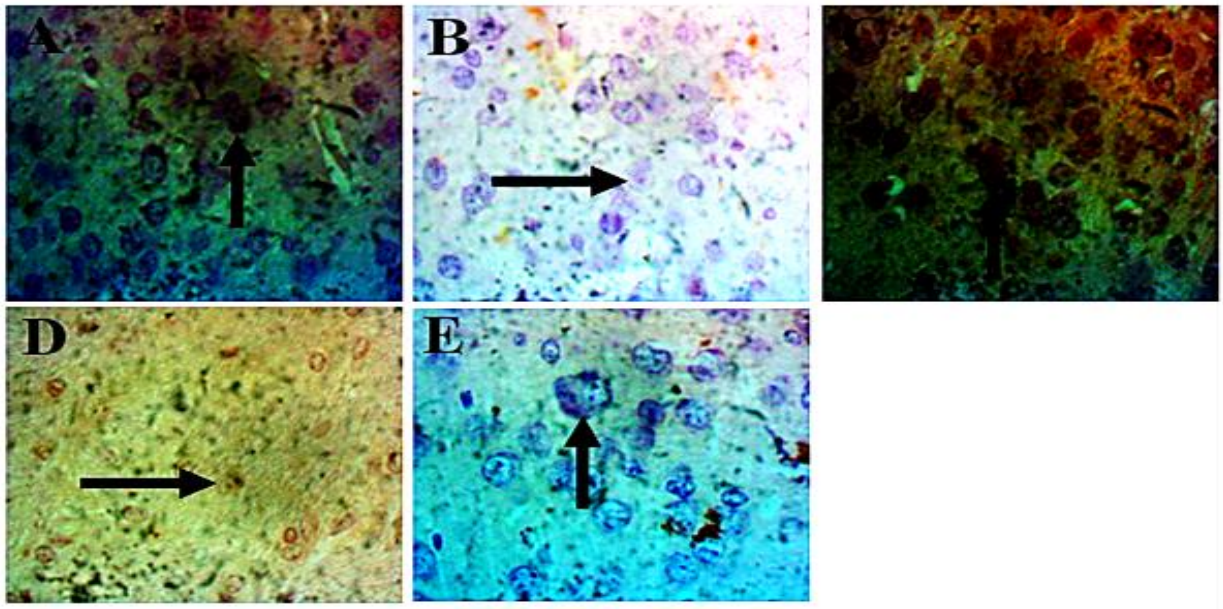
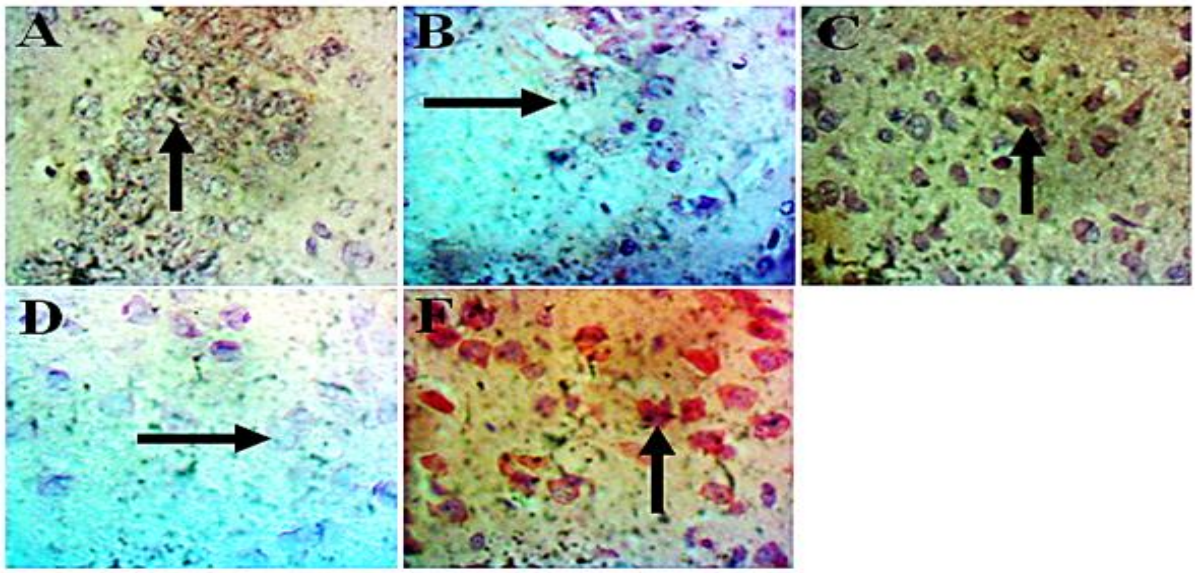


Plate 4.14: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of BDNF immunopositive cells in the *prefrontal cortex* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

A



B

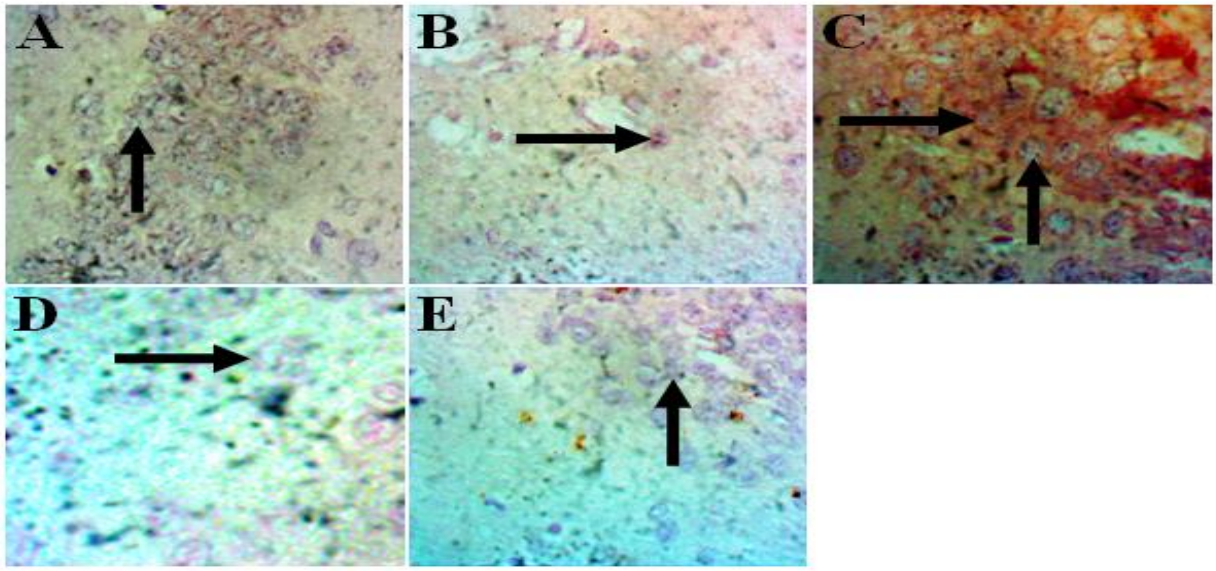


Plate 4.15: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of BDNF immunopositive cells in the CA1 region of the *hippocampus* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

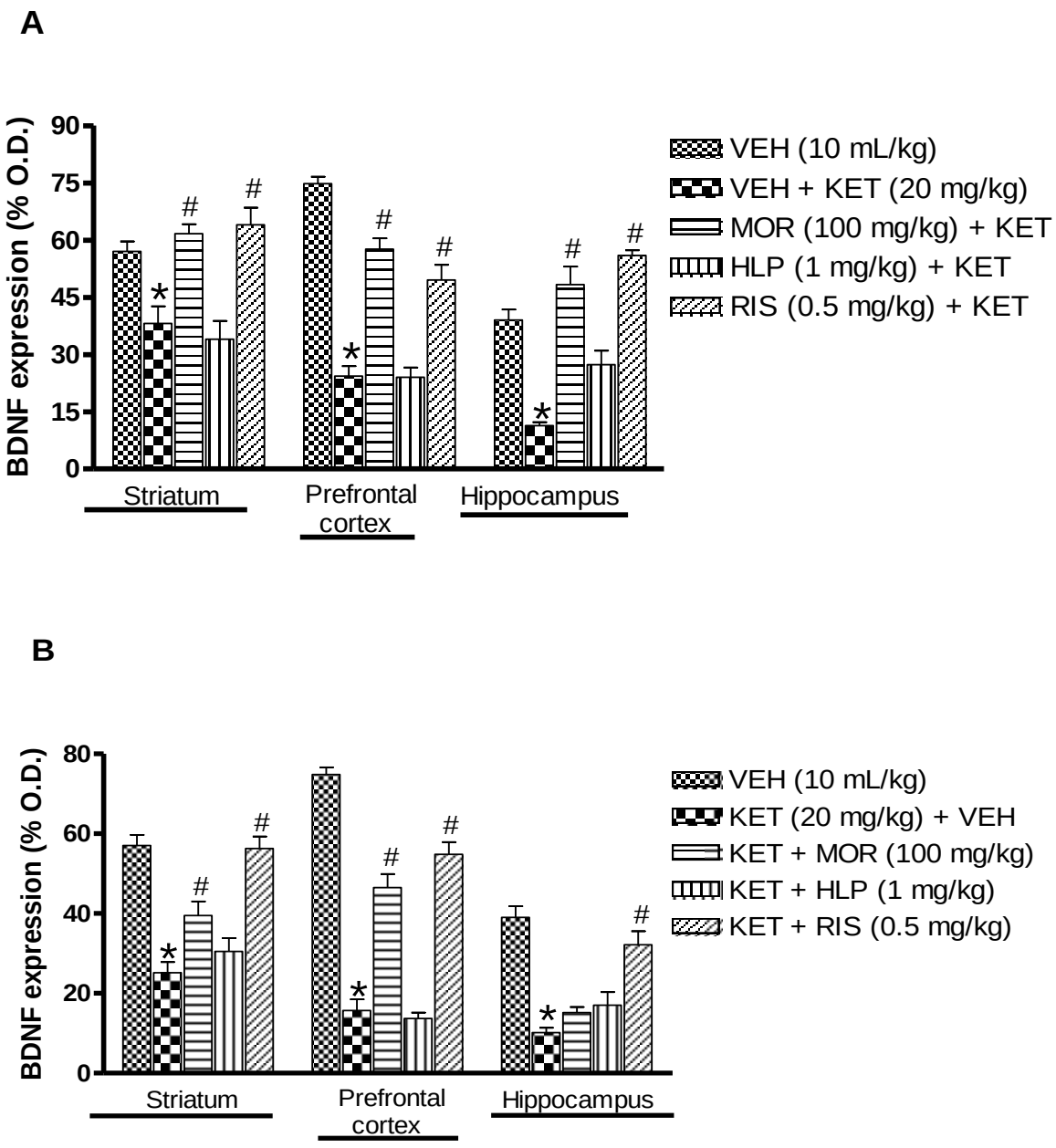


Figure 4.41: Effect of morin on expression of BDNF immunopositive cells of the striatum, prefrontal cortex and hippocampus (CA1) of mice brains in the preventive (A) and reversal (B) treatments with ketamine

Bars represent the mean ± S.E.M of 3 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

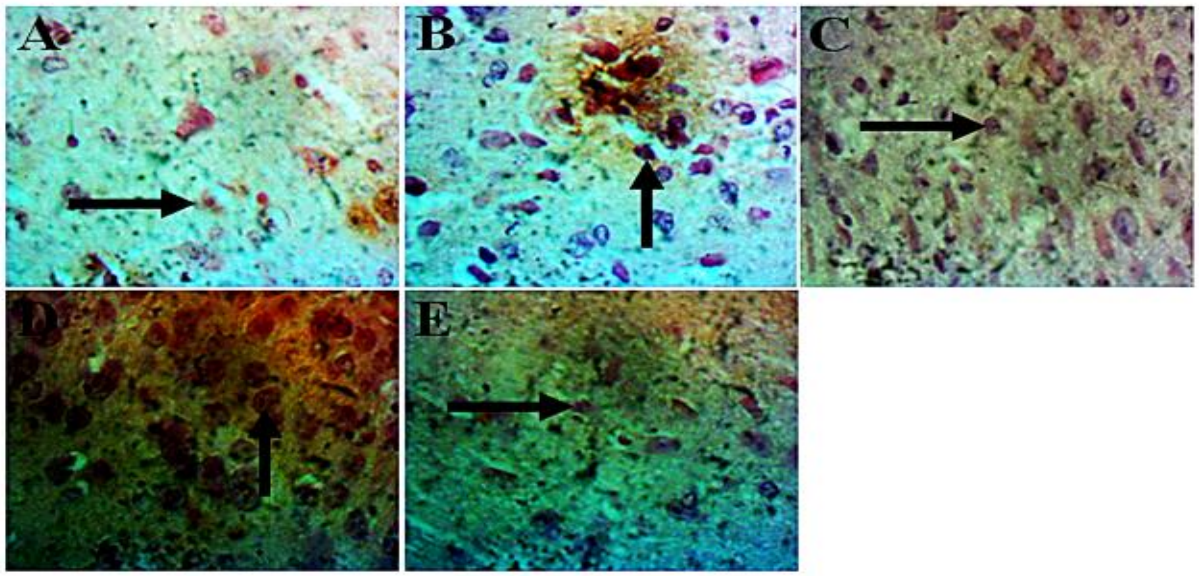
VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.51 Effect of morin on Nox-2 expressions in the striatum, prefrontal cortex and hippocampus in the preventive and reversal treatments with ketamine

The photomicrographs of the effect of MOR on KET-induced immunohistochemical changes and expressions of Nox-2 [nicotinamide adenine dinucleotide phosphate-oxidase-2 (NADPH oxidase-2) gp91 phox subunit] immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatments are shown in Plates 4.16 (A and B), 4.17 (A and B), 4.18 (A and B) and Figure 4.42 (A and B). Chronic intraperitoneal injection of KET (20 mg/kg) did not cause any marked immunohistochemical changes of Nox-2 in the CA1 region of the hippocampus (Plate 4.18A), it produced significant ($p < 0.05$) immunohistochemical alterations in the striatum (Plate 4.16A) and prefrontal cortex (Plate 4.17A), as revealed by increased % O.D. expressions of striatal and cortical Nox-2 immunopositive cells in the preventive treatment (Figure 4.42A) in comparison with vehicle-treated mice, suggesting increased production of superoxide free radical. MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented KET-induced expressions of Nox-2 immunopositive cells in the striatum (Plate 4.16A and Figure 4.42A) and prefrontal cortex (Plate 4.17A and Figure 4.42A) in the preventive treatment when compared to KET-treated mice, which suggests antioxidant property. Pretreatment with HLP (1 mg/kg, i.p.) failed to prevent KET-induced immunohistochemical changes and expressions of Nox-2 immunopositive cells in the striatum and prefrontal cortex when compared to KET-treated mice brains. HLP caused a significant increase in the expressions of immunopositive cells of Nox-2 in the hippocampus relative to vehicle-treated mice (Figure 4.42A).

In the reversal treatment, KET significantly ($p < 0.05$) induced immunohistochemical alterations and increased expressions of Nox-2 immunopositive cells in all three sub-regions of mice brain relative compared to vehicle-treated group (Plates 4.16B, 4.17B and 4.18B). Treatments with MOR (100 mg/kg, i.p.) and RIS significantly ($p < 0.05$) decreased the expressions of Nox-2 positive cells in the striatum, prefrontal cortex and hippocampus when compared with KET-treated mice (Figure 4.42B). HLP (1 mg/kg, i.p.) failed to decrease striatal, prefrontal cortical and hippocampal (CA1 region) expressions of Nox-2 positive cells when compared with KET-treatment alone (Figure 4.42B).

A



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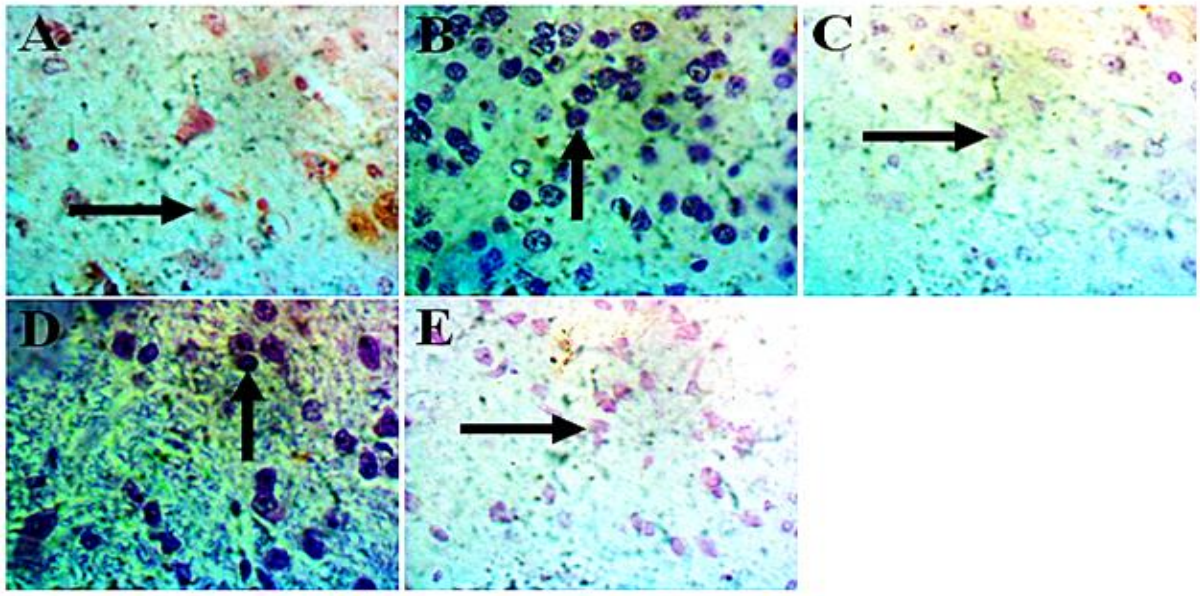


Plate 4.16: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of Nox-2 immunopositive cells in the *striatum* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

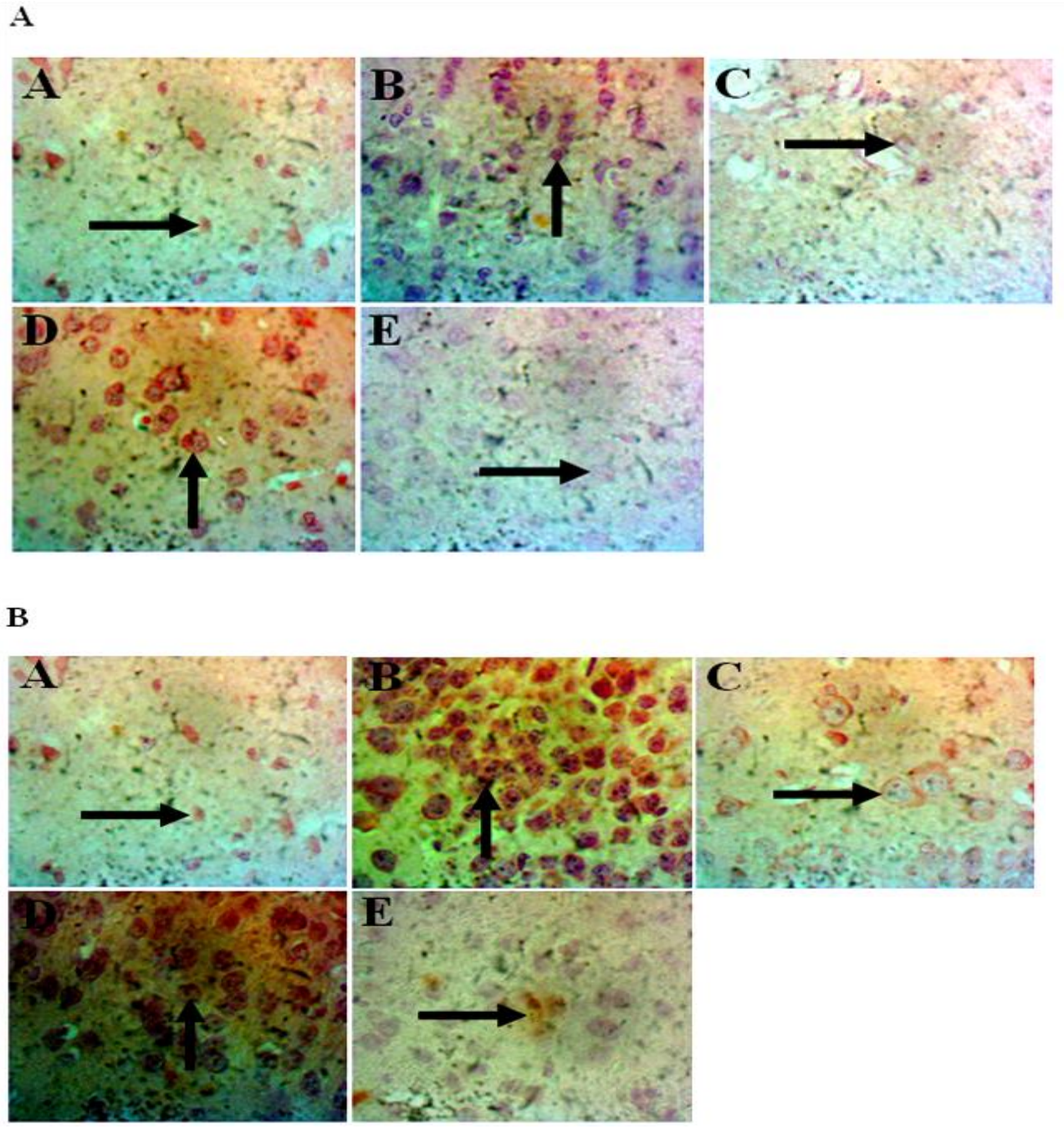
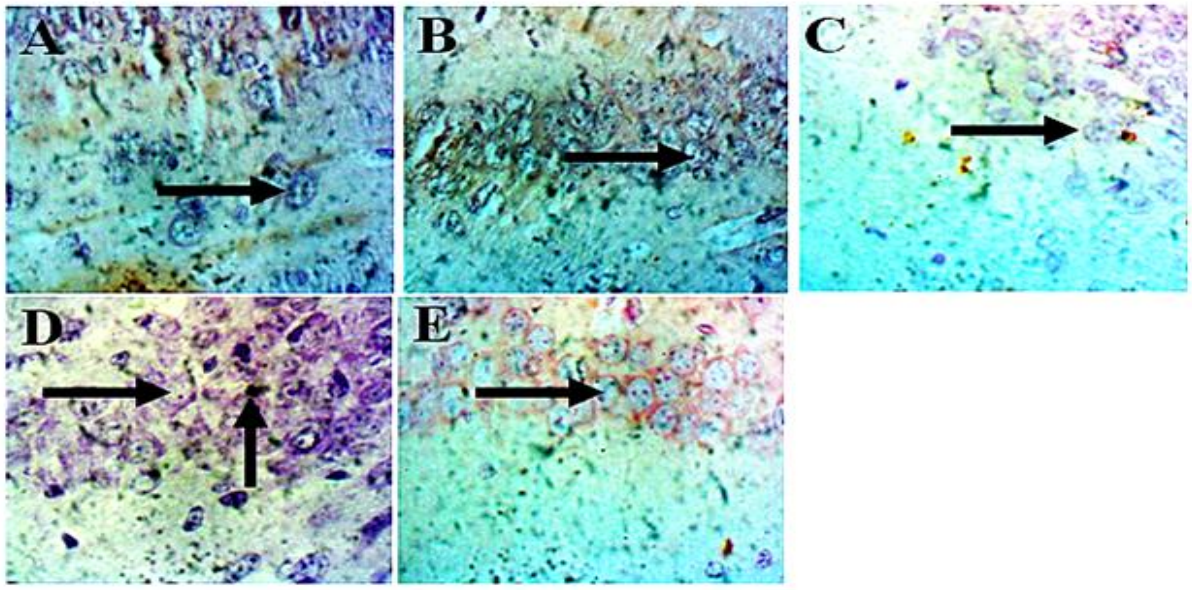


Plate 4.17: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of Nox-2 immunopositive cells in the *prefrontal cortex* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

A



B

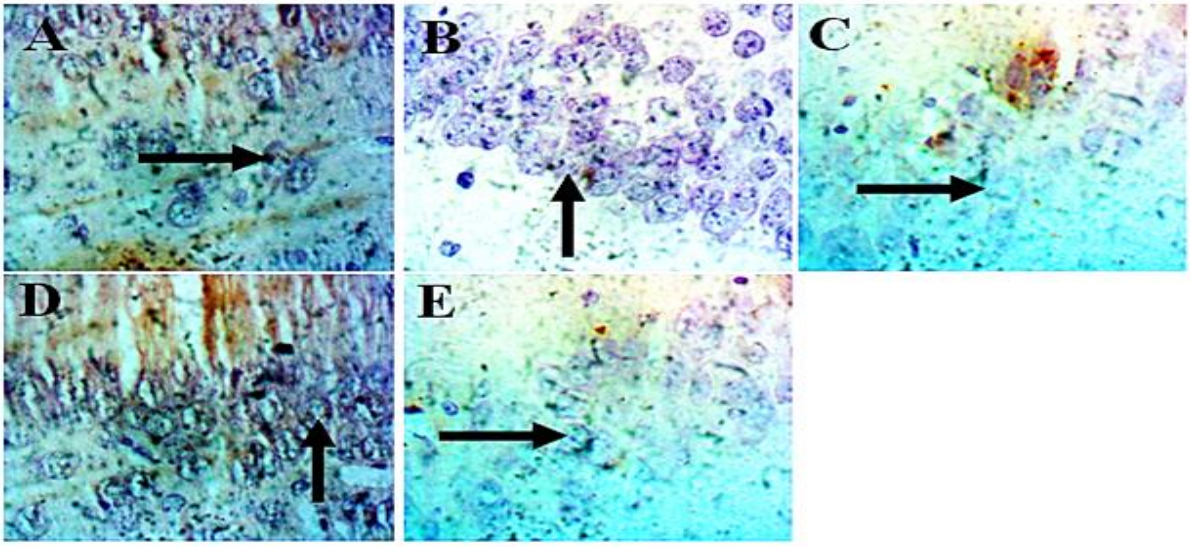


Plate 4.18: Representative photomicrographs of the effect of morin on ketamine-induced immunohistochemical changes and expressions of Nox-2 immunopositive cells in the CA1 region of the *hippocampus* of mice brains in the preventive (A) and reversal (B) treatments. A = Vehicle 10 mL/kg, B = Ketamine 20 mg/kg, C = Morin 100 mg/kg, D = Haloperidol 1 mg/kg, and E = Risperidone 0.5 mg/kg

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

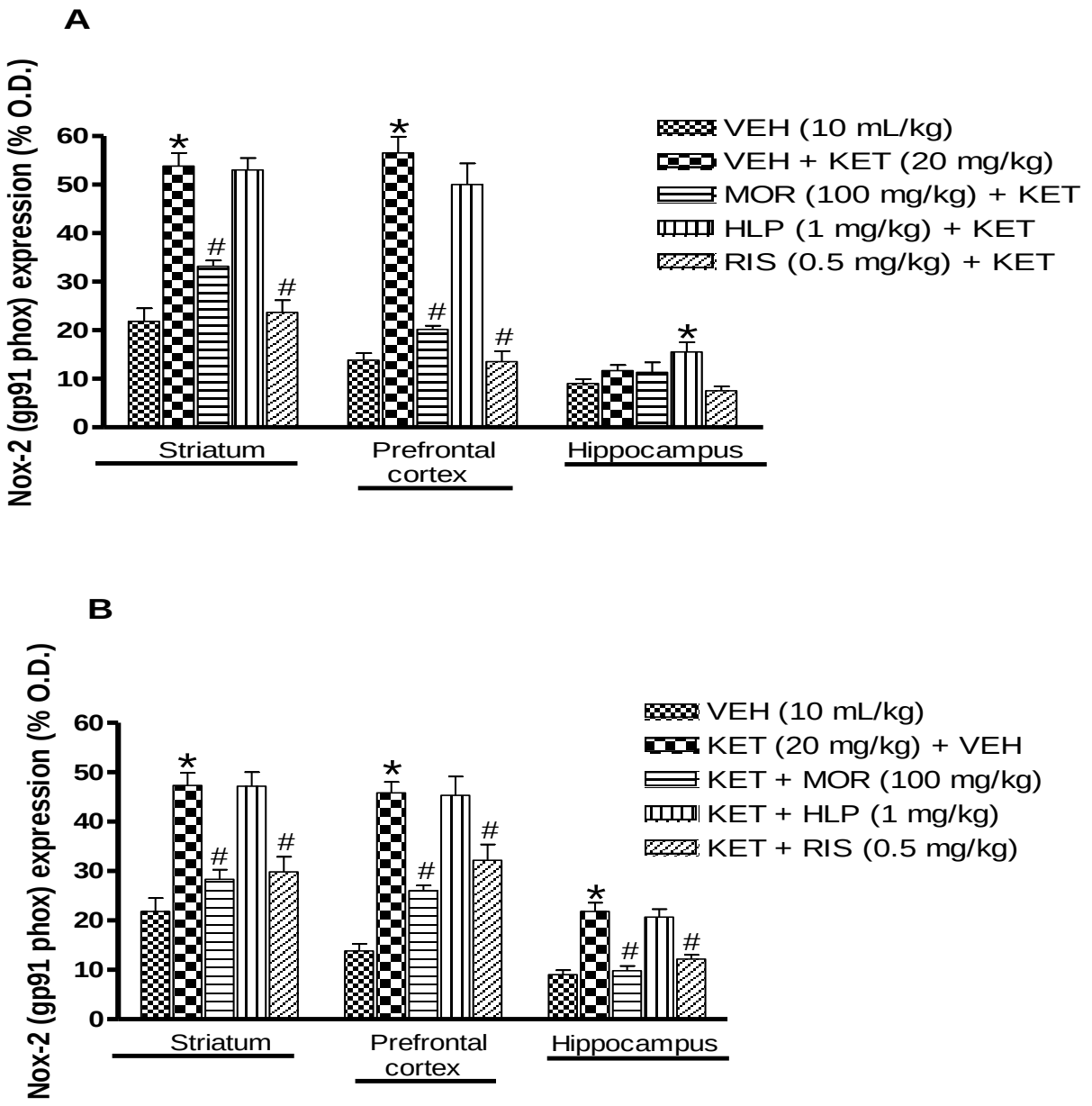


Figure 4.42: Effect of morin on expression of Nox-2 (gp91 phox) immunopositive cells of the striatum, prefrontal cortex and hippocampus (CAI) of mice brains in the preventive (A) and reversal (B) treatments with ketamine

Bars represent the mean ± S.E.M of 3 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.52 Effect of morin on lipopolysaccharide-ketamine-induced stereotypy

The effect of MOR on LPS-KET-induced stereotypy, as assessed by repetitive behaviours such as head movements, intermittent sniffing, chewing and intense licking in mice, using the total stereotypy score is shown in Figure 4.43. Intraperitoneal administration of LPS (0.1 mg/kg) for 14 days followed by KET (20 mg/kg) treatment from the 8^{th} to the 14^{th} day once daily produced marked ($p < 0.05$) increase in head movements, intermittent sniffing, chewing and intense licking in mice, which suggests stereotyped behaviour. However, tretreatment of MOR (100 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) prevented the stereotyped behaviours by LPS and KET when compared with LPS plus KET-treated mice. Similarly, treatments with the standard drugs, HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated the stereotyped behaviour in comparison with LPS (0.1 mg/kg, i.p.) plus KET (20 mg/kg, i.p.) treatment alone (Figure 4.43).

4. 53 Effect of morin on lipopolysaccharide-ketamine-induced hyperlocomotion

The effect of MOR on LPS-KET-induced hyperlocomotion in open field test (OFT) is shown in Figure 4.44. Pretreatment of LPS (0.1 mg/kg) for 14 days prior to KET (20 mg/kg) treatment from the 8^{th} to the 14^{th} day once daily, significantly ($p < 0.05$) increased the number of line crossing when compared with vehicle-treated group, suggesting hyperlocomotion. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) decreased LPS plus KET-induced hyperlocomotion when compared with LPS plus KET-treated mice (Figure 4.44). HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced the number of line crossings (hyperlocomotion) in comparison with LPS plus KET treatment alone (Figure 4.44).

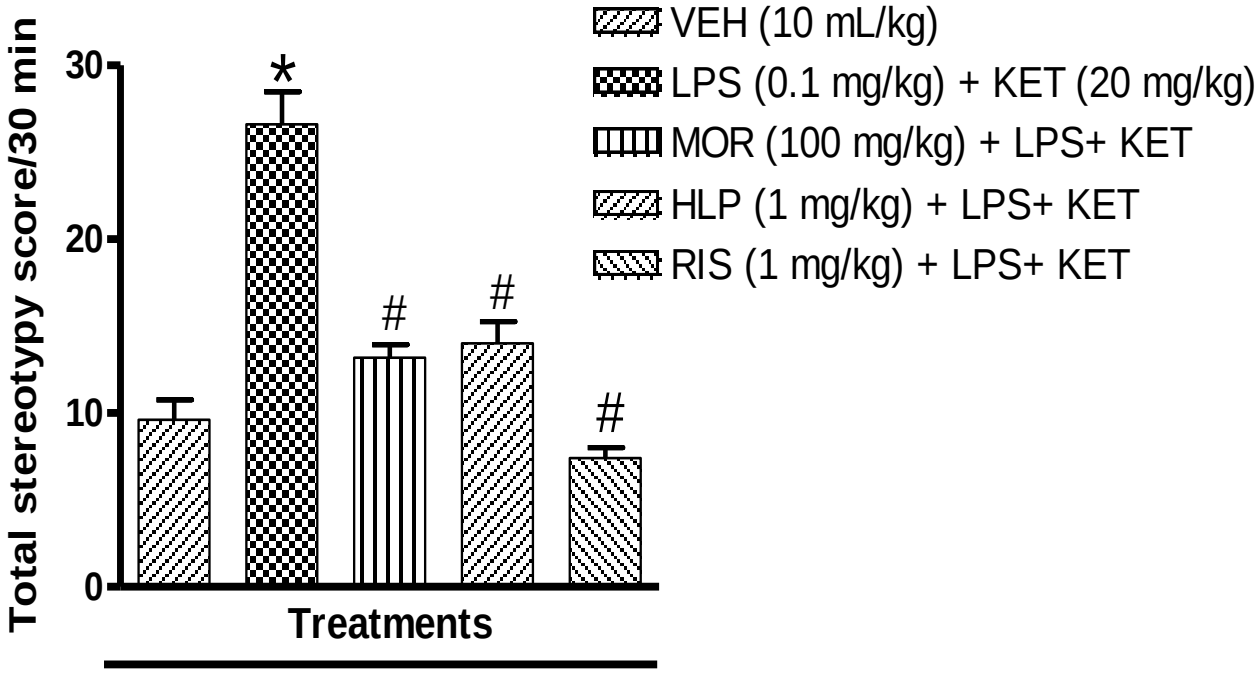


Figure 4.43: Effect of morin on lipopolysaccharide-ketamine-induced stereotypy in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

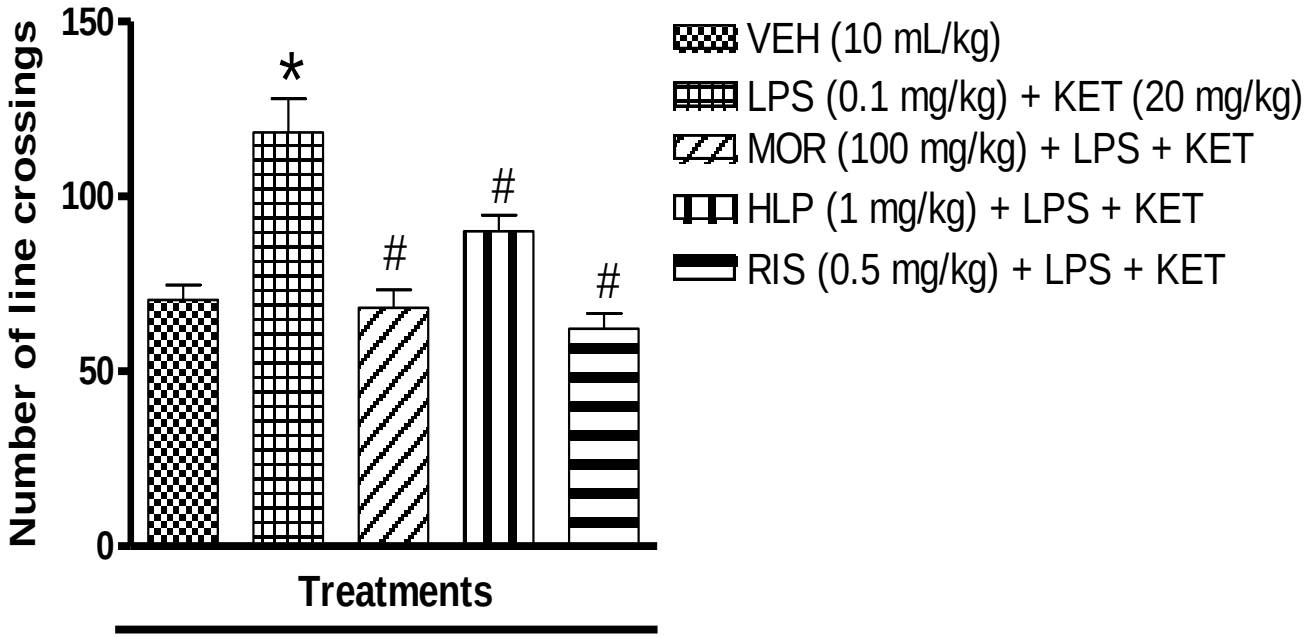


Figure 4.44: Effect of morin on lipopolysaccharide-ketamine-induced hyperlocomotion in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.54 Effect of morin on lipopolysaccharide-ketamine-induced alteration in spatial working memory

Intraperitoneal injection of LPS (0.1 mg/kg) for 14 days in combination with KET (20 mg/kg) treatment from the 8^{th} to the 14^{th} day once daily markedly impaired spatial working memory performances, as it significantly ($p < 0.05$) decreased % correct alternations or navigations of mice in the Y-maze test (YMT) in comparison to vehicle treatment (Figure 4.45). However, pretreatment with MOR (100 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) decreased LPS plus KET-induced spatial working memory impairment when compared with LPS plus KET-treated mice, suggesting memory enhancing property via anti-inflammatory activity (Figure 4.45). RIS (0.5 mg/kg, i.p.) demonstrated significant ($p < 0.05$) increase in the % correct navigations in the YMT in LPS plus KET-treated mice when compared with LPS plus KET-treated mice alone. HLP (1 mg/kg, i.p.) failed to prevent the decrease in % correct alternations by LPS plus KET, indicating lack of memory enhancing property via anti-inflammatory activity in mice (Figure 4.45).

4.55 Effect of morin on lipopolysaccharide-ketamine-induced alteration in non-spatial working (recognition) memory

The effect of MOR on LPS-KET-induced alteration on non-spatial working memory, as measured by discrimination index in the novel object recognition test (NORT) is shown in Figure 4.46. Intraperitoneal injection of LPS (0.1 mg/kg) for 14 days in combination with KET (20 mg/kg) treatment from the 8^{th} to the 14^{th} day once daily, demonstrated significant ($p < 0.05$) decrease in non-spatial working memory impairment in the NORT when compared with vehicle treated animals. MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) pretreatment once daily for 14 days reversed LPS plus KET-induced non-spatial working memory impairment, as it significantly ($p < 0.05$) increased the DI when compared with LPS plus KET-treated mice, which is also suggestive of psychotropic memory enhancing property via anti-inflammatory activity (Figure 4.46). HLP (1 mg/kg, i.p.) did not reverse non-spatial memory performance by LPS and KET in the NORT in mice (Figure 4.46).

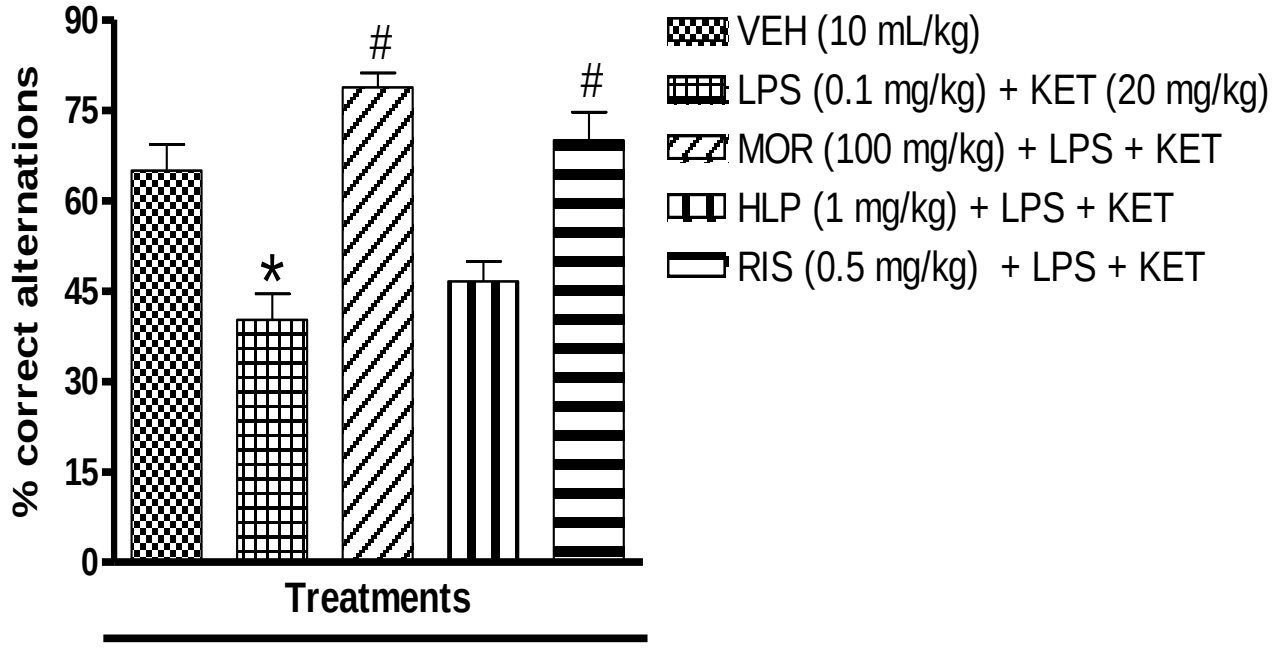


Figure 4.45: Effect of morin on lipopolysaccharide-ketamine-induced alteration in spatial working memory in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

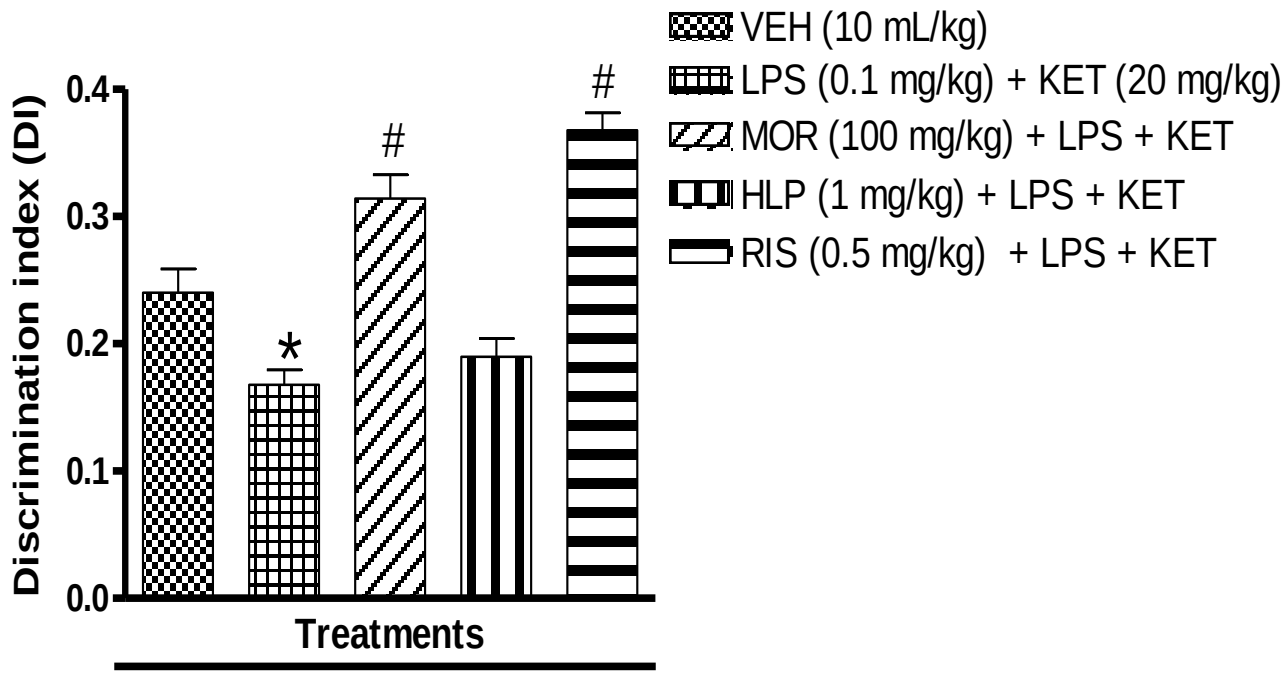


Figure 4.46: Effect of morin on lipopolysaccharide-ketamine-induced alteration in non-spatial working (recognition) memory in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.56 Effect of morin on lipopolysaccharide-ketamine-induced alteration in social interaction

The effect of MOR on LPS-KET-induced alteration on social interaction, as assessed by the % social preference using the social interaction test (SIT) is shown in Figure 4.47. Administration of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day once daily, significantly ($p < 0.05$) decreased the % social preferences in mice, suggesting impairment of social behaviour. However, pretreatment with MOR (100 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) decreased LPS plus KET-induced social performance impairment when compared with LPS plus KET-treated mice (Figure 4.47). RIS (0.5 mg/kg, i.p.), but not HLP (1 mg/kg, i.p.), significantly ($p < 0.05$) reduced social behavioural impairment by LPS (0.1 mg/kg, i.p.) plus KET (20 mg/kg, i.p.) in comparison with LPS plus KET treatment alone (Figure 4.47).

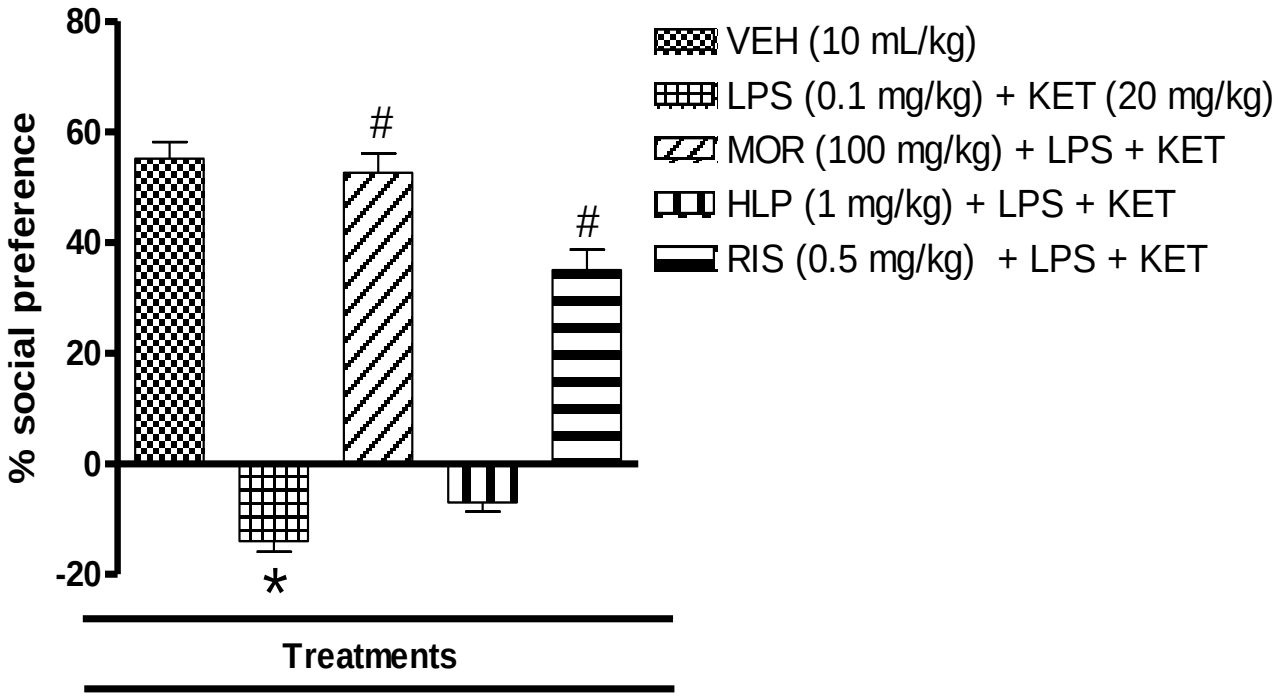


Figure 4.47: Effect of morin on lipopolysaccharide-ketamine-induced alteration in social interaction in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.57 Effect of morin on lipopolysaccharide-ketamine-induced alteration in social recognition memory

The effect of MOR on LPS-KET-induced social recognition memory impairment, as measured by decrease in social investigation time following inter-trial interval re-exposure is shown in Figure 4.48. Intraperitoneal injection of LPS (0.1 mg/kg) for 14 days in combination with KET (20 mg/kg, i.p.) treatment caused a decrease in social memory discriminative capability, with no significant ($p < 0.05$) differences in the duration of social investigative time of re-exposed conspecific juvenile companion mice at inter-trial interval of 30 min (short-term social memory) and 24 hr (long-term social memory) relative to social investigative time of initial exposure; suggesting social recognition memory impairment. However, MOR (100 mg/kg, i.p.) reversed LPS and KET-induced social recognition memory impairment, by significantly ($p < 0.05$) decreasing the social investigative time upon re-exposure of conspecific companion mice at 30 min and 24 hr in comparison with investigation time of initial exposure respectively, suggesting enhancement of short- and long-term social recognition memories, respectively. Similarly, RIS (0.5 mg/kg, i.p.), but not HLP, demonstrated significant ($p < 0.05$) prevention of social recognition memory impairment by LPS and KET, as indicated by decreases in the social investigation time of conspecific mice following inter-interval re-exposure at 30 min and 24 hr when compared with investigation time of initial exposure (Figure 4.48).

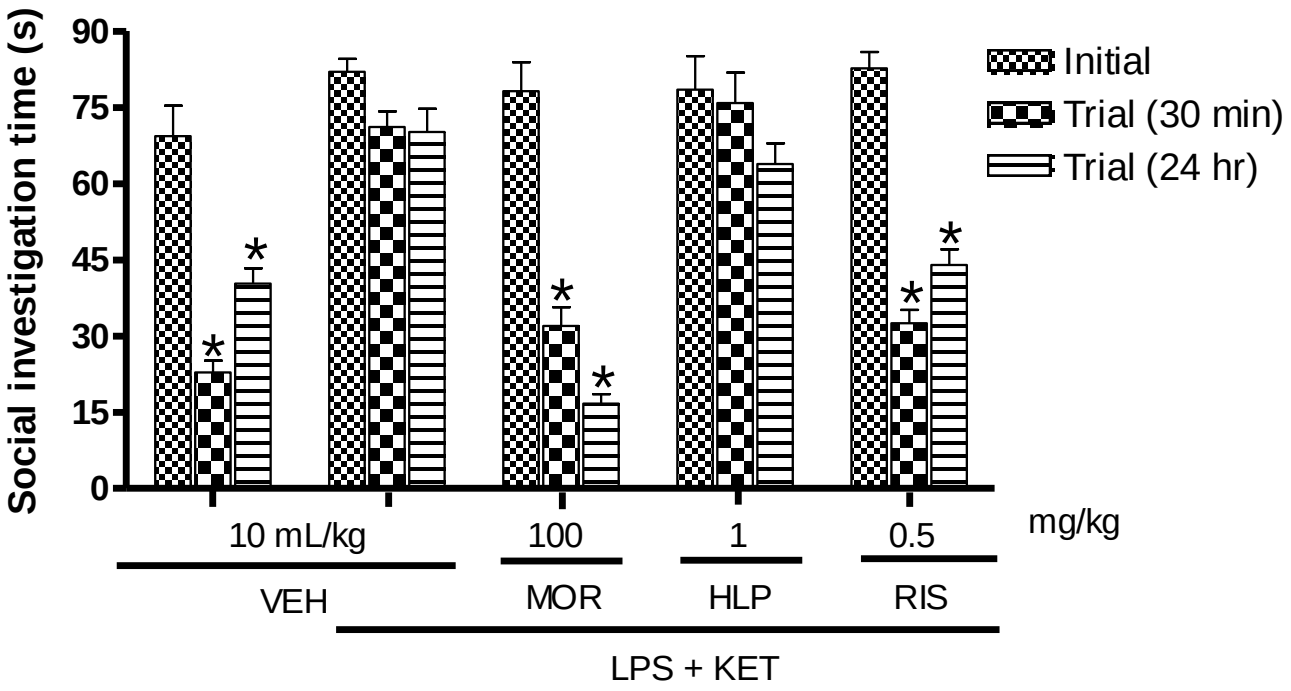


Figure 4.48: Effect of morin on lipopolysaccharide-ketamine-induced alteration in social recognition memory in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to initial exposure (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin, **HLP =** Haloperidol, **RIS** = Risperidone

4.58 Effect of morin on lipopolysaccharide-ketamine-enhanced immobility in forced swim test

The effect of MOR on LPS-KET-enhanced immobility in forced swim test (FST) is shown in Figure 4.49. Intraperitoneal injection of LPS (0.1 mg/kg) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day once daily, significantly ($p < 0.05$) enhanced the duration of immobility in the FST in mice, which is suggestive behavioural despair. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) decreased LPS plus KET-induced behavioural despair when compared with LPS plus KET-treated mice (Figure 4.49). RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) diminished LPS (0.1 mg/kg, i.p.) and KET (20 mg/kg, i.p.) enhanced immobility time in comparison with LPS plus KET treatment alone. However, HLP (1 mg/kg, i.p.) failed to attenuate the enhancement of immobility time in the FST by LPS and KET relative to LPS plus KET-treated mice (Figure 4.49).

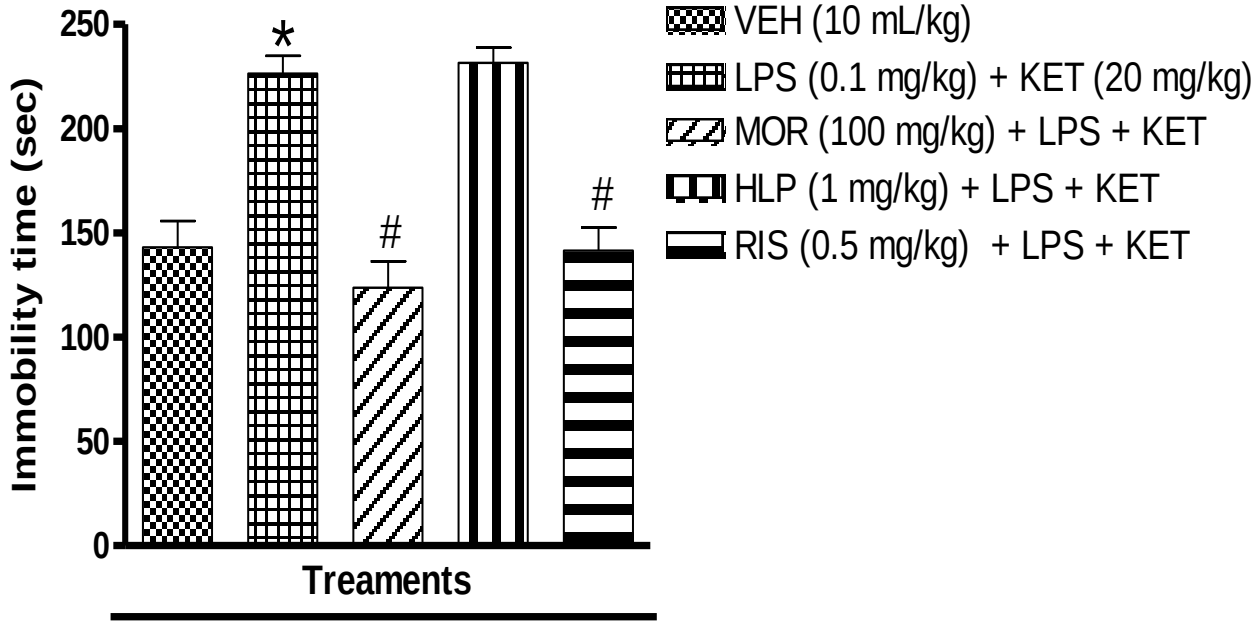


Figure 4.49: Effect of morin on lipopolysaccharide-ketamine-enhanced immobility in forced swim test in mice

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.59 Effect of morin on lipopolysaccharide-ketamine-induced alteration in acetylcholinesterase activity in the striatum, prefrontal cortex and hippocampus of mice brains

The effect of MOR on AChE emzyme activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.50. Repeated administration of LPS (0.1 mg/kg, i.p.) once daily for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day significantly ($p < 0.05$) increased brain AChE enzyme activity in the prefrontal cortex and hippocampus relative to vehicle-treated mice, suggesting decreased prefrontal cortical and hippocampal cholinergic transmissions, with no significant change in AChE activity in the striatum was observed (Figure 4.50). MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) did not demonstrate any significant change in AChE activity in the striatum, but significantly ($p < 0.05$) decreased LPS plus KET-induced enhancement of AChE activity in the prefrontal cortex and hippocampus when compared with LPS plus KET-treated mice, suggesting increase in prefrontal cortical and hippocampal cholinergic transmissions. HLP (1 mg/kg, i.p.) did not prevent the increase in AChE enzyme activity sequel to the administration of LPS and KET in mice (Figure 4.50).

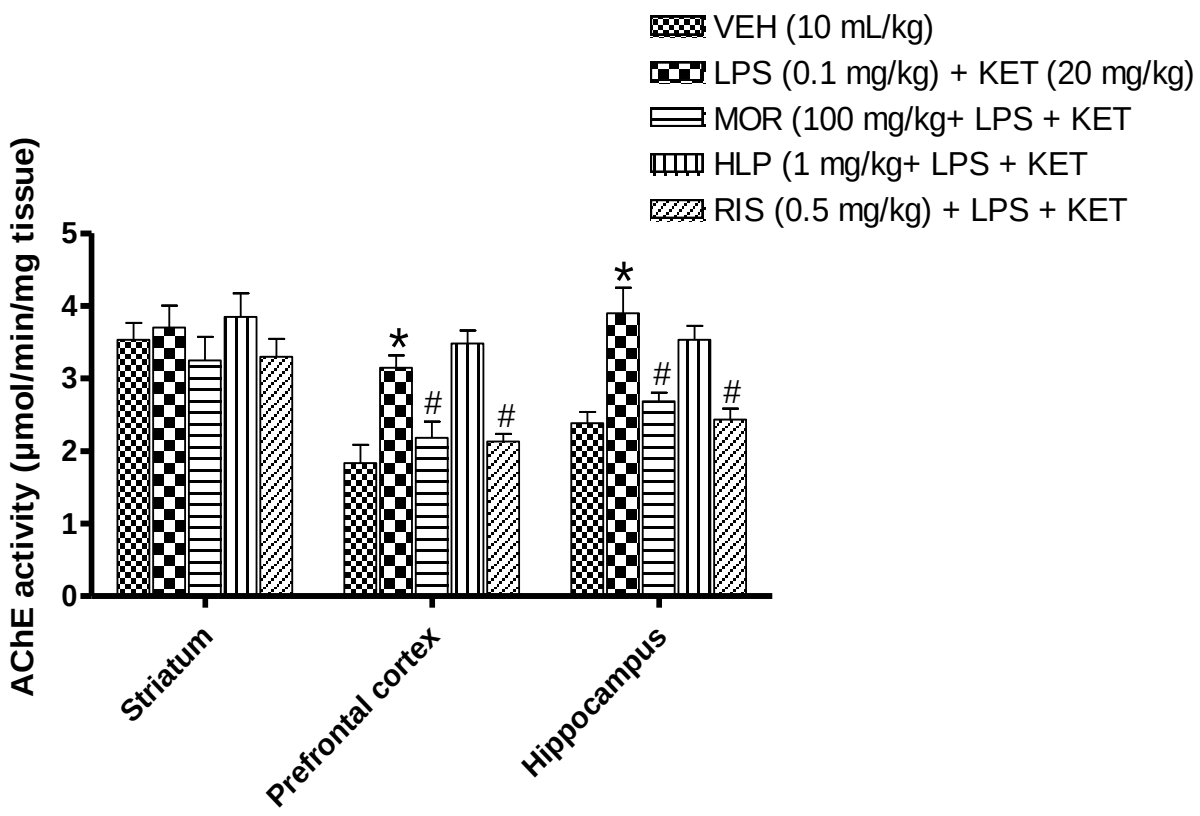


Figure 4.50: Effect of morin on AChE activities in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.60 Effect of morin on glutathione concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on GSH concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.51. Intraperitoneal injection of LPS (0.1 mg/kg) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day once daily, significantly ($p < 0.05$) decreased GSH concentration in the striatum, prefrontal cortex and hippocampus relative to vehicle control group. MOR (100 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) reversed LPS plus KET-induced decreases in GSH concentrations in the striatum, prefrontal cortex and hippocampus when compared with LPS plus KET-treated mice (Figure 4.51). Also, RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented LPS (0.1 mg/kg, i.p.) and KET (20 mg/kg, i.p.) decreases in GSH concentrations in the three brain regions of mice brain in comparison with LPS plus KET treatment alone. HLP (1 mg/kg, i.p.) once daily for 14 days failed to prevent the effect of LPS and KET on GSH concentrations in the striatum, prefrontal cortex and hippocampus when compared with LPS and KET-treated mice alone (Figure 4.51).

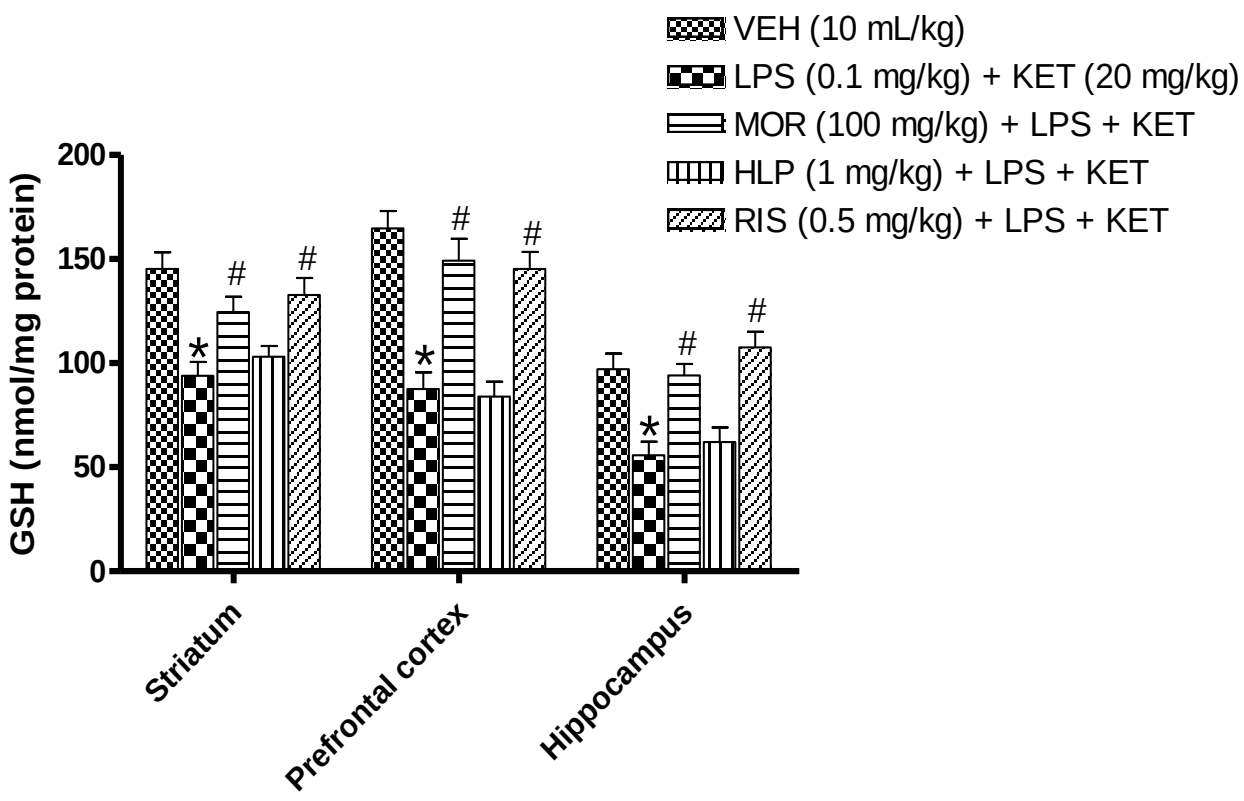


Figure 4.51: Effect of morin on GSH concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.61 Effect of morin on superoxide dismutase activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on LPS and KET-induced alterations on SOD activity in the striatum, prefrontal cortex and hippocampus of mice brains is shown in Figure 4.52. Administration of LPS (0.1 mg/kg, i.p.) for 14 days along with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day once daily caused a significant ($p < 0.05$) decreases in SOD activity in the striatum and prefrontal cortex when compared with vehicle-treated mice, suggesting cortical and sub-cortical oxidative stress. Meanwhile, no significant change in hippocampal SOD activity was observed when compared with LPS plus KET treatment alone (Figure 4.52). MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) increased SOD activity in the striatum and prefrontal cortex in LPS and KET-treated mice relative to LPS plus KET-treated group alone, suggesting increased cortical and sub-cortical antioxidant activity. However, HLP (1 mg/kg, i.p.) did not prevent the alterations in SOD activities in the striatum and prefrontal cortex by LPS and KET in mice (Figure 4.52).

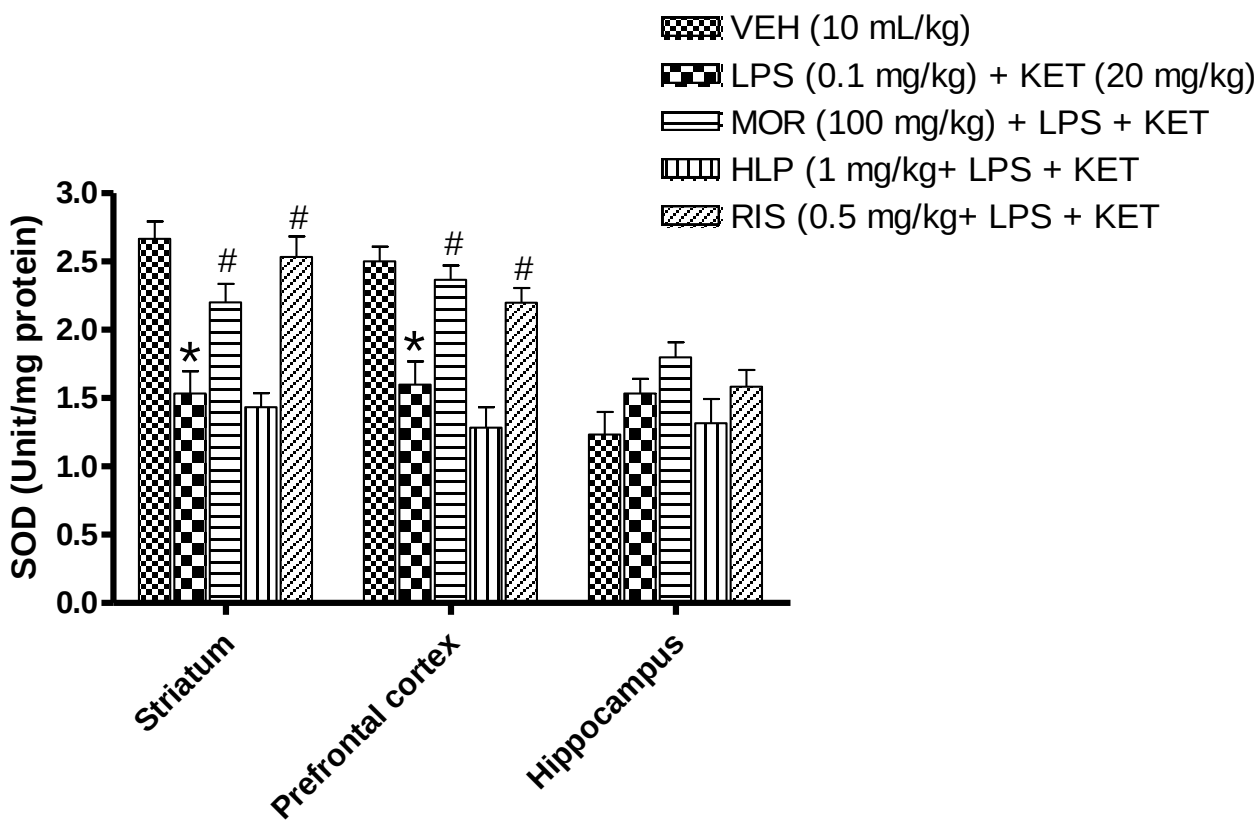


Figure 4.52: Effect of morin on SOD activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.62 Effect of morin on catalase activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on CAT activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.53. Chronic injection of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day once daily significantly ($p < 0.05$) decreased CAT activity in the striatum and prefrontal cortex, but not in the hippocampus relative to vehicle-treated mice. However, MOR (100 mg/kg, i.p.) once daily for 14 days significantly ($p < 0.05$) prevented LPS plus KET-induced decreases in CAT activity in the striatum and prefrontal cortex when compared with LPS plus KET-treated mice (Figure 4.53). RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented LPS plus KET-induced decreases in striatal and prefrontal cortical CAT activity in mice brain in comparison with LPS plus KET treatment alone. HLP (1 mg/kg, i.p.) once daily for 14 days, failed to prevent the effect of LPS plus KET on CAT activity in the striatum and prefrontal cortex when compared with LPS and KET-treated group (Figure 4.53).

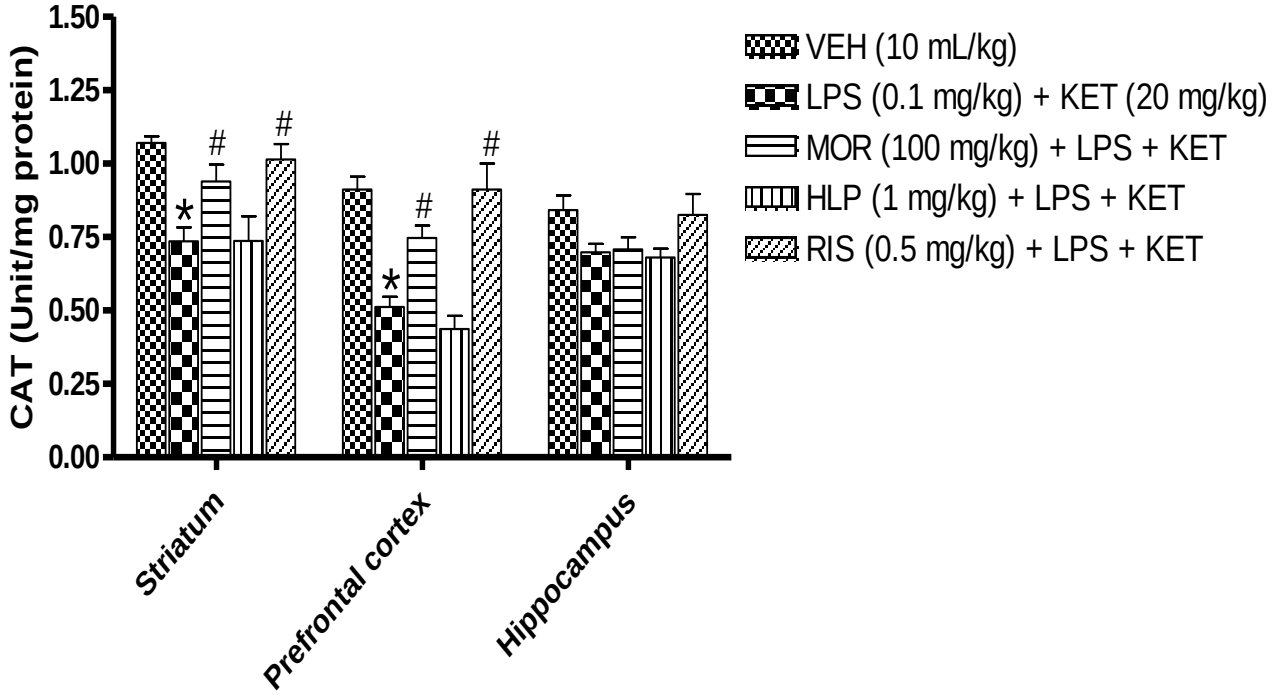


Figure 4.53: Effect of morin on CAT activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.63 Effect of morin on malondialdehyde concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on MDA concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.54. Repeated injection of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8th to the 14th day once daily significantly ($p < 0.05$) increased MDA concentration in the striatum, prefrontal cortex and hippocampus in comparison with vehicle control group, suggesting increase in lipid peroxidation. However, treatment with MOR (100 mg/kg, i.p.) given once daily for 14 days significantly ($p < 0.05$) reduced LPS plus KET-induced increases in MDA concentrations in the striatum, prefrontal cortex and hippocampus when compared with LPS plus KET-treated mice (Figure 4.54). RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented LPS plus KET-induced increases in MDA concentrations in the striatum, prefrontal cortex and hippocampus of mice brain in comparison with LPS plus KET treatment. HLP (1 mg/kg, i.p.) once daily for 14 days, failed to inhibit the effect of LPS and KET on lipid peroxidations in the striatum, prefrontal cortex and hippocampus when compared with LPS and KET-treated mice alone (Figure 4.54).

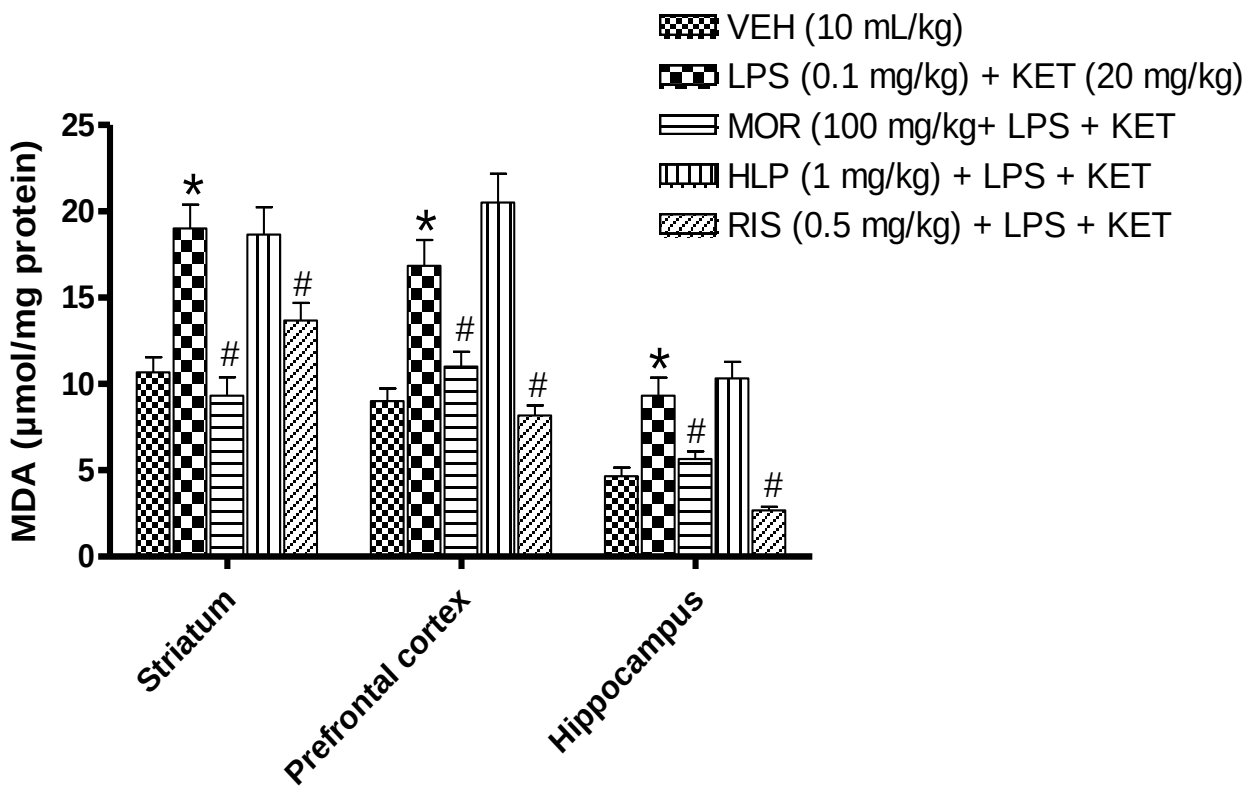


Figure 4.54: Effect of morin on MDA concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.64 Effect of morin on nitrite concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on nitrite concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.55. Chronic injection of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day of treatment, significantly ($p < 0.05$) increased nitrite level in the striatum, however caused a profound depletion of nitrite concentrations in the prefrontal cortex and hippocampus when compared with vehicle-treated mice. MOR (100 mg/kg, i.p.) given once daily for 14 days significantly ($p < 0.05$) reduced brain nitrite concentration in the striatum relative to LPS plus KET-treated animals (Figure 4.55). Also, MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated the effect of LPS plus KET on nitrite levels in the prefrontal cortex and hippocampus in comparison with LPS plus KET-treated mice. HLP (1 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated nitrite concentrations in the striatum when compared with LPS plus KET treatment alone, but did not cause any significant changes in nitrite concentrations in the prefrontal cortex (Figure 4.55). RIS (0.5 mg/kg, i.p.) increased brain nitrite levels in the hippocampus in a significant ($p < 0.05$) manner, HLP (1 mg/kg, i.p.) did not show any significant increase in hippocampal nitrite level when compared with LPS and KET-treated group (Figure 4.55).

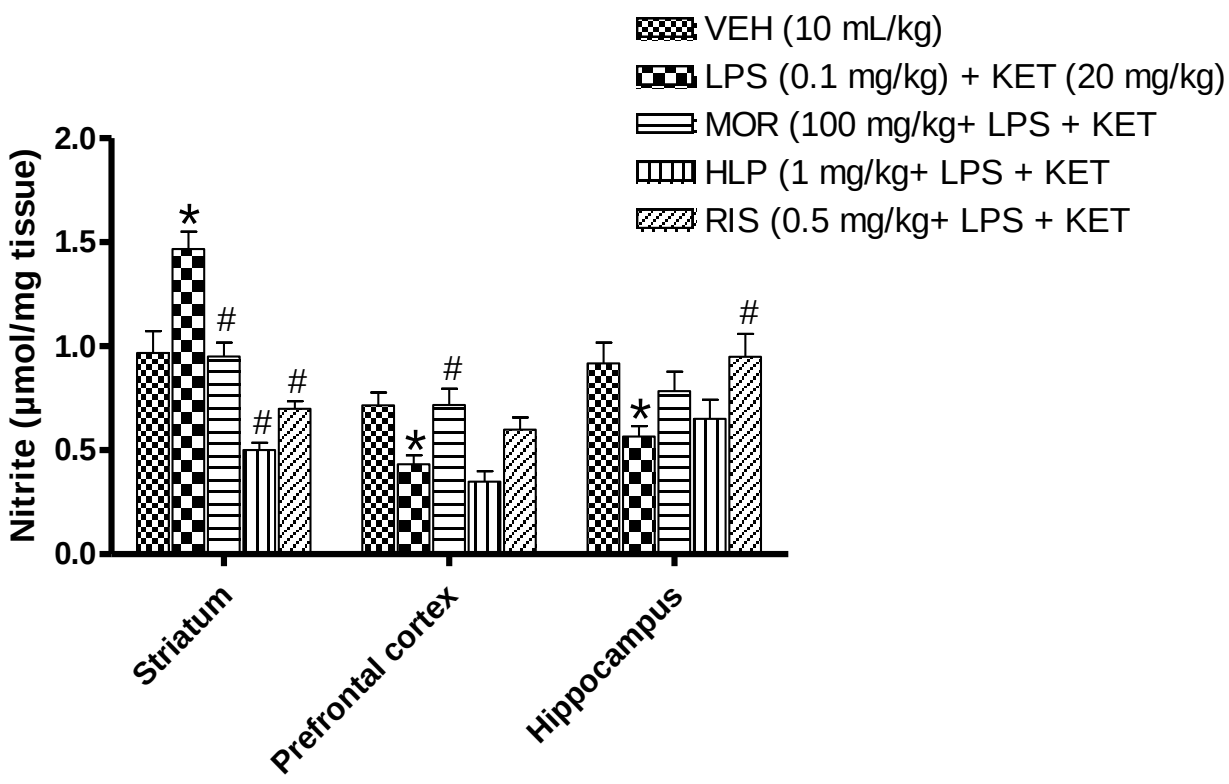


Figure 4.55: Effect of morin on nitrite concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.65 Effect of morin on myeloperoxidase activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on MPO activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.56. Chronic administration of LPS (0.1 mg/kg, i.p.) once daily for 14 days in combination with KET (20 mg/kg, i.p.) treatment from the 8^{th} to the 14^{th} day, significantly ($p < 0.05$) increased brain MPO activity in the striatum, prefrontal cortex and hippocampus in comparison with vehicle-treated mice (Figure 4.56). Treatments with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated LPS plus KET-induced increases in MPO activity in the striatum and prefrontal cortex in comparison with LPS plus KET-treated mice. However, RIS did not show any significant effect on hippocampal MPO activity, while MOR significantly ($p < 0.05$) reduced MPO activity in the hippocampus when compared with LPS plus KET-treated group. HLP (1 mg/kg, i.p.) failed to prevent the effect of LPS plus KET on MPO activity in the three sub-regions of the mice brains when compared with LPS plus KET in mice (Figure 4.56).

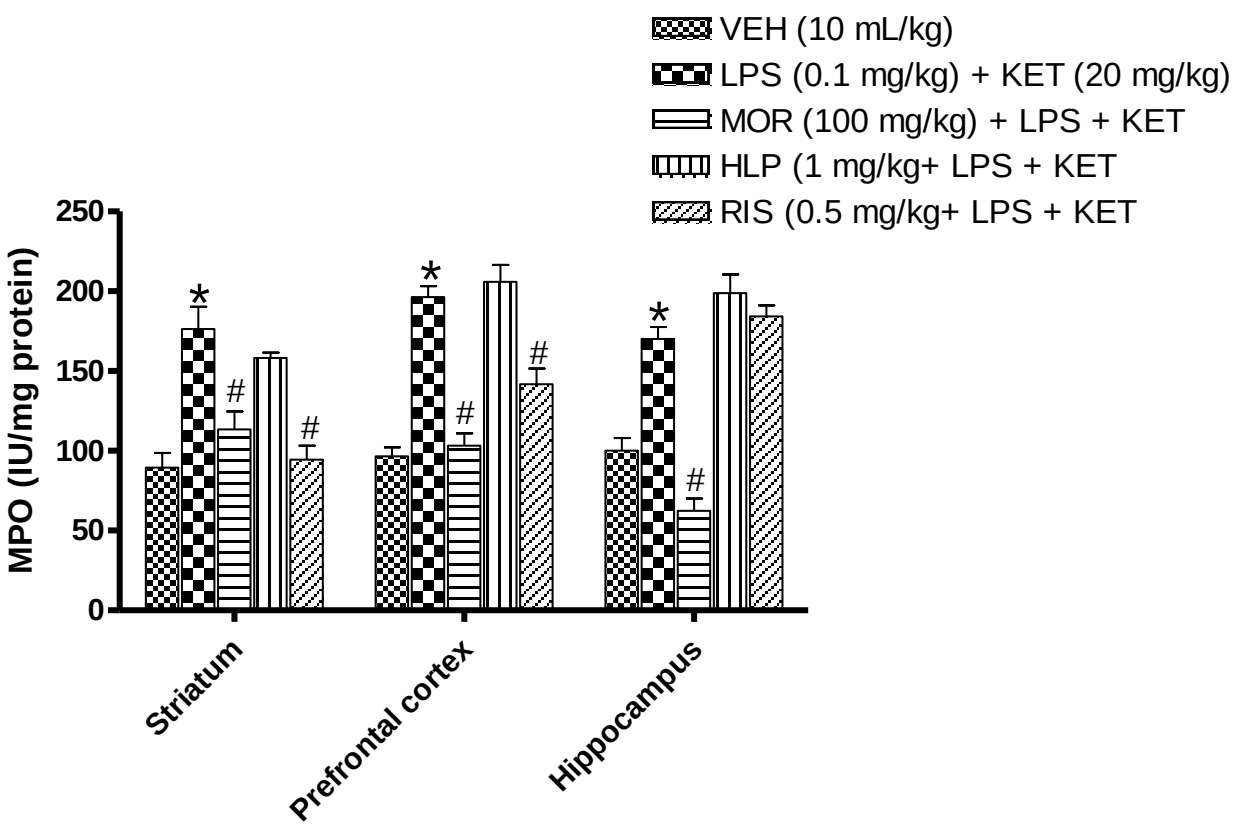


Figure 4.56: Effect of morin on MPO activity in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.66 Effect of morin on TNF-α concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on TNF-α concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS and KET is shown in Figure 4.57. Repeated exposure of LPS (0.1 mg/kg, i.p.) for 14 days together with KET (20 mg/kg, i.p.) injection from the 8^{th} to the 14^{th} day of treatment significantly ($p < 0.05$) increased the levels of TNF-α in the striatum and prefrontal cortex when compared with vehicle control group; although did not cause any significant change in the hippocampus (Figure 4.57). MOR (100 mg/kg, i.p.) given once daily for 14 days significantly ($p < 0.05$) reduced brain concentrations of TNF-α in the striatum and prefrontal cortex relative to LPS and KET-treated animals, suggesting anti-neuroinflammatory activity. No significant change in the hippocampal TNF-α level was observed when compared with vehicle- and/or LPS and KET-treated mice. RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated LPS plus KET-induced increases in TNF-α concentrations in the striatum and prefrontal cortex in comparison with LPS and KET-treated animals. However, HLP (1 mg/kg, i.p.) did not show any anti-neuroinflammatory protection, as it failed to decrease TNF-α levels in the striatum and prefrontal cortex when compared with LPS plus KET-treated mice (Figure 4.57).

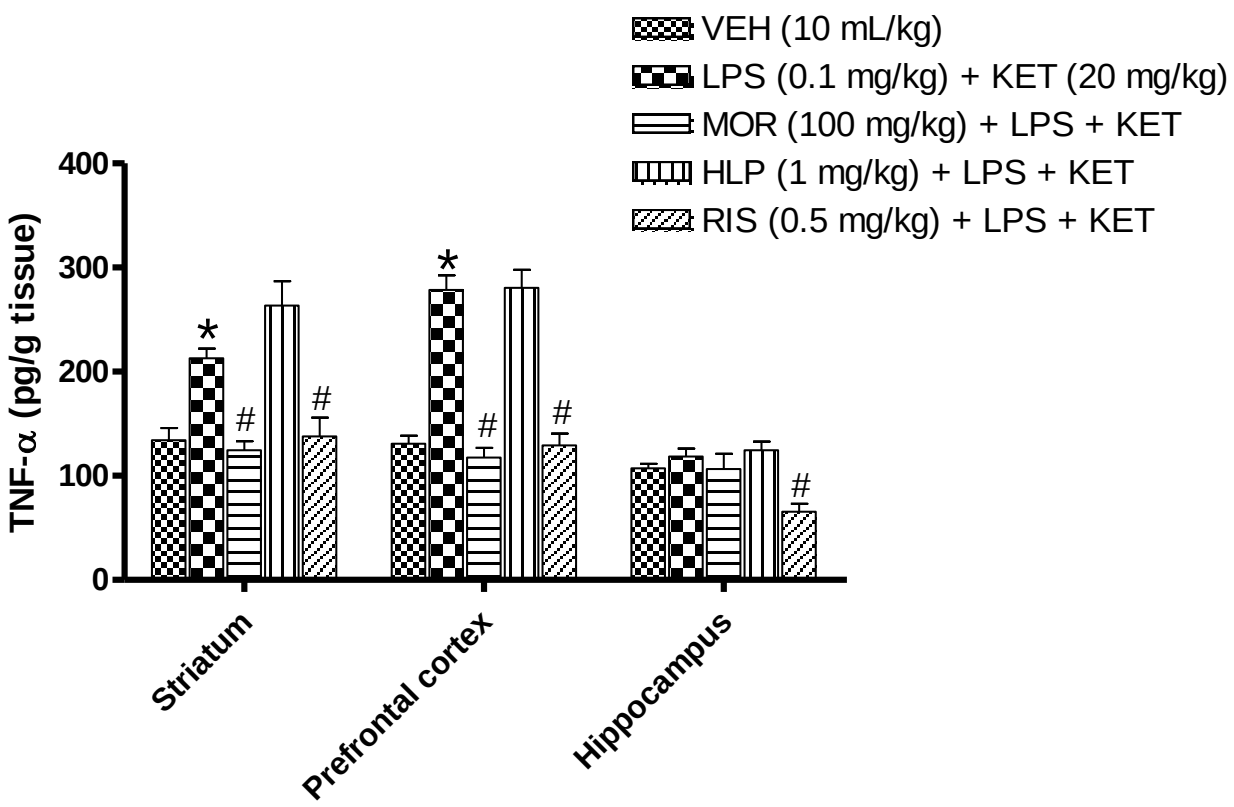


Figure 4.57: Effect of morin on TNF-α concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.67 Effect of morin on IL-6 concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The effect of MOR on LPS and KET-induced changes on interleukin-6 (IL-6) concentrations in the striatum, prefrontal cortex and hippocampus of mice brains is shown in Figure 4.58. Although chronic administration of LPS (0.1 mg/kg, i.p.) for 14 days together with KET (20 mg/kg, i.p.) injection from the 8^{th} to the 14^{th} day of treatment did not cause any change in IL-6 concentration in the striatum, LPS plus KET treatments significantly ($p < 0.05$) increased in IL-6 levels in the prefrontal cortex and hippocampus when compared with vehicle-treated control group, suggesting neuroimmune activation (neuroinflammation) (Figure 4.58). Treatments with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.), given once daily for 14 days significantly ($p < 0.05$) reduced the effect of LPS plus KET on IL-6 concentrations in the prefrontal cortex and hippocampus when compared with LPS and KET-treated mice, which further suggests anti-neuroinflammatory property. However, HLP (1 mg/kg, i.p.) failed to reverse LPS plus KET-induced increases in prefrontal cortical and hippocampal IL-6 concentrations in comparison to LPS and KET-treated mice (Figure 4.58).

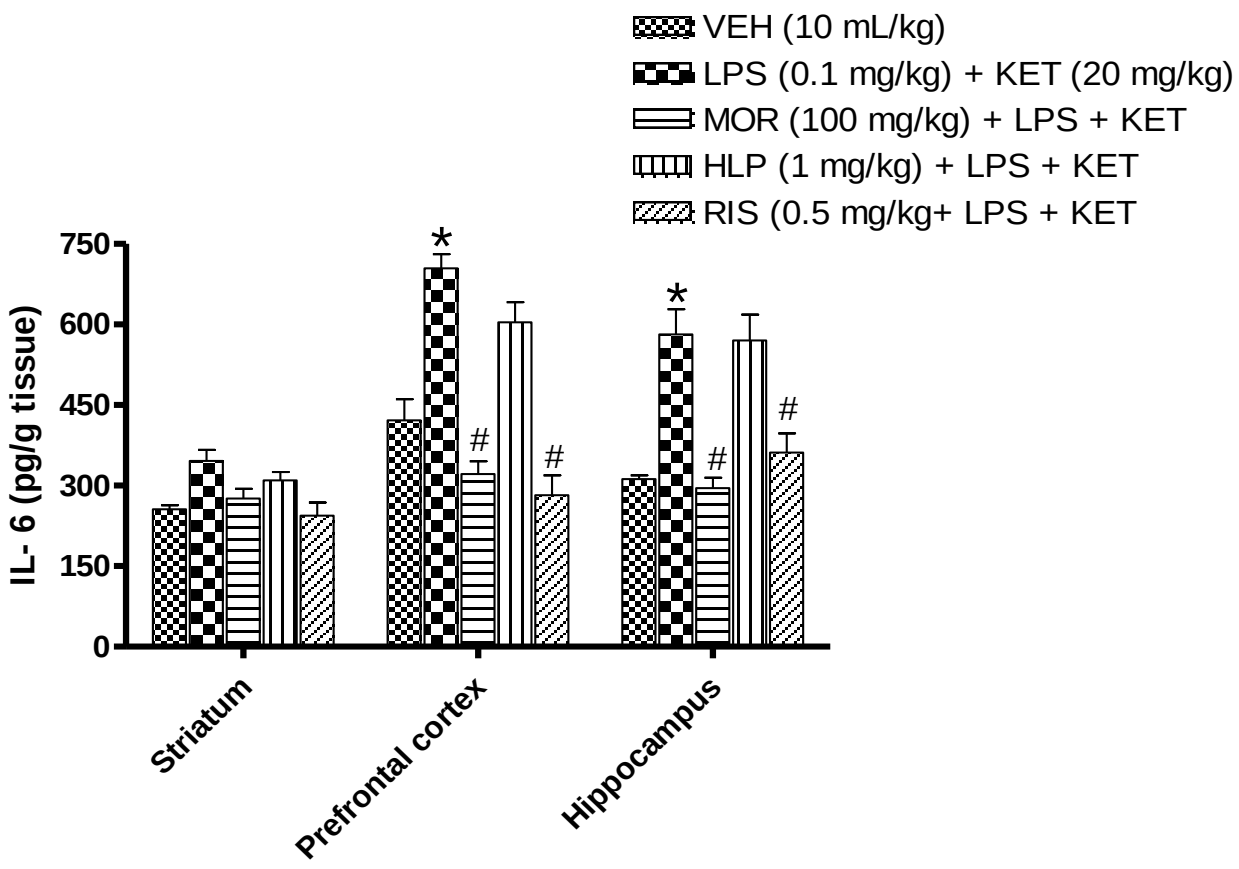


Figure 4.58: Effect of morin on IL-6 concentrations in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.68 Neuroprotective effect of morin in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of morin on LPS plus KET-induced histological and histomorphological (density of viable cells) changes in the striatum, prefrontal cortex and hippocampus of mice brains are shown in Plates 4.19, 4.20, 4.21 and Figure 4.59. The H&E staining showed that intraperitoneal injection of LPS (0.1 mg/kg, i.p.) for 14 days together with KET (20 mg/kg, i.p.) from the 8^{th} to the 14^{th} day of treatment produced a significant cytoarchitectural changes in the striatum (Plate 4.19) and prefrontal cortex (Plate 4.20) but not in the CA1 region of the hippocampal (Plate 4.21); as revealed by increased population of highly condensed (pyknotic) neuronal cells and decreased viable neuronal cells (optical densitometry count) in the striatum and prefrontal cortex (Figure 4.59) relative to vehicle-treated mice brain. Pretreatments with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly reduced these histomorphological alterations induced by LPS plus KET in the striatum (Plate 4.19) and prefrontal cortex (Plate 4.20). MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) also significantly ($p < 0.05$) decreased the loss of viable neuronal cells of the striatum and prefrontal cortical regions when compared with LPS plus KET-treated mice brains (Figure 4.59). HLP (1 mg/kg, i.p.) did not prevent LPS plus KET-induced neuropathological changes and loss of viable neuronal cells in the striatum (Plate 4.19 and Figure 4.59) and prefrontal cortex (Plate 4.20 and 4.59) when compared with LPS plus KET-treatment alone.

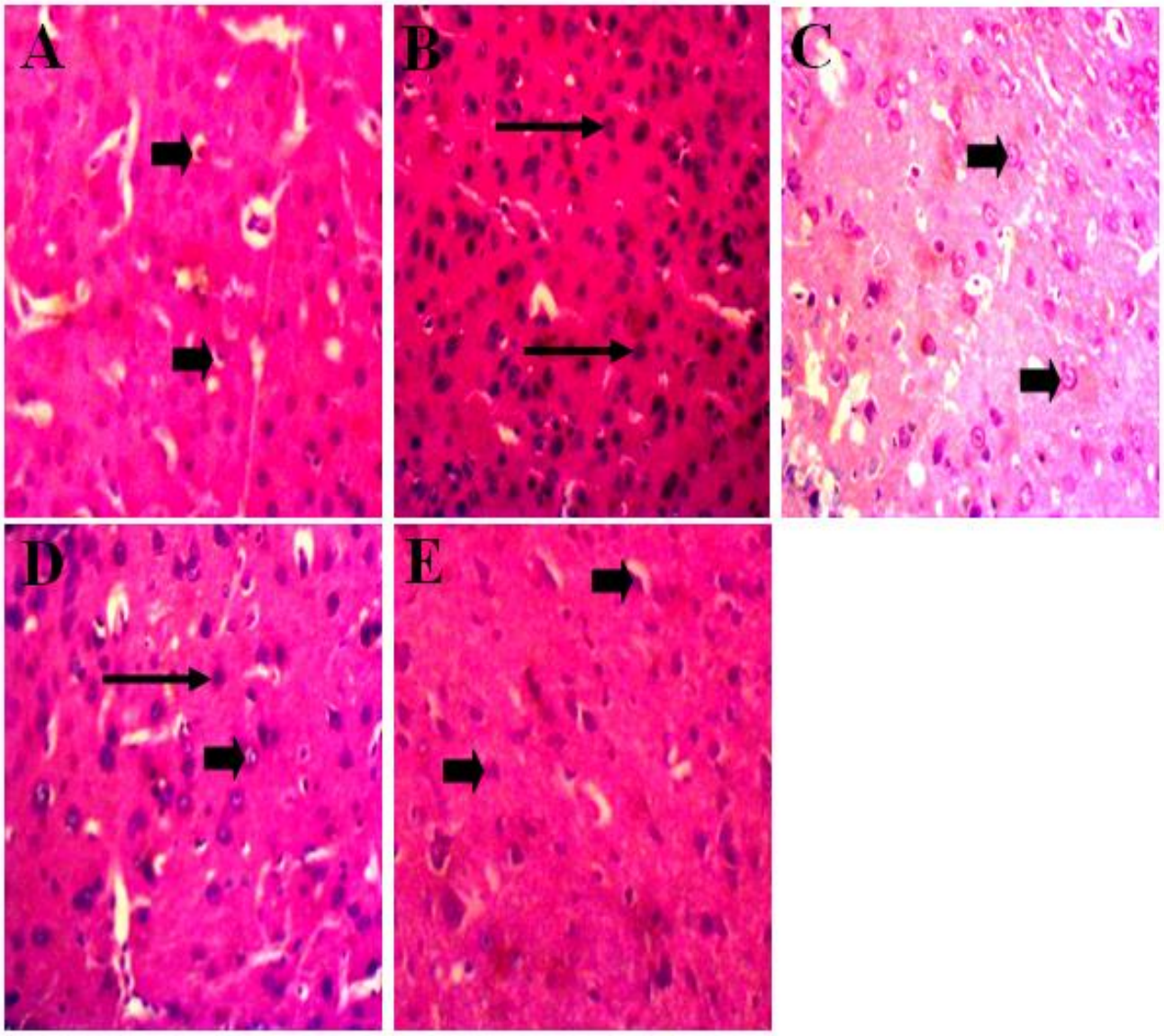


Plate 4.19: Representative stained sections of the effects of morin on the *striatum* of mice brains treated with lipopolysaccharide and ketamine. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET. Slide A shows normal multiple neuronal cells with hyperchromatic nuclei. Slide B revealed neurons with highly condensed, pyknotic and angulated cells. Slide C shows increased open chromatin nuclei and normal cells. Slide D reveals few normal neurons with angulated cells. Slide E showed a few ghost neuronal cells and a few normal cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

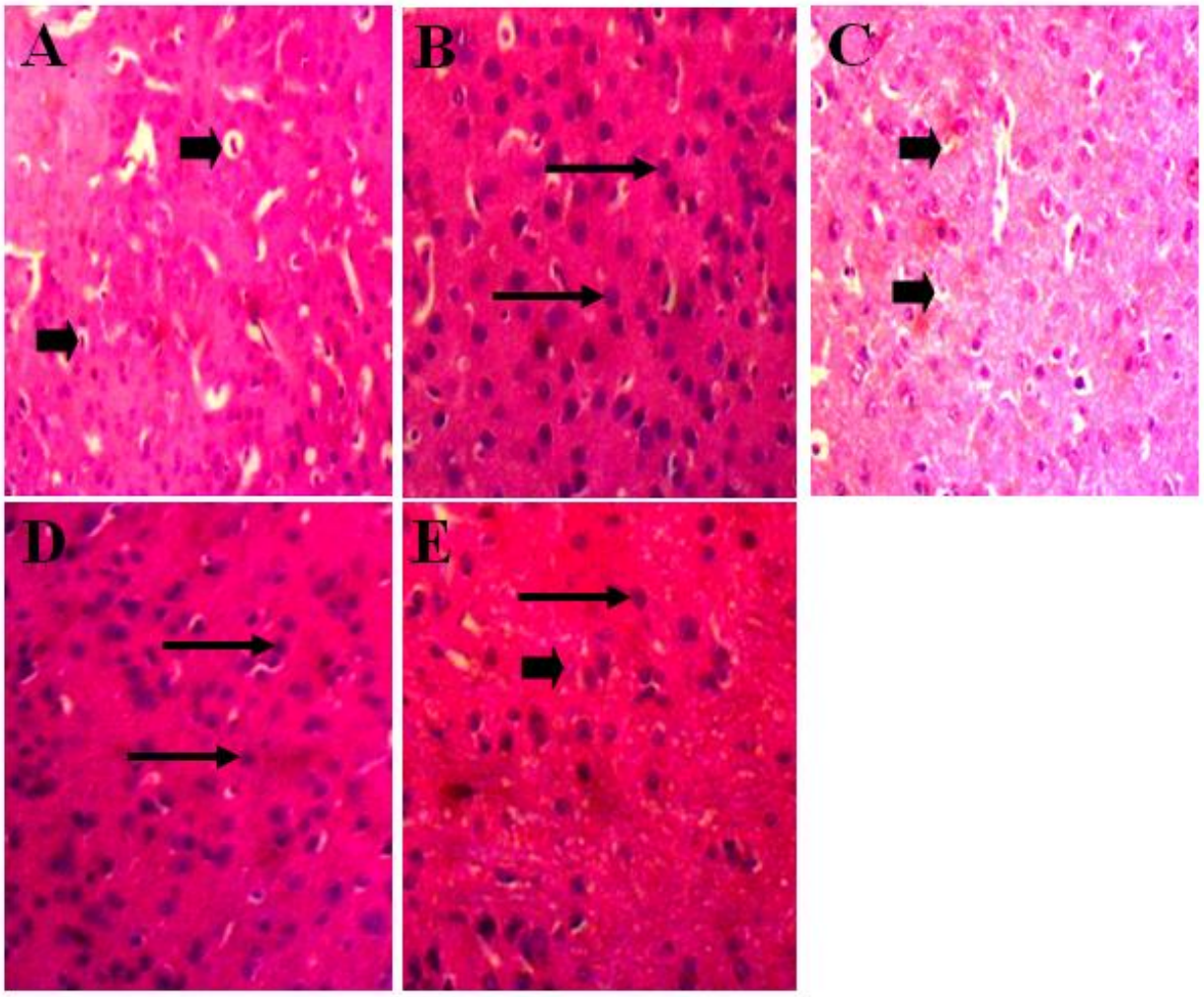


Plate 4.20: Representative stained sections of the effects of morin on the *prefrontal cortex* of mice brains treated with lipopolysaccharide and ketamine. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET. Slide A shows normal multiple neuronal cells with open chromatin nuclei. Slide B revealed neurons with highly condensed, pyknotic and angulated cells. Slide C shows increased population of hyperchromatin nuclei and normal cells. Slide D reveals neurons with condensed and angulated cells. Slide E showed a few ghost neuronal cells and a few normal cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

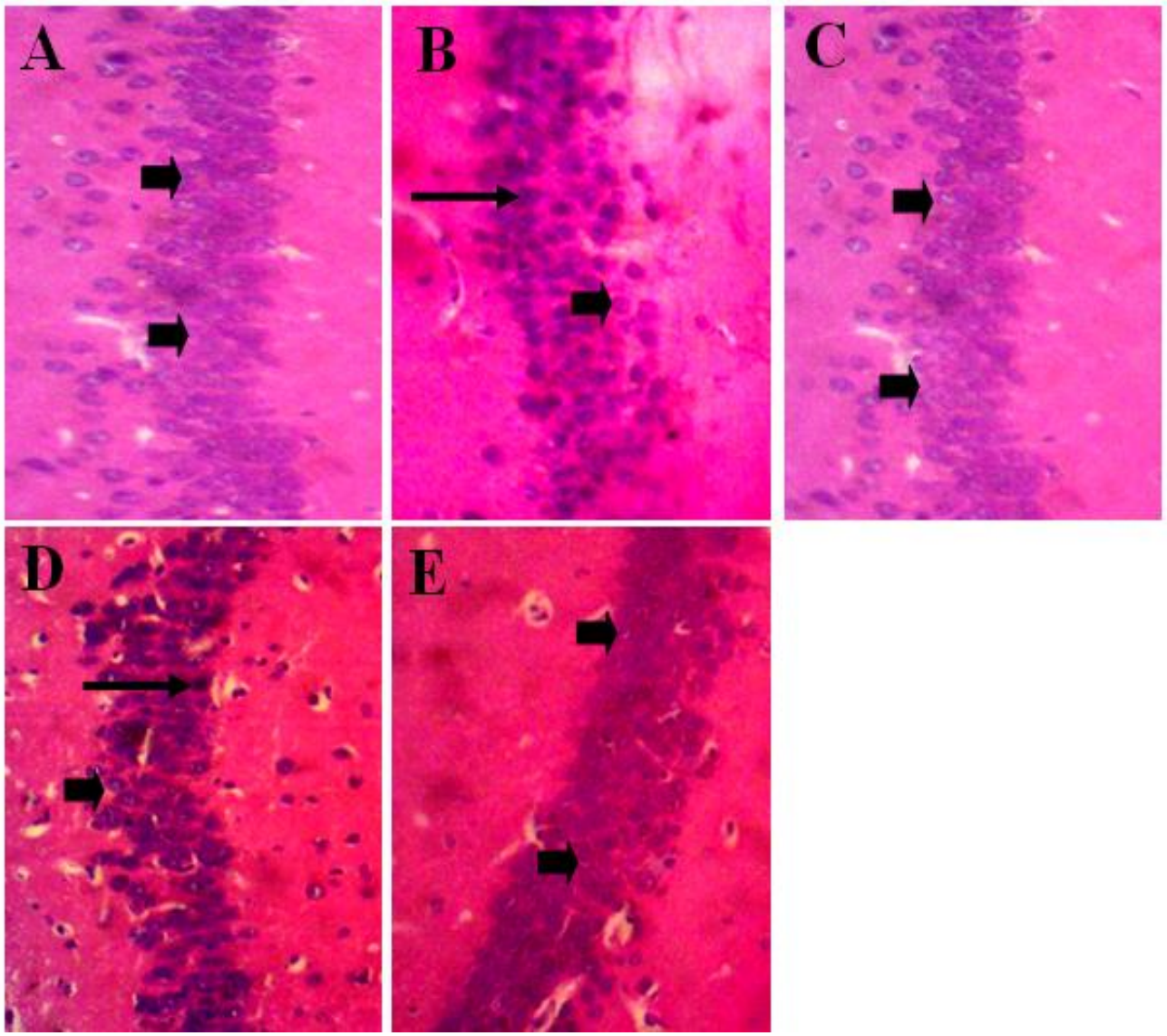


Plate 4.21: Representative stained sections of the effects of morin on the *hippocampus* (CA1) of mice brains treated with lipopolysaccharide and ketamine. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET. Slide A shows normal multiple neuronal cells with open chromatin nuclei with normal cytoarchiture. Slide B revealed neurons with few condensed and pyknoti cells. Slide C shows increased population of open chromatin nuclei and normal cells. Slide D reveals neurons with dark chromatin nuclei with relative few normal cells. Slide E showed a few ghost neuronal cells and a few normal cells. Arrow head – normal neuron; arrow - dark neurons. Haematoxylin-eosin stain: x400. Calibration bar = 0.01 mm (10 µm) for all figures.

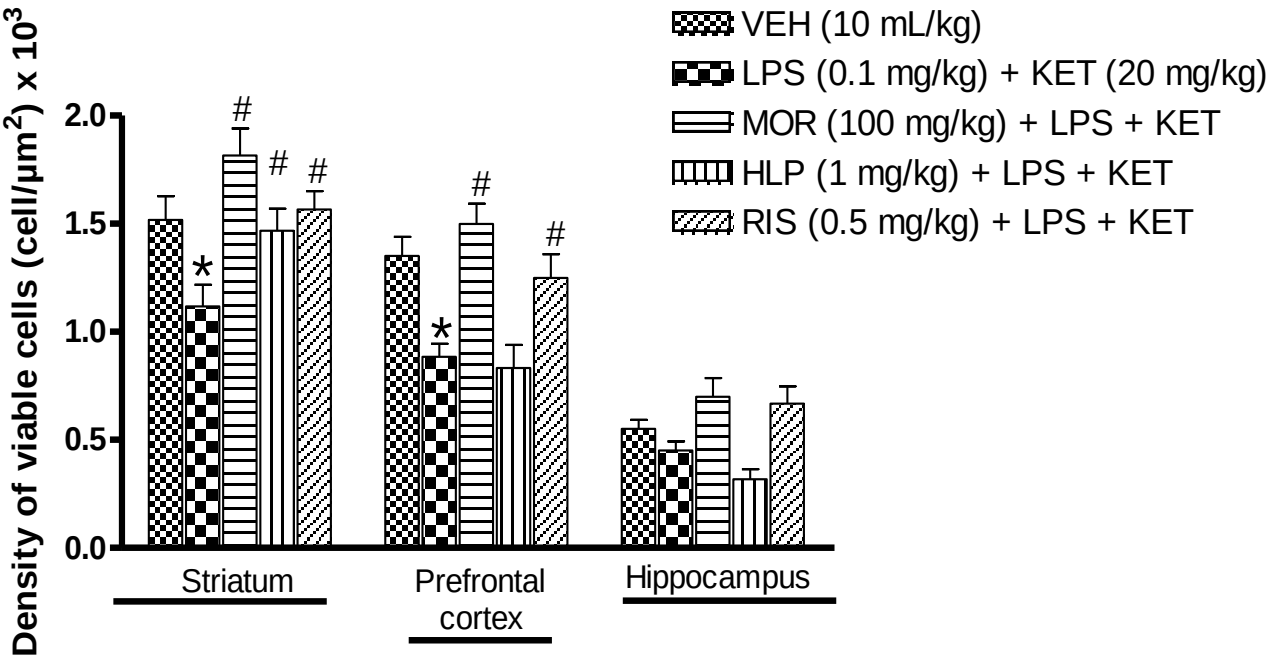


Figure 4.59: Effect of morin on the density of viable neuronal cells of the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 3 animals / group. *p < 0.05 compared to vehicle group and #P < 0.05 compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.69 Effect of morin on COX-2 expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS plus KET-induced immunohistochemical changes and expressions of COX-2 (Cyclooxygenase-2) immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains are shown in Plates 4.22, 4.23, 4.24 and Figure 4.60. Repeated injections of LPS (0.1 mg/kg, i.p.) for 14 days together with KET (20 mg/kg, i.p.) from the 8^{th} to the 14^{th} day of treatment caused a marked immunohistochemical changes in the striatum (Plate 4.22), prefrontal cortex (Plate 4.23) and CA1 region of the hippocampus (Plate 4.24) and a significant ($p < 0.05$) increase in the expressions of COX-2 immunopositive cells in all three brain regions based on percentage optical density (% O.D.) (Figure 4.60) when compared with vehicle-treated group, suggesting neuroinflammatory immunoreactivity. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) prevented immunohistochemical changes and decreased expressions of COX-2 immunopositive cells induced by LPS plus KET in the striatum (Plate 4.22 and Figure 4.60), prefrontal cortex (Plate 4.23 and Figure 4.60) and hippocampus (Plate 4.24 and Figure 4.60) relative to LPS plus KET-treated mice brains, which indicates anti-neuroinflammatory activity. RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) attenuated LPS plus KET-induced increased expressions of COX-2 immunopositive cells in the striatum, prefrontal cortex and hippocampus (CA1 region) when compared with LPS and KET-treated mice brains. However, HLP (1 mg/kg, i.p.) failed to prevent the effect of LPS plus KET on COX-2 expressions in the striatum (Plate 4.22 and Figure 4.60), prefrontal cortex (Plate 4.23 and Figure 4.60) and hippocampus (Plate 4.24 and Figure 4.60) in comparison with LPS and KET-treated mice brains.

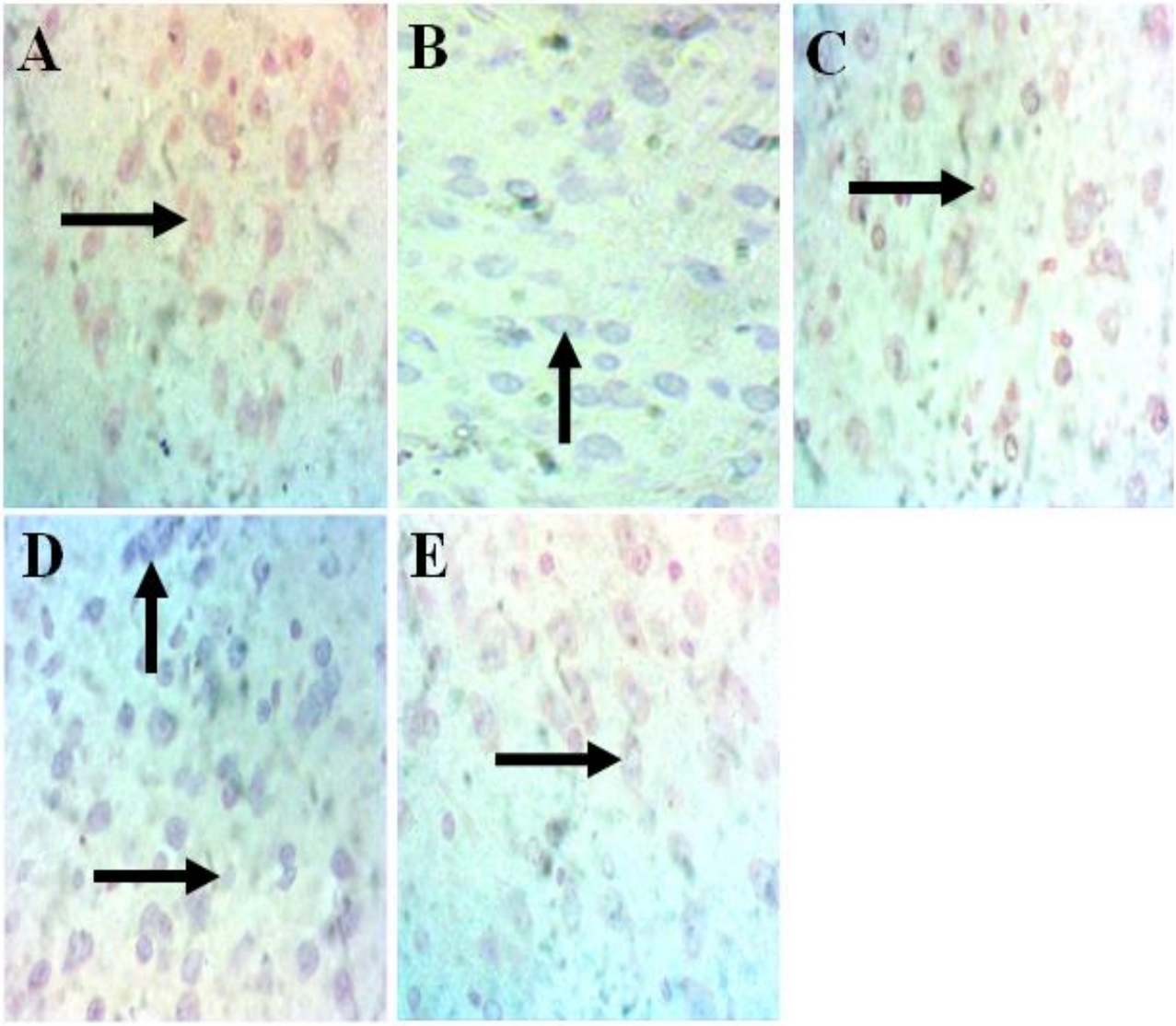


Plate 4.22: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of COX-2 immunopositive cells in the *striatum* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

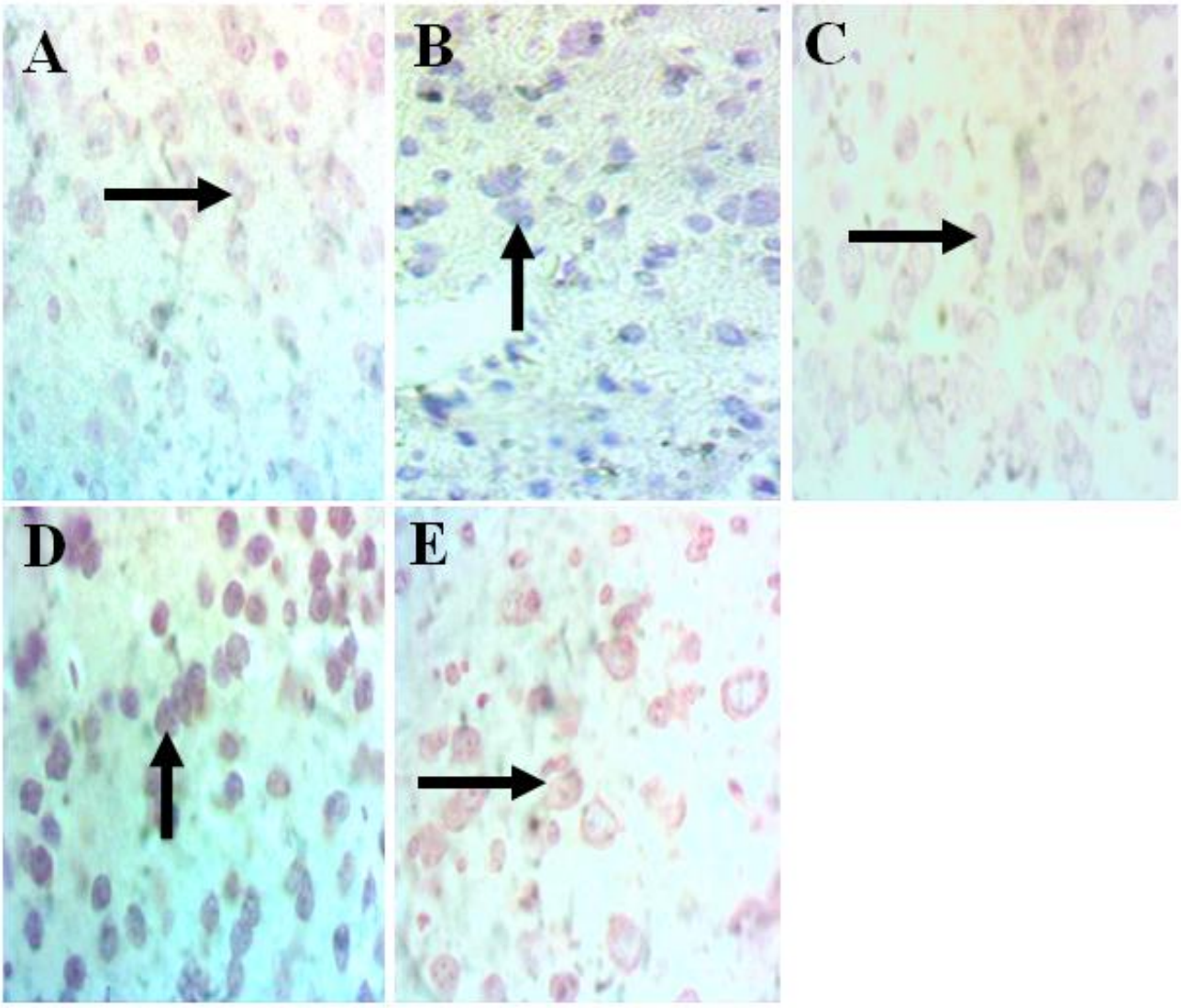


Plate 4.23: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of COX-2 immunopositive cells in the *prefrontal cortex* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

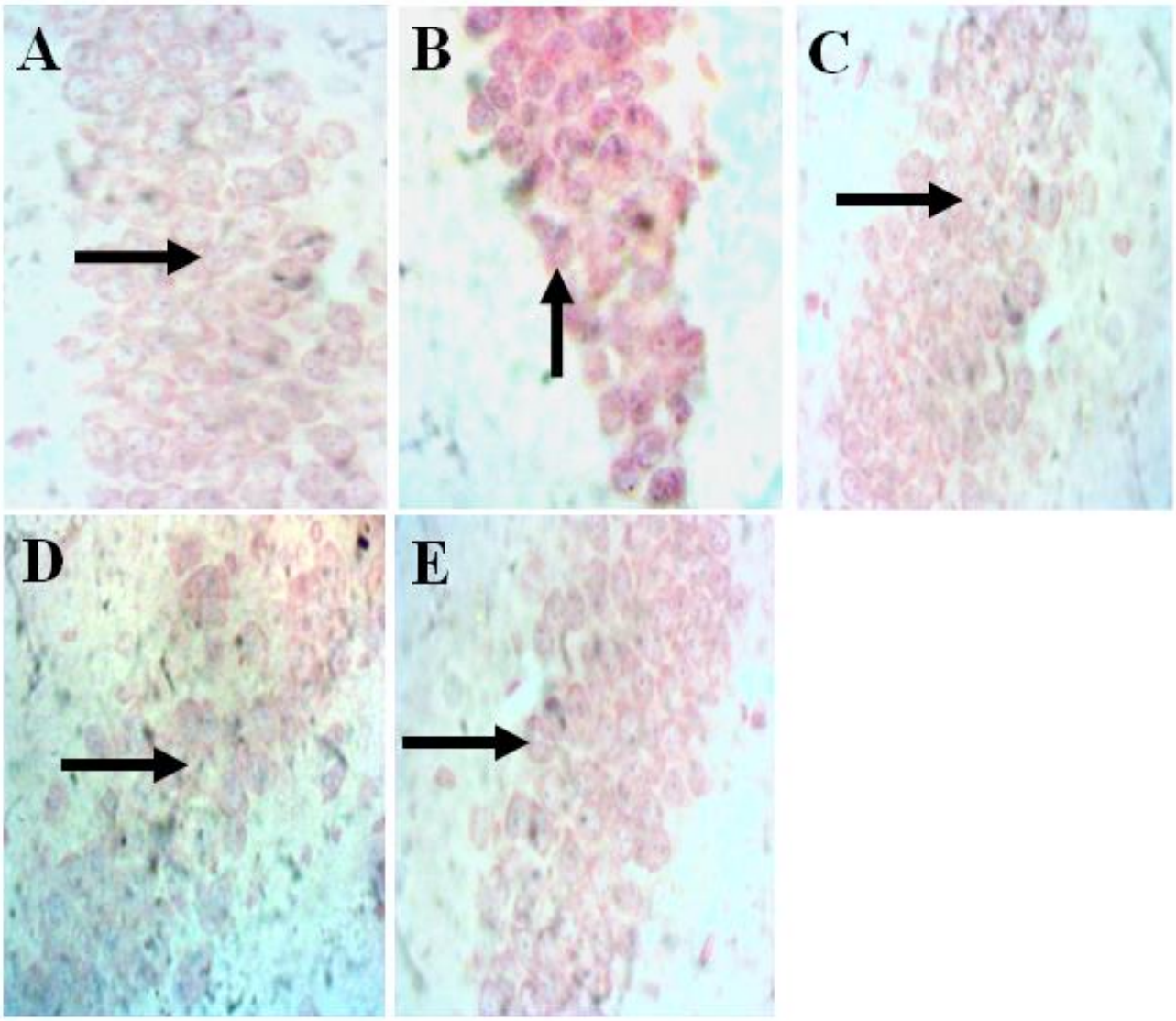


Plate 4.24: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of COX-2 immunopositive cells in the CA1 region of the *hippocampus* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

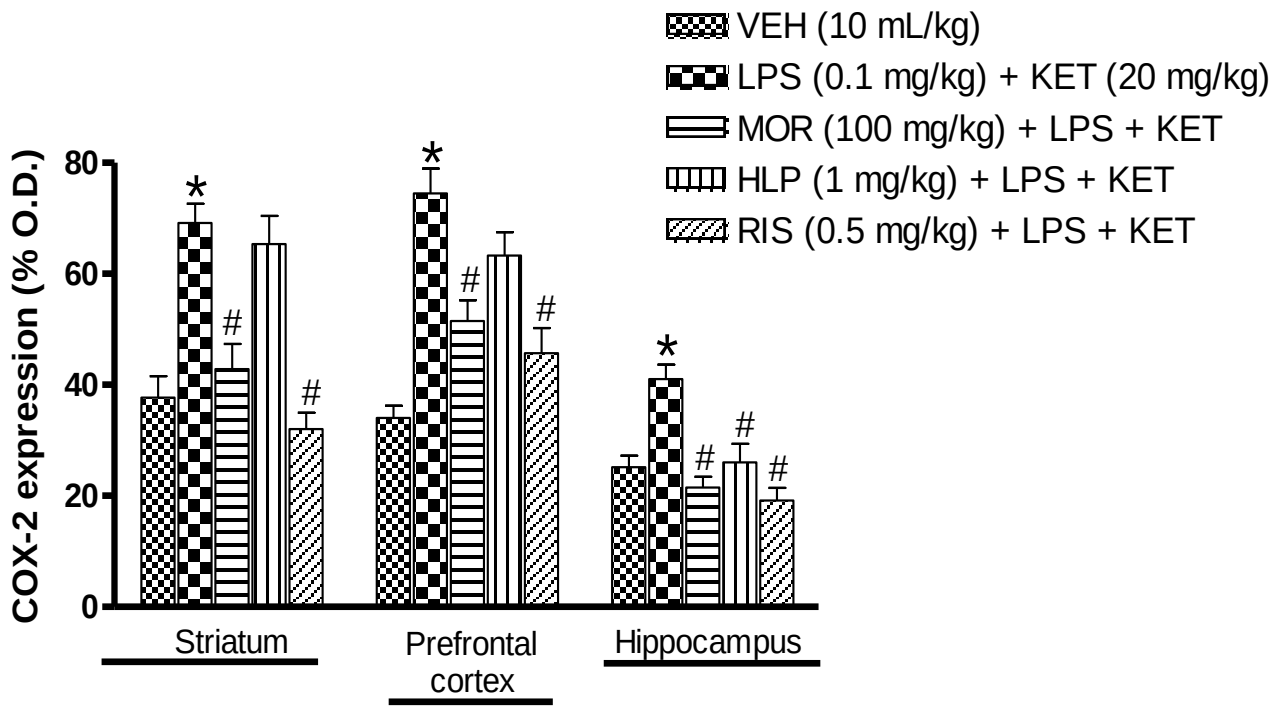


Figure 4.60: Effect of morin on COX-2 expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.70 Effect of morin on iNOS expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS plus KET-induced immunohistochemical changes and expressions of iNOS (Inducible nitric oxide synthase) immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains are shown in Plates 4.25, 4.26, 4.27 and Figure 4.61. Repeated injections of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) from the 8^{th} to the 14^{th} day of treatment produced a profound immunohistochemical changes and significant ($p < 0.05$) increase in the expressions of iNOS immunopositive cells in the striatum (Plate 4.25), prefrontal cortex (Plate 4.26) and CA1 region of the hippocampus (Plate 4.27) of immunopositive cells (Figure 4.61) when compared with vehicle-treated group. However, MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reduced immunohistochemical changes and decreased expressions of iNOS immunopositive cells induced by LPS plus KET in the striatum (Plate 4.25 and Figure 4.61), prefrontal cortex (Plate 4.26 and Figure 4.61) and CA1 region of the hippocampus (Plate 4.27 and Figure 4.61) in comparison with LPS plus KET-treated mice brains. HLP (1 mg/kg, i.p.) did not prevent the effect of LPS plus KET on iNOS expressions in the striatum, prefrontal cortex and hippocampus when compared with LPS plus KET-treated mice brains.

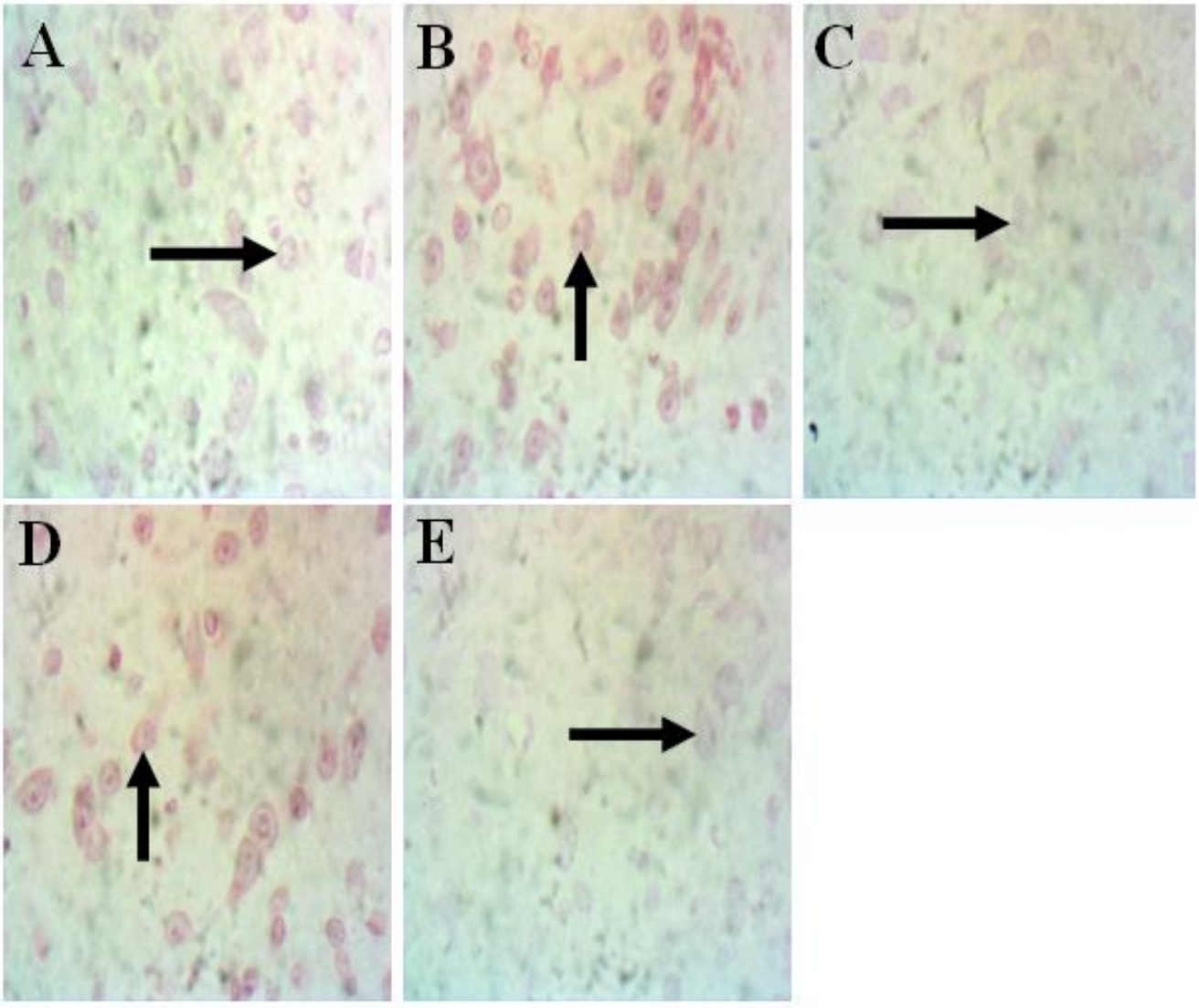


Plate 4.25: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of iNOS immunopositive cells in the *striatum* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

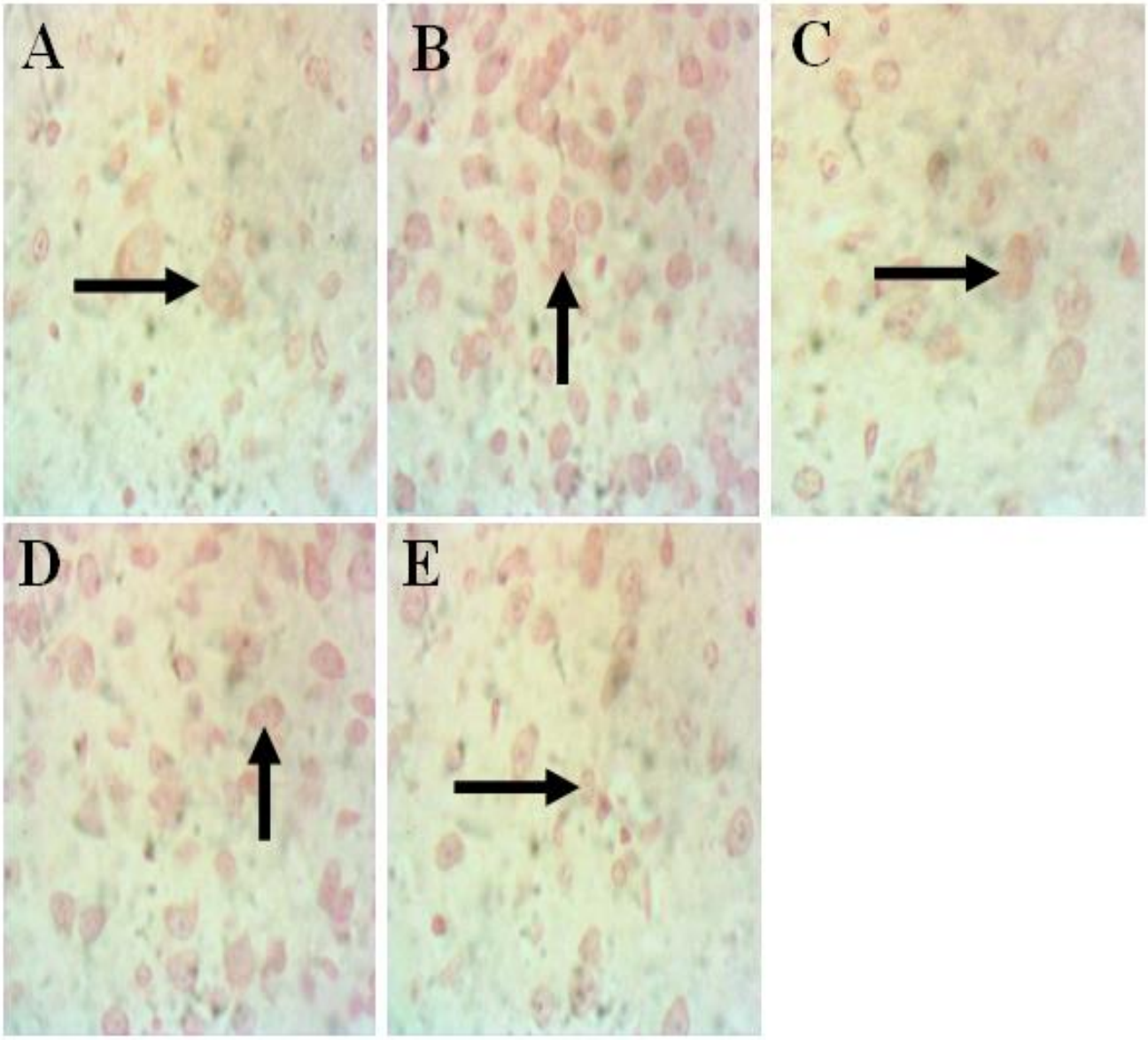


Plate 4.26: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of iNOS immunopositive cells in the *prefrontal cortex* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

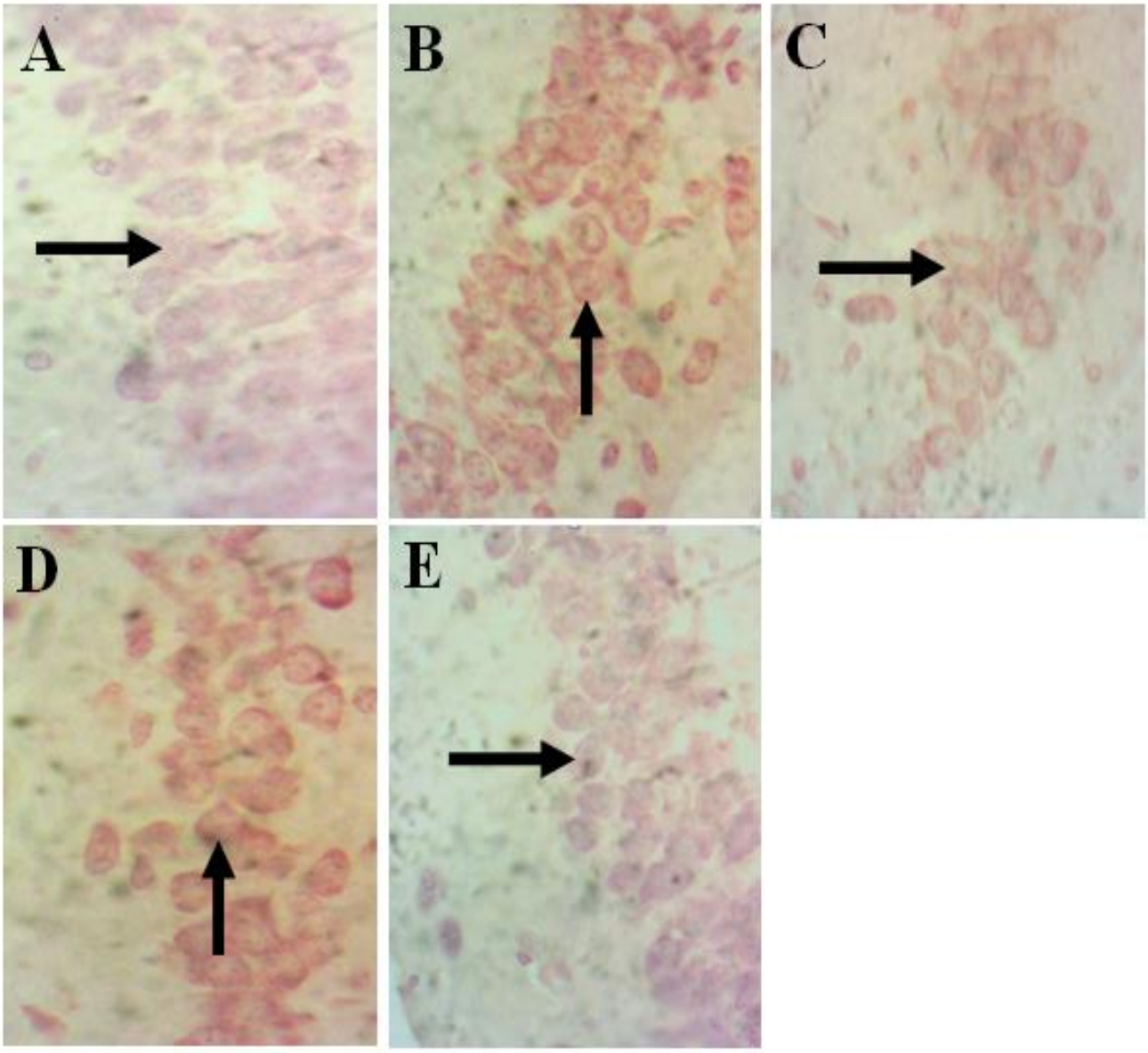


Plate 4.27: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of iNOS immunopositive cells in the CA1 region of the *hippocampus* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

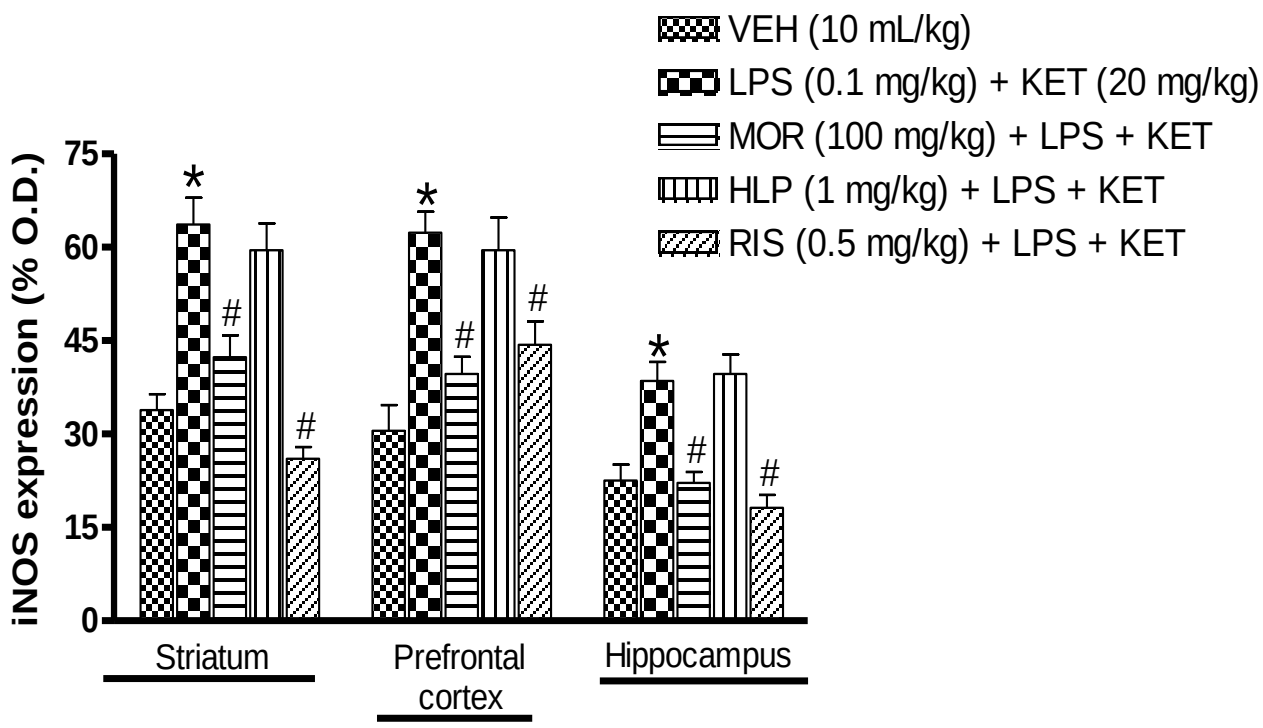


Figure 4.61: Effect of morin on iNOS expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.71 Effect of morin on NF-κB expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS plus KET-induced immunohistochemical changes and expressions of NF-κB (Nuclear factor kappa-B) immunopositive cells in the striatum, prefrontal cortex and hippocampus of mice brains are shown in Plates 4.28, 4.29, 4.30 and Figure 4.62. Chronic injections of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg) from the 8^{th} to the 14^{th} day of treatment significantly ($p < 0.05$) produced immunohistochemical changes and increased in the expressions of NF-κB immunopositivity in the striatum (Plate 4.28), prefrontal cortex (Plate 4.29) and CA1 region of the hippocampus (Plate 4.30), based on percentage optical density of immunopositive cells (Figure 4.62) when compared with vehicle-treated group, suggesting increased expressions of neuroinflammatory signaling. Pretreatment with MOR (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p < 0.05$) prevented immunohistochemical alteration, by decreasing expressions of NF-κB immunopositive cells induced by LPS plus KET treatments in the striatum (Plate 4.28 and Figure 4.62), prefrontal cortex (Plate 4.29 and Figure 4.62) and CA1 region of the hippocampus (Plate 4.30 and Figure 4.62) in comparison with LPS plus KET-treated mice brains, which indicates anti-neuroinflammatory property. HLP (1 mg/kg, i.p.) did not also prevent the effect of LPS plus KET on NF-κB expressions in the three brain regions examined relative to LPS plus KET-treated mice brains.

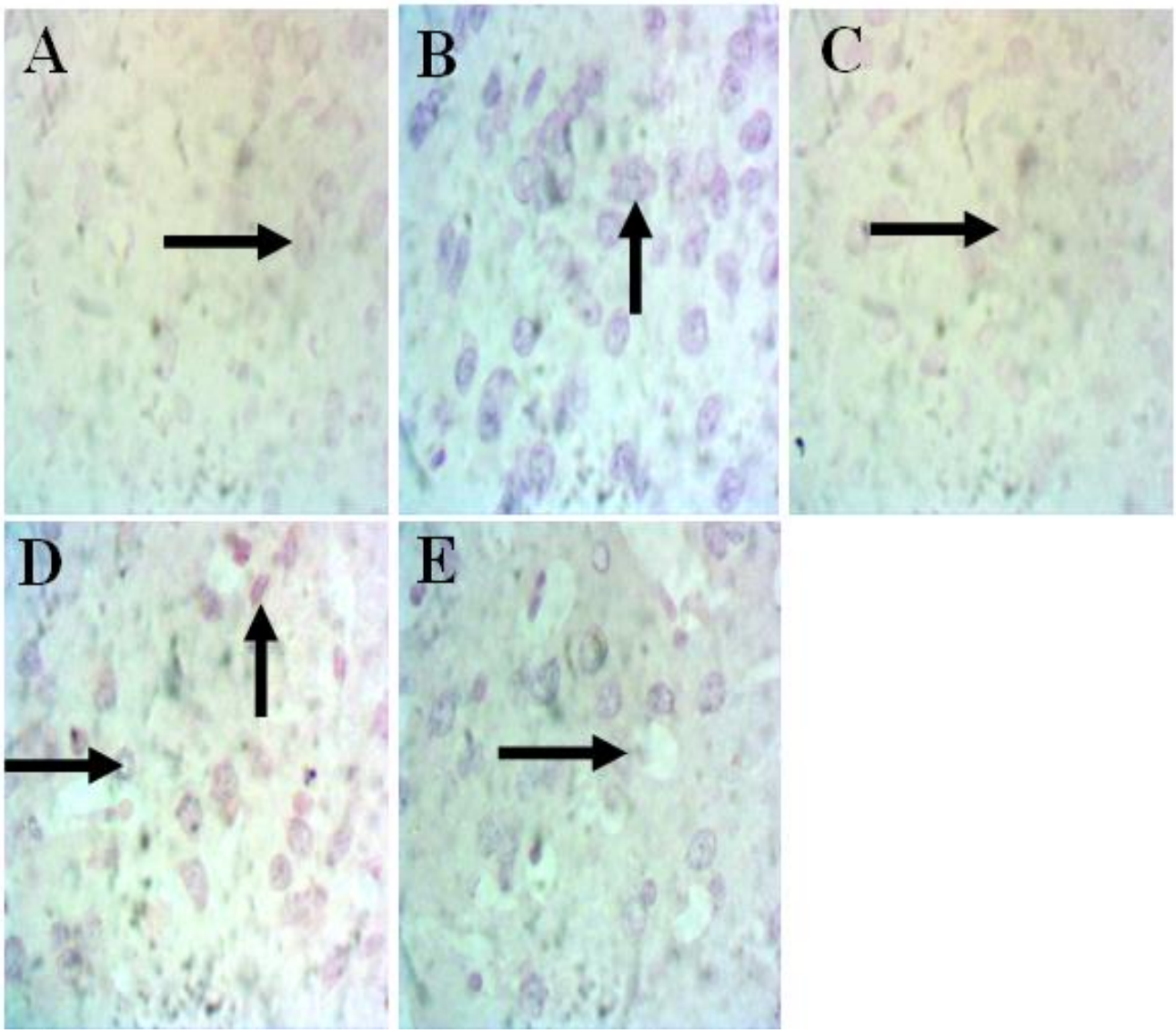


Plate 4.28: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of NF-κB immunopositive cells in the *striatum* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

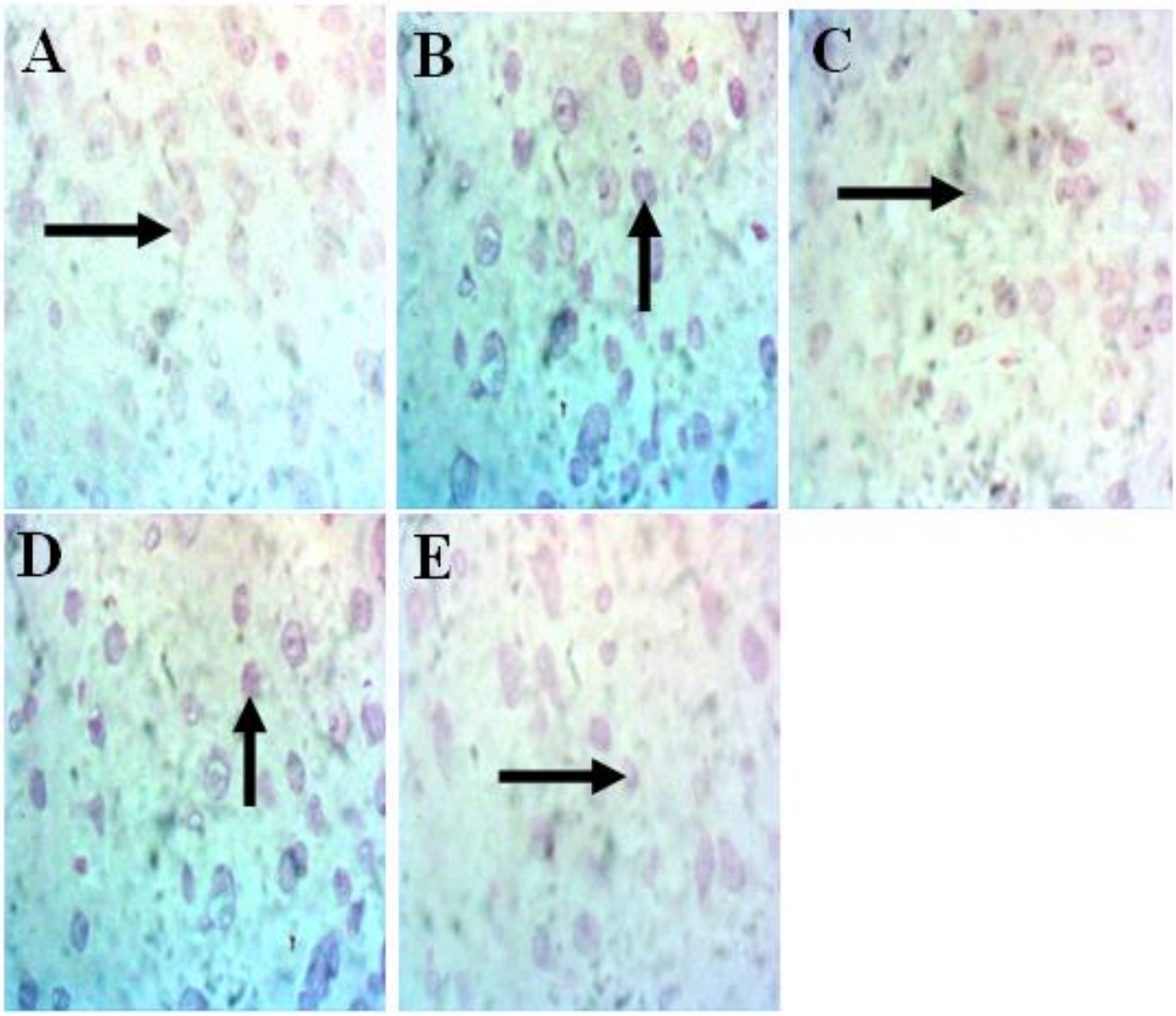


Plate 4.29: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of NF-κB immunopositive cells in the *prefrontal cortex* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

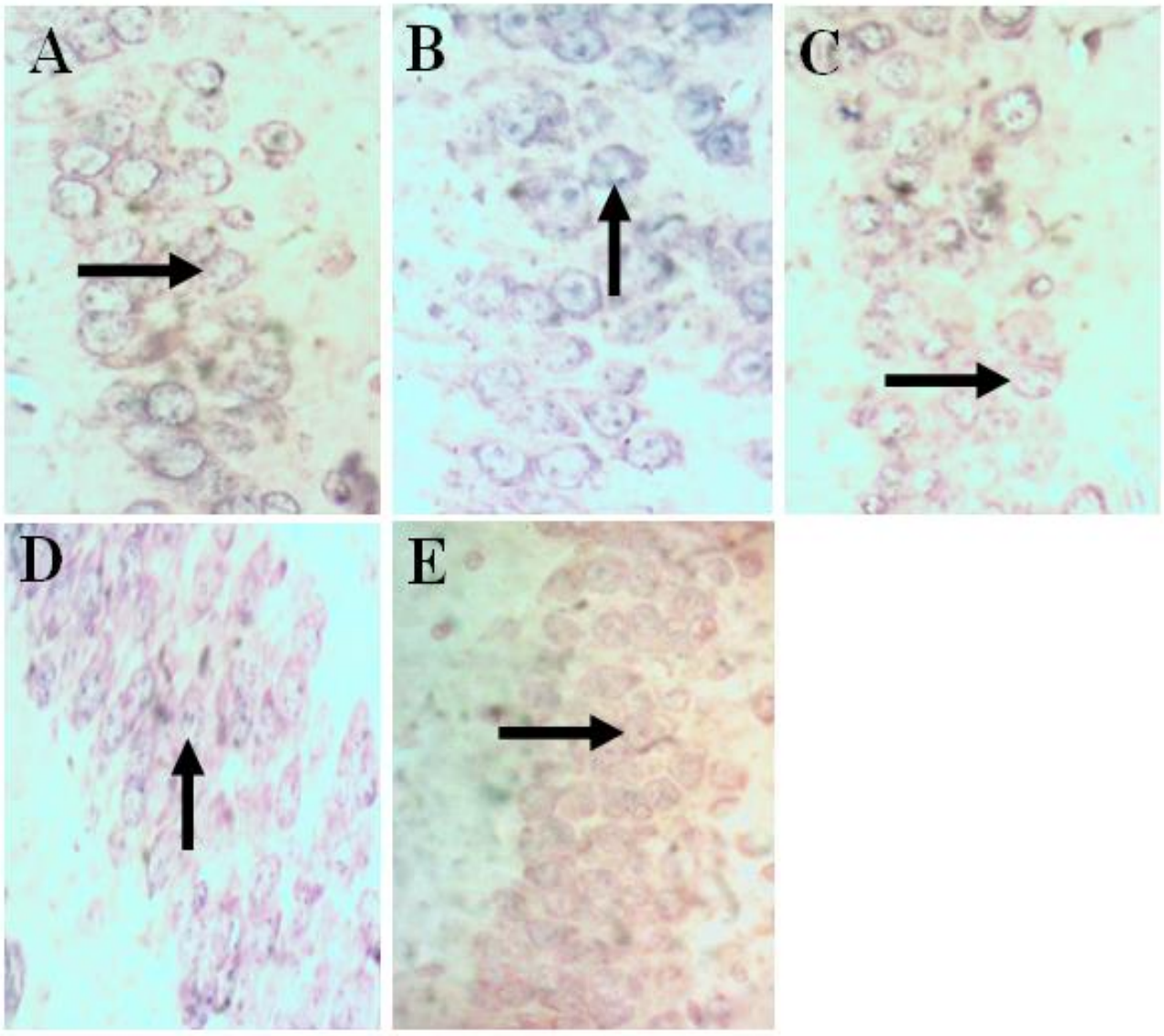


Plate 4.30: Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of NF-κB immunopositive cells in the CA1 region of the *hippocampus* of mice brains. A = Vehicle (10 mL/kg), B = LPS (0.1 mg/kg) plus KET (20 mg/kg), C = MOR (100 mg/kg) + LPS + KET, D = HLP (1 mg/kg) + LPS + KET, and E = RIS (0.5 mg/kg) + LPS + KET.

Vertical arrow indicates: High immunopositive cell expression

Horizontal arrow indicates: Low immunopositive cell expression

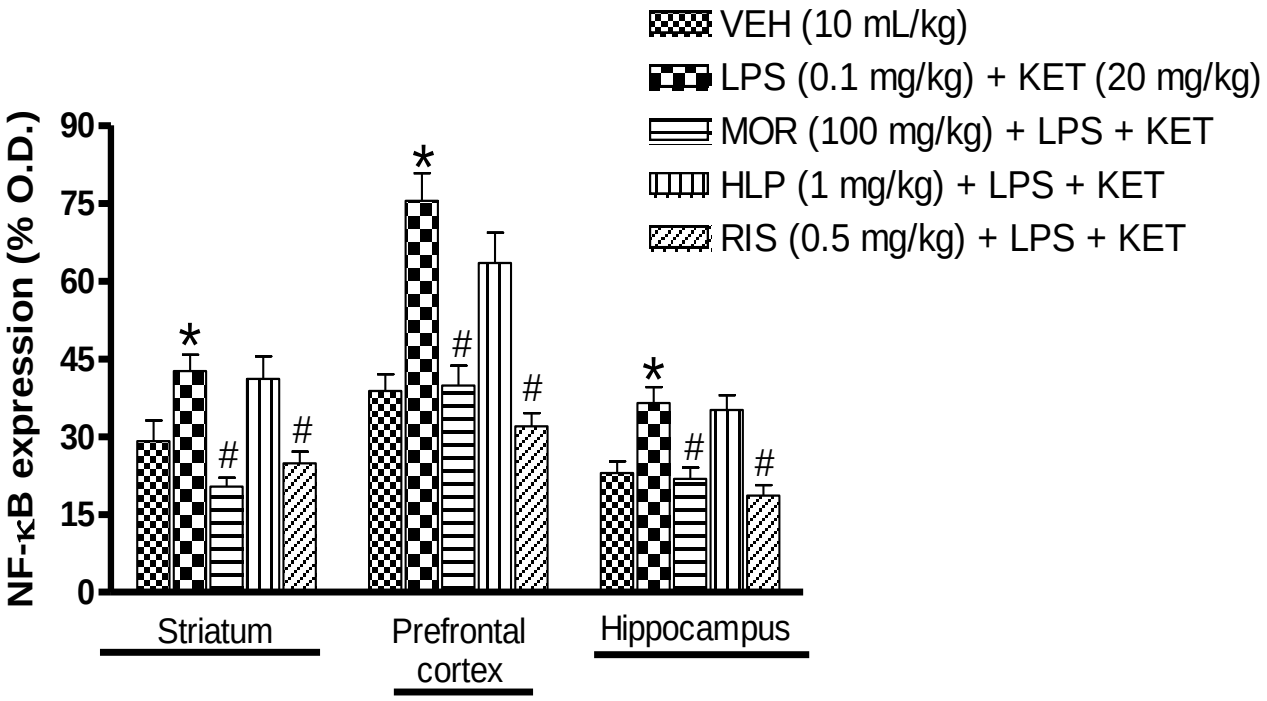


Figure 4.62: Effect of morin on NF-κB expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 5 animals / group. **p* < 0.05 compared to vehicle group and #*P* < 0.05 compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

4.72 Effect of morin on soma size and dendritic spine arborization of pyramidal neurons of the prefrontal cortex of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS and KET-induced alterations of soma size and dendritic spine arborization of the pyramidal neurons of prefrontal cortex of mice brains is shown in Plate 4.31 and Figure 4.63 (A and B). Repeated injections of LPS (0.1 mg/kg, i.p.) for 14 days in combination with KET (20 mg/kg, i.p.) from the 8^{th} to the 14^{th} day of treatment significantly ($p < 0.05$) produced a significant ($p < 0.05$) decrease in dendritic arborization (Plate 4.31), somata of pyramidal neuron (soma size) (Figure 4.63A) and number of dendritic spines (Figure 4.63B) of pyramidal neurons of the prefrontal cortex when compared with vehicle-treated group, suggesting reduced neuronal network/connectivity between neurons and altered cortical inhibition and excitation of the prefrontal cortex. MOR (100 mg/kg, i.p.) significantly ($p < 0.05$) increased dendritic arborization (Plate 4.31), soma size (Figure 4.63A) and number of dendritic spines (Figure 4.63B) of pyramidal neurons of the prefrontal cortex in comparison with LPS and KET-treated mice pyramidal neurons. RIS (0.5 mg/kg, i.p.) did not increase ($p > 0.05$) the soma size of the pyramidal neuron (Figure 4.63A), but significantly ($p < 0.05$) increased dendritic pyramidal neuronal arborization (Plate 4.31) and number of dendritic spines (Figure 4.63B) relative to LPS and KET-treatment. However, HLP (1 mg/kg, i.p.) caused a significant loss of prefrontal cortical pyramidal neurons, as evidenced by absence of dendritic aborization (Plate 4.31), soma body (Figure 4.63A) and dendritic spines (Figure 4.63B) of the pyramidal neurons in comparison with LPS and KET-treated cortical mice brains.

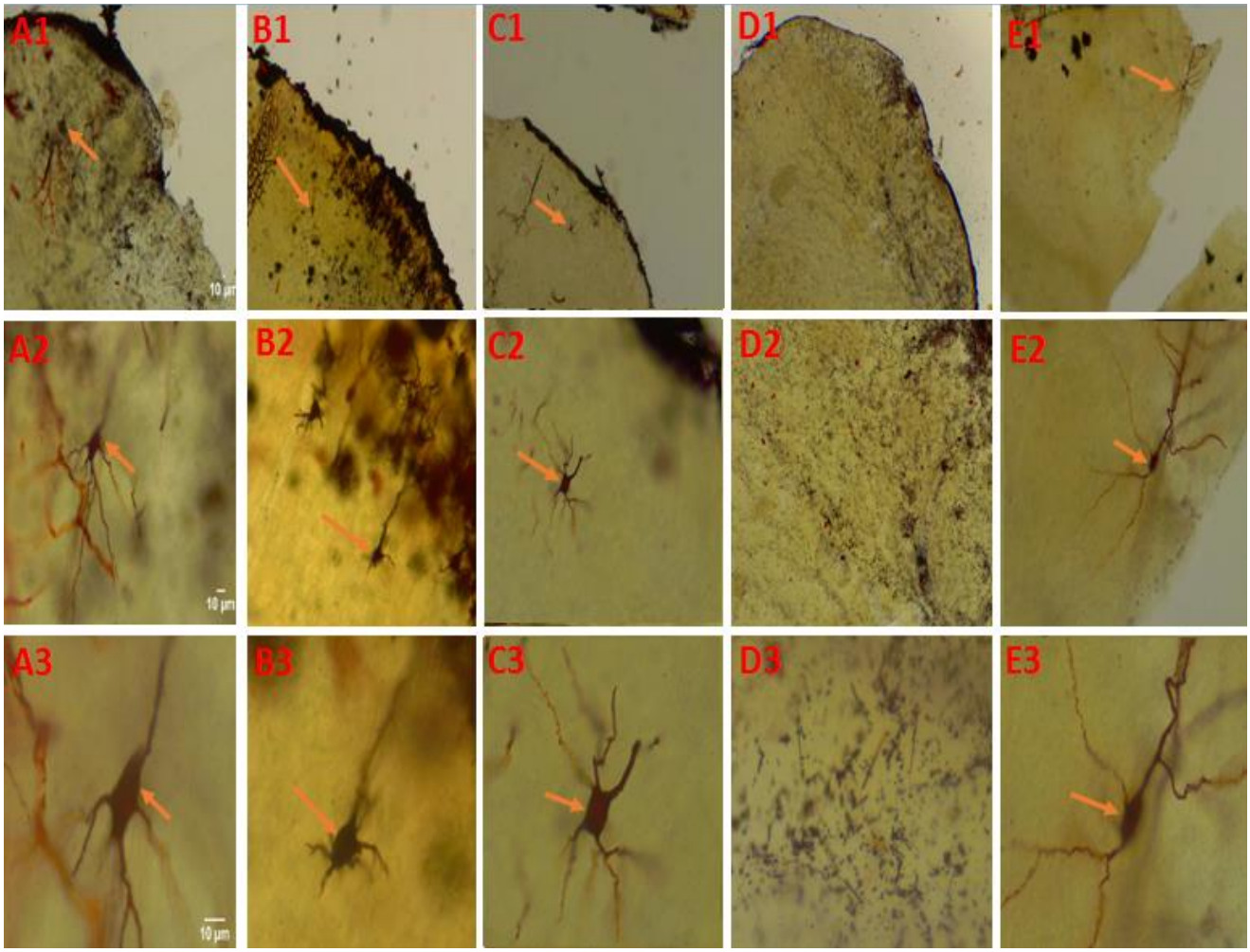


Plate 4.31: Representative photomicrographs (Golgi-silver stained sections) of the effect of morin on lipopolysaccharide and ketamine-induced alterations of soma size and dendritic spine arborization of pyramidal neurons in the *prefrontal cortex* of mice brains. A (A1-A3) = Vehicle (10 mL/kg) revealed normal soma size and dendritic aborization, B (B1-B3) = LPS (0.1 mg/kg) plus KET (20 mg/kg) showed decreased soma size and dendritic spine projections, C (C1-C3) = MOR (100 mg/kg) + LPS + KET revealed increased soma size and dendritic spines, D (D1-D3) = HLP (1 mg/kg) + LPS + KET showed showed the absence of both soma body and dendritic spines of pyramidal neurons, and E (E1-E3) = RIS (0.5 mg/kg) + LPS + KET showed increased soma and dendritic aborizations. Upper panel, A1-E1 represents groups at ×100 magnification; middle panel, A2-E2 is at ×400 magnification while the bottom panel, A3-E3 is at ×1000 magnification. Scale bar for all figures = 10 µm.

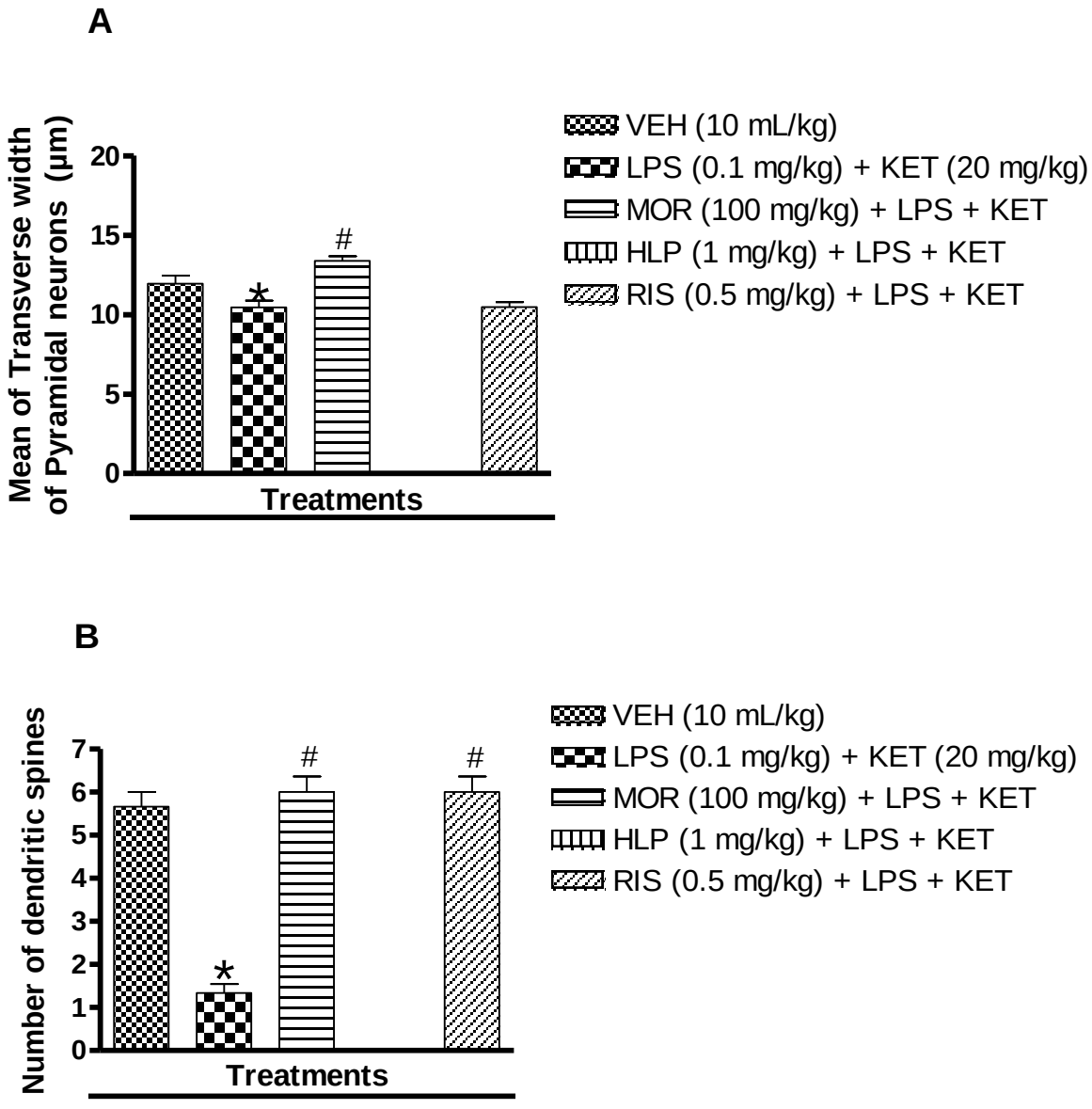


Figure 4.63: Effect of morin on soma size (A) and number of dendritic spines (B) of pyramidal neurons of the prefrontal cortex of mice brains treated with lipopolysaccharide and ketamine

Bars represent the mean ± S.E.M of 3 animals / group. *$p < 0.05$ compared to vehicle group and #$P < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test).

VEH = Vehicle, **LPS =** Lipopolysaccharide, **KET** = Ketamine, **MOR** = Morin,
HLP = Haloperidol, **RIS** = Risperidone

CHAPTER FIVE

5.0. DISCUSSION

Despite the substantial progress made in the management of psychiatric disorders, the treatment of schizophrenia still remains a significant therapeutic challenge. Currently available typical and atypical antipsychotic drugs neither provide cure nor prevent relapses (Linck *et al.,* 2011). Moreover, treatments with these antipsychotics are often accompanied by debilitating adverse reactions including extrapyramidal effects, hyperlipideamia, hyperglyceamia, agranulocytosis, weight gain etc. Hence, there is a compelling need for search of better pharmacological agents to overcome these barriers. Antioxidants with psychotropic properties are the major targets in search of new drugs and lead compounds for the management of these psychiatric disorders. The results of this study showed that morin, a neuroactive flavonol attenuates schizophrenia-like behavioural manifestations (positive, negative and cognitive symptoms) induced by APO and KET respectively, as well as ameliorated the neuropathological biochemical alterations relevant to the pathophysiological basis of schizophrenia by KET or LPS plus KET in mice.

Morin significantly reduced spontaneous motor activity and attenuated KET-induced hyperlocomotion in mice; suppressed APO- and KET-induced stereotypes characterized by head movements, intermittent sniffing, chewing and intense licking in mice. The increase in immobility in forced swim test and social withdrawal due to KET and/or LPS plus KET administrations were reduced by morin in a significant manner. The results of this study also revealed that morin significantly improved memory performance in mice treated via intraperitoneal injection of KET and/or LPS plus KET. On the other hand, morin demonstrated significant antipsychotic effects devoid of extrapyramidal side effects, as evidenced by decreased descent latency, and time of performance and/or no of falls of mice in the wood block catalepsy test and rota rod test, respectively. One significant finding in this study is the observations that morin similarly to risperidone, but not haloperidol modulates psychotropic biogenic amines, cholinergic, neurotrophic, inflammatory, oxidative and nitrergic pathways in different part of mice brains (striatum, prefrontal cortex and hippocampus) to mediate its antipsychotic-like activity.

Animal models used for the study of schizophrenia is increasingly becoming popular and include both models of the full syndrome and models of specific signs of symptoms (Geyer and

Maghaddam, 2002; Chatterjee *et al.,* 2012a). Biochemical data have shown that different neurotransmitters such as dopamine, glutamate, 5-HT, GABA and acetylcholine are involved in the different symptoms of psychosis and their neuropathologies (Stahl *et al.,* 2007; Chatterjee *et al.,* 2015). Spontaneous motor activity (SMA) is one of the novelty-induced behaviours characterized by an increased number of lines crossing in the OFT (Aderibigbe *et al.,* 2010). It is used as a measure of central nervous system (CNS) activity for assessing the effects of novel compounds on behavioural excitation or depression (Aderibigbe *et al.,* 2010). The ability of a novel agent to increase or decrease SMA in rodents has been linked to increased CNS excitation or depression following modulation of excitatory or inhibitory neurotransmitter(s) (Chatterjee *et al.,* 2012a). Indeed, previous studies have confirmed the involvement of dopamine and glutamate on SMA (Chatterjee *et al.,* 2012a; Chatterjee *et al.,* 2015). The finding that morin attenuates SMA in the OFT, suggests a beneficial role against conditions associated with behavioural hyperactivity such as schizophrenia (Gottlieb *et al.,* 2006). Administration of morin significantly suppressed APO- and KET-induced stereotypies as well as KET-induced hyperlocomotion, which suggest antipsychotic-like activity.

Stereotyped behaviour which presents itself as continuous, ritualistic and functionless motor behaviours is one of the most prominent positive symptoms of schizophrenia, and it usually manifest in humans in the form of repetitive performance of a set of strange gestures or making the same kind of comments (Annafi *et al.,* 2017). In animal studies, it manifests itself as: sideways movement of the head, intermittent sniffing, intense chewing and intense licking behaviours; the degree of which are usually heightened when induced by amphetamine and dopamine agonist like apomorphine (Omogbiya *et al.,* 2013), or glutamatergic NMDA receptor antagonist like KET (Yamamoto *et al.,* 1997; Chatterjee *et al.,* 2015). This behaviour is susceptible to the blocking effect of traditional antipsychotic drugs (Davis *et al.,* 1991). According to dopamine hypothesis, a well documented hypothesis on the pathophysiology of schizophrenia (Davis *et al.,* 1991), blockade of dopamine receptors is a common mechanism of action of traditional antipsychotic agents (Meltzer, 2010). The antagonism of stereotyped behaviours induced by APO is an accepted animal model for screening the antipsychotic effect of novel compounds on schizophrenia-like behaviour (Taïwe *et al.,* 2012; Gupta *et al.,* 2012). The neurochemical pathway involved in the mediation of apomorphine-induced stereotyped behaviours has been well characterized and shown to be mediated through the stimulation of

dopamine D_2 receptors (Geyer and Moghaddam, 2002); which is the major molecular target implicated in schizophrenia and drug treatment of the disease (Chatterjee *et al.,* 2012a). In this study, APO produced a significant increase in the stereotyped behaviour, suggesting an enhancement of dopamine transmission (Taïwe *et al.,* 2012; Gupta *et al.,* 2012). The ability of morin to significantly attenuate APO-induced stereotyped supports it's utility in the treatment of schizophrenia-like behavioural hyperactivity.

The antipsychotic activity of morin was also investigated using stereotypy and hyperlocomotion induced by acute and chronic administrations of KET as well as LPS enhanced KET-induced psychosis. Ketamine-induced psychosis is increasingly becoming popular as animal model for mimicking most of the major symptoms of schizophrenia and for delineation of the molecular culprits associated with the pathophysiology of the disease (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013; Vasconcelos *et al.,* 2015; Annafi *et al.,* 2017). More particularly, KET-induced stereotypy and hyperlocomotion are well-recognized animal models currently used for the screening of novel compounds with antipsychotic-like activity against positive symptoms of schizophrenia (Monte *et al.,* 2013; Vasconcelos *et al.,* 2015; Annafi *et al.,* 2017). It is based on the ability of suspected neuroleptic agents to reduce the repetitive, ritualistic functionless motor movements and increased number of line crossings of the animals by pro-psychotic agents e.g., KET (Yamamoto *et al.,* 1997; Monte *et al.,* 2013). The increase in stereotypy scores and hyperlocomtion (hyperactivity) induced by KET have been ascribed to indirectly activate dopaminergic pathways through blockade of NMDA receptors located on the inhibitory GABAergic neurons in the mesolimbic brain regions (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013). Thus, resulting to increased sub-cortical neuronal excitations; congruent to behavioural disinhibition (Stahl *et al.,* 2007) partially due to decreased GABAergic inhibitory regulation of NMDA-induced limbic-striatal dopamine and glutamate release (Chatterjee *et al.,* 2012a). Studies also revealed that KET may present as a potent agonist to D_2 receptor (Vasconcelos *et al.,* 2015), which further explains the basis of KET-induced behavioural stimulation (Monte *et al.,* 2013).

In this study, acute and chronic injections KET alone or KET plus LPS were found to provoke a significant increase in stereotyped behaviours, which was inhibited by morin in a dose-dependent manner. Furthermore, acute and repeated administrations of KET produced a significant increase in locomotion, which agrees with previous investigations that KET increased behavioural

activity and repetitive behaviours of rodent in the OFT (Chatterjee *et al.,* 2012b; Monte *et al.,* 2013; Vasconcelos *et al.,* 2015). In addition, endotoxeamia-induced psychosis-relevant behaviour by LPS in combination with KET model was also found to cause an increased locomotor activity (da Silva *et al.,* 2017). However, morin significantly decreased hyperlocomotion produced by acute and chronic injections of KET or/and KET plus LPS, suggesting antipsychotic property in mice (Davis *et al.,* 1991). This observation further supports previous studies, showing that morin inhibits behavioural hyperactivity (Gottlieb *et al.,* 2006). Hyperactivity produced by KET in rodents is related to the psychotic agitation seen in patients with schizophrenia. Hyperactivity and displays of aggressive behaviours are central components of psychomotor excitement commonly seen in most patients with schizophrenic disorders (Mullen, 2006). As such, patients in this state of psychomotor excitement require the use of drugs with a tranquilizing effect. Therefore, the findings that morin inhibited KET-induced hyperlocomotion further indicates its potential usefulness as an antipsychotic agent in the management of positive symptoms of schizophrenia, which are known to be related to excess of normal human behaviours (Davis *et al.,* 1991).

Acute or repeated administrations of morin were devoid of sedative effect, which may serve as a source of advantage of morin as potential antipsychotic agent. Indeed, marked sedative effect has been reportedly linked to possible tendencies of extrapyramidal side effects (EPSs) of neuroleptic drugs; as extrapyramidal symptoms are believed to result from excessive blockade of dopamine activity in the striatum (Leite *et al.,* 2008; Omogbiya *et al.,* 2013). Therefore, preferential action or blockade of novel agents against D_2 receptors or spontaneous motor activity has been reportedly linked to serve as predictive indicator of little or no tendency of novel antipsychotic agents to induce EPSs (Porsolt *et al.,* 2010; Omogbiya *et al.,* 2013). Although first-generation or typical antipsychotics such as haloperidol inhibit apomorphine-induced stereotypies, the atypical antipsychotic counterparts such as risperidone are known to be less effective against apomorphine-induced stereotypies (Castagné *et al.,* 2009). These observations further confirm the notion that atypical antipsychotics show preferential action on D_2 receptors in the limbic system (Leite *et al.,* 2008). Thus, the findings that morin attenuates both APO- and KET-induced stereotypy as well as KET-induced hyperlocomotion comparably with risperidone suggest an action that may resemble those of the atypical antipsychotic drugs. Moreover, drug-induced ptosis showed that morin demonstrates preferential dose-dependent

ptosis induction, suggesting moderate suppression of the monoaminergic system (Bourin *et al.,* 1983).

Acute and chronic intraperitoneal administrations morin did not impair sensorimotor performance or produce EPSs as evidenced by decreased descent latency in the wood block catalepsy test in mice, suggesting an action that may be different from typical (e.g., haloperidol) antipsychotic drugs (Leite *et al.,* 2008; Omogbiy *et al.,* 2013). Moreover, morin demonstrated significant increased motoric coordination and performances, as indicated by increased time of performance and reduced the number of falls in the rota rod test in mice treated with i.p. injection of morin alone or in combination with KET. Catalepsy test is a paradigm established in rodents that reveals the tendency of antipsychotics such as haloperidol to induce EPSs (Omogbiya *et al.,* 2013; Chatterjee *et al.,* 2012b). Also, the rota rod test is a widely test for evaluating the motor coordination of rodents (Arruda *et al.,* 2008). The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning. The advantage of this test is that it creates a discretely measurable continuous variable (length of time) effect of test compounds (Arruda *et al.,* 2008).

The clinical manifestations of psychotic disorders have been characterized by clusters of positive, negative and cognitive symptoms (Yee and Singer, 2013; Vasconcelos *et al.,* 2015). The negative symptom being increasingly recognized as a major feature of schizophrenia and is thought to be intrinsic to its pathogenesis (Chindo *et al.,* 2012; Monte *et al.,* 2013). They are often present in the early stage of the disease and continue throughout the entire course of the disease even during periods of remission. They are suggested to be a major marker of the amount of disadvantages patients with schizophrenia experience in social and occupational function and independent living (Green *et al.,* 2000). It is becoming increasingly common that the efficiency of antipsychotic drugs against negative symptoms, particularly of typical antipsychotic drugs is associated with therapeutic failures (Meltzer, 2010). The pertinence of the latter observation is underscored by body of evidence showing that existing animal models such as amphetamine- and apomorphine-induce psychosis formerly developed on the basis of central hyperdopaminergic hypothesis are known to replicate only the positive symptoms of schizophrenia (Davis *et al.,* 1991; Yee and Singer, 2013; Omogbiya *et al.,* 2013). However, repeated KET injection, but not acute administration has been shown to replicate the entire positive, negative, and cognitive symptoms associated with the disease (Chatterjee *et al.,* 2012a;

Vasconcelos *et al.,* 2015). Thus, chronic KET administration has gained increased popularity as a suitable animal model that closely mimics the symptoms of this disease (Chindo *et al.,* 2012; Hołuj *et al.,* 2015).

Repeated KET treatment is known to up-regulate 5-HT_{2A} binding sites in the frontal and parietal cortex, as well as interact with phencyclidine allosteric NMDA channel complex and dopamine D_2 receptor binding sites at the hippocampus (Becker and Grecksch, 2004; Chindo *et al.,* 2012). Blockade of NMDA receptor channel complex in the ventral tegmental area has been found to promote a decrease in dopamine release in the prefrontal cortex and increase in cortical extracellular brain levels of 5-HT leading to dopaminergic hypofrontality which may be partly responsible for the negative symptoms (Neill *et al.,* 2010). Blockade of 5-HT receptors in the prefrontal cortex has been reportedly linked to restore cortical dopamine function and this is compatible with the ability of antipsychotic agents to treat negative symptoms (Chindo *et al.,* 2012; Monte *et al.,* 2013). For example, the efficacy of 5-HT_{2A} and 5-HT_7 antagonists such as risperidone and lurasidone in KET-induced negative symptomology has at least, in part been attributed to their 5-HT_{2A} and 5-HT_7 receptor blocking activities (Chindo *et al.,* 2012; Hołuj *et al.,* 2015). In this study, KET induced social withdrawal, suggesting negative symptoms, which is in agreement with findings of previous investigations (Monte *et al.,* 2013; Vasconcelos *et al.,* 2015). Also, pretreatment with LPS prior to KET injection caused a significant enhancement of KET-induced social withdrawal when compared with vehicle treatment group. However, treatment with morin and risperidone, but not haloperidol significantly prevented and reversed social deficits by KET or KET plus LPS in a dose-dependent manner. Moreover, repeated administrations of morin alone demonstrated significant increase in percentage social performance.

The effect of morin on social preference was further assessed in mice using social recognition memory (SRM), also known as social novelty discrimination; indicative of negative-cognitive symptoms. In humans, the ability to socially interact appropriately requires the capability to construct mental representations about oneself and others, termed social cognition (Adolphs, 2001). The SRM is an important component of survival in animal groups and is based on relational learning of complex stimuli in a social environment that enables them discriminate between conspecific as familiar versus unfamiliar individuals (Thor and Holloway, 1982; Lai *et al.,* 2005). Animals recognize each other on the basis of multimodal sensory characteristics,

conjunctively encoded (Thor and Holloway, 1982; Dluzen and Kreutzberg, 1993). Under laboratory conditions, social learning and memory in animals can be studied by exposing rodents to each other for an initial meeting, then testing their conspecific recognition as a function of time. When two rodents are exposed to each other for a specified period of time, they engage in a series of social investigative behaviours to acquaint with each other conspecifically and re-exposure of the two animals at a subsequent episode is characterized by a shorter investigation time. This foreshortened time is taken to represent the social recognition memory (Thor and Holloway, 1982; Dluzen and Kreutzberg, 1993). In this study, pretreatment of LPS prior KET caused a significant decrease in the social memory discriminative capability in mice, indicating impairment of social recognition memory, which is consistent with previous investigation (Gao *et al.,* 2009). Thus, the ability of morin to significantly reverse the increase in social investigative time caused by LPS and KET, as evidenced by decreased social investigative time of conspecific familiar mice following re-exposure, further suggests it might offer beneficial effect in psychotic patients with negative-cogonitive symptoms.

Furthermore, the effect of morin on negative symptoms was also assessed on KET-enhanced duration of immobility in FST paradigm in mice. Ketamine-enhanced duration of immobility reflects the negative symptom of schizophrenia (Chatterjee *et al.,* 2011). Depression is one of the major negative symptoms of schizophrenia (Chindo *et al.,* 2012) and forced swim-induced immobility in rodent is an acceptable animal model of depression (Porsolt, 2001), that reflects a state of despair in rodents and reduction in the immobility time serves as a specific and selective index of antidepressant activity (Chindo *et al.,* 2012). Therefore, an increase in immobility time in FST following repeated administration of a sub-anaesthetic dose of KET indicates an increase in the state of behavioural despair that connotes depression, which is an important characteristic of the negative symptom of schizophrenia (Porsolt, 2001). In this study, sub-acute or chronic injections of KET produced a significant increase in the duration of immobility in FST, which corroborates with observations from previous studies (Chatterjee *et al.,* 2012a; Chindo *et al.,* 2012). Similarly, endotoxeamia-mediated psychosis by LPS pretreatment in combination with KET model was also found to cause accentuation of immobility time. However, morin significantly attenuated the increase in duration of immobility caused by KET or LPS plus KET comparably with risperidone, as shown by decreased immobility time, which offers beneficial effect in psychotic patients with negative symptoms.

Remarkably, repeated administrations of morin and risperidone alone significantly decreased the duration of immobility and social interaction in the FST and SIT respectively. However, repeated administration of haloperidol alone produced marked increase in the duration of immobility in FST, decreased the percentage social preference for social exploration in SIT, and also failed to prevent and reverse KET-induced enhanced immobility and social withdrawal in mice. This therapeutic failure has been ascribed to lack of 5HT-$_2$ receptor blocking activity (Chindo *et al.*, 2012) and also due to impaired motor function in experimental mice due to excessive dopaminergic D_2 receptor blockade in the substantia nigra (Chatterjee *et al.*, 2012b, 2015). Moreover, haloperidol has been reported to exert a central depressant effect on general explorative behaviour in rodents (Spielewoy *et al.*, 2000; Onaolapo *et al.*, 2016), effects which were also apparent in this study. Thus, the differential effect of risperidone and haloperidol in this study further confirms the results from previous findings (Chindo *et al.*, 2012; Chatterjee *et al.*, 2012b, 2015), which showed the inability of typical antipsychotic agents (e.g., haloperidol) to ameliorate negative symptoms of schizophrenia. Thus, the ability of morin to alleviate KET or LPS plus KET-induced social withdrawal, social memory impairment and -enhanced behavioural despair in a similar manner to risperidone, however in contrast to haloperidol, therefore suggests its closeness to atypical antipsychotic agents in the management of negative symptoms of this disease.

Cognitive impairments such as deficits in attention, executive function, short-term (working) and long-term memories simulate the cognitive symptoms of schizophrenia (Glahn *et al.*, 2003; Barch and Sheffield 2014). Learning and memory impairments are known to be particularly severe and affects approximately one-third of patients with the illness, and the severity of the cognitive symptoms is often associated with the course of the illness as well as the patients' function and quality of life (Green *et al.*, 2000; Glahn *et al.*, 2003; Barch and Sheffield 2014). Remarkably, the efficiency of antipsychotic drugs, particularly of typical antipsychotics on cognitive symptom of this disease remains disputable and induces memory deficits with repeated use (Xu *et al.*, 2012; Onaolapo *et al.*, 2016).

The ability of morin to improve cognitive functions was assessed in mice chronically treated with KET using Y-maze and Novel object recognition tests. The YMT is a well-recognized, noninvasive, forced alternation animal model used routinely as an alternative to Morris water maze for screening compounds with memory-enhancing effect in rodents (Lee *et al.*, 2009).

However, the YMT does not involve swimming and therefore considered to be less anxiogenic than the Morris water maze (Casadesus *et al.,* 2006). The YMT is based on the ability of rodents to enter as many different arms as possible while trying to remember the sequence of arms entry, usually known as spontaneous alternations; tendency to choose a different option than one previously chosen (Casadesus *et al.,* 2006). The pattern of arms visitation and the last arm visited is believed to be held in online memory, commonly referred to as spatial working memory (Lee *et al.,* 2009). This action thereby enebles the animal to prevent revisiting the previous arm through arm choice alternation, thus making the YMT a suitable model for short-term memory (Casadesus *et al.,* 2006; Lee *et al.,* 2009). The results of this study showed that KET produced a significant decrease in alternation behaviour in Y-maze model, which suggests cognitive dysfunction. Also, chronic intraperitoneal pretreatment with LPS prior to KET significantly decreased spontaneous alternation behaviour. These findings further support previous investigations, which showed that KET and LPS impaired memory performance of rodents in YMT (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013; Ming *et al.,* 2015). However, treatment with morin significantly prevented and reversed KET or LPS plus KET induced memory impairments, evidenced by increased percentage alternation behaviour in the Y-maze test.

The Novel object recognition memory (NORT) was also utilized in this study to validate the effect of morin on cognitive performance (non-spatial working memory) in mice. The primary behavioural phenotype indicative of cognitive performance in NORT is based on the natural tendency of rodents to explore its environment and natural preferences for novel objects based on visual memory (Ennaceur *et al.,* 2010; Zhu *et al.,* 2014). The preference for novel object over the familiar one is an indication of the existence of the familiar object in the memory of the animal, thus serving as a measure of memory function (Ennaceur *et al.,* 2010). Enhancement of memory performance in NORT is commonly appraised by increases in discrimination index (DI), which is the difference in time of exploration of the novel and familiar objects divided by the total amount of time spent with both objects. The extent of exploration of novel object is dependent on the amount of residual memory of the familiar objects. Agents with memory impairing effect are known to decrease DI (exploratory preference) (Ennaceur *et al.,* 2010 Wischhof *et al.,* 2015), which shows the reduction of motivation for a novel object and concentration ability as well as withdrawal symptoms, congruent to the negative-cognitive deficits of schizophrenia (Fujita *et al.,* 2008). In this study, chronic injection of KET, and/or LPS plus KET was found to produce a

significant decrease in the discrimination index (i.e., decreased preference for novel object) in comparison with vehicle control, which indicates memory impairment. This finding further supports previous investigations, which showed that KET and LPS impaired memory performance of rodents in the NORT (Venâncioa *et al.,* 2011; Zhu *et al.,* 2014). Thus, the ability of morin to significantly prevent and reverse the decreases in the preference for novel object caused by KET and/or LPS plus KET, as evidenced by increased discrimination index in a similar manner to risperidone but not haloperidol, which further suggests that the antipsychotic-like effect of morin that may be beneficial to memory impairment seen in most patients with psychotic disorders. Moreover, repeated administration of morin was found to enhance cognitive performance of intact mice in both tests (YMT and NORT) in a dose-dependent manner when compared with vehicle-treated animals, although risperidone in this contest did not produce any significant change in Y-maze and Novel object recognition tests. However, treatment with haloperidol significantly impaired memory performances of intact mice relative to vehicular treatment.

The effects of risperidone on cognitive functions in schizophrenia are conflicting. Although previous study have reported the worsening effects of risperidone on working and spatial memory of intact rodents and humans (Ihalainen *et al.,* 2016), there are some preclinical and clinical evidence demonstrating the null or improving effects of risperidone on cognitive performance (Addy and Levin, 2002; Monte *et al.,* 2013; Rogaz and Kaminska, 2016). In this study, repeated administration with risperidone did not produce any significant change on cognitive performances in both YMT and NORT relative to vehicle controls. Previous studies have demonstrated impairment of learning and memory with haloperidol (Zirnheld *et al.,* 2004; Abdel-Salam and Nada, 2011; Onaolapo *et al.,* 2016**)**. Haloperidol worsened the immediate and delayed free and cued recall deficits produced by Delta-9-tetrahydrocannabinol (D'Souza *et al.,* 2008). The dopaminergic system plays an important role in memory processes (Costa *et al.,* 2008; Bäckman *et al.,* 2011). In support of this notion is the finding that dopamine transmission is abnormal in the brains of patients with schizophrenia and working memory deficit is a core dysfunction in schizophrenia (Tanaka, 2006). Accordingly, chronic blockade of dopamine D_2 receptors by antipsychotic drugs (e.g., haloperidol), down-regulates D_1 receptors in the prefrontal cortex and produces severe impairments in working memory, which could be reversed by Dopamine D_1 receptor stimulation (Castner *et al.,* 2000). It has also been suggested that

inhibition of 5-$HT_{2A/2C}$ receptors mediates the cognitive improvement of antipsychotic-treated schizophrenic patients (Meltzer *et al.,* 2003). However, while haloperidol has been reported to present as partial agonist to 5-HT receptors (Roth *et al.,* 1992), riperidone has shown to be an antagonist to 5-$HT_{2A/2C}$ receptors (Bubenikova-Valesova *et al.,* 2008). In this study, haloperidol induced memory impairment and worsening of negative symptoms (Zirnheld *et al.,* 2004; Chindo *et al.,* 2012). This may in part, be related to primary blockade of D_2 receptor and the lack of 5-$HT_{2A/2C}$ receptor blocking activity. Thus, this suggestion further highlights the differences between typical (e.g., haloperidol) and atypical (e.g., risperidone) antipsychotic drugs, their relationships or affinity for dopaminergic D_2 and 5-$HT_{2A/2C}$ receptors blocking activity, and the correlations to their ability to ameliorate cognitive impairment seen in schizophrenia patients (Bubenikova-Valesova *et al.,* 2008; Meltzer, 2010). Hence, the ability of morin to improve memory performance in a similar manner to risperidone in KET-treated mice suggests an action related to attenuation of central 5-hydrotryptaminergic neurotransmission through 5-$HT_{2A/2C}$ receptor blocking activity and 5-$HT_{2A/2C}$-dependent actions. Thus, the result of this study indicates that morin may be a potential agent for the treatment of certain (positive, negative and cognitive) symptoms of this disease caused by impairment of central dopaminergic, 5-hydroxytryptaminergic, glutamatergic and GABAergic systems that often characterized schizophrenia.

In the light of this and in accordance with previous studies (Ayoka *et al.,* 2006; Aderibigbe *et al.,* 2010), the neurotransmitter hypothesis of schizophrenia predicts that the underlying pathophysiologic basis of schizophrenia is at least, in part, an increase in the levels of dopamine, 5-HT and noradrenaline, and/or decrease in GABA and acetylcholine in the CNS. These hypothesized pathophysiologies appear to be supported by mechanism of action of antipsychotic drugs that decreases or increases the levels of these neurotransmitters in the brain. In this context, it is well documented in literatures that dopaminergic system is closely implicated in the pathophysiology of schizophrenia and in the mechanism of action of antipsychotics (Davis *et al.,* 1991; Chatterjee *et al.,* 2012a; Omogbiya *et al.,* 2013). Most of the antipsychotic drugs in clinical use promote a decrease in dopamine availability directly affecting turnover in the brain, and also interacting with D_2 as well as a considerable affinity for D_3 and D_4 (Davis *et al.,* 1991; Leysen *et al.,* 1992; Ayoka *et al.,* 2006). In this context, literature data have shown that pretreatment with haloperidol and sulpiride (D_2, D_3 and D_4 receptor antagonists) decreases

dopaminergic neurotransmission and prevents the antipsychotic-like effects of novel compounds (Ayoka *et al.,* 2006; Aderibigbe *et al.,* 2010). Accordingly, the result of this study revealed that that the antipsychotic effect of morin in terms of spontaneous motor activity in the OFT, but not immobility time in the FST and alternation behaviour in the YMT was prevented by pretreatment of mice with haloperidol and sulpiride, suggesting that the dopaminergic system is probably implicated in the antipsychotic effect induced by morin in open field test relatively to its effects on positive symptoms.

Also, it is a well-known fact in literature that the deficiency of GABAergic system plays an important role in the pathophysiology of schizophrenia (Lewis *et al.,* 2008), as depletion of GABAergic neurotransmission also alters the effects of some antipsychotic drugs (Ayoka *et al.,* 2006; Chatterjee *et al.,* 2012a). Interestingly, enhancement of GABAergic neurotransmissions within the sub-cortical regions of the brain has been proposed to encourage the actions of antipsychotic drugs, thereby decreasing mesolimbic neuronal excitability and behavioural disinhibition (Nunes *et al.,* 2012; Monte *et al.,* 2013). In line with this notion, there is compelling evidence in literature for the role of $GABA_A$ receptor in the pathogenesis of schizophrenia, as the blockade of $GABA_A$ receptors mimics schizophrenic states, which are associated with GABAergic deficiency (Hinton and Graham, 2008; Ahn *et al.,* 2011). Appropraitely, flumazenil served as a pharmacological tool for elucidation of the involvement of GABAergic pathway in antipsychotic actions of novel drugs (Ayoka *et al.,* 2006). An antagonism of the action of antipsychotic drugs by flumazenil therefore suggests interaction with GABAergic system. Accordingly, the anti-immobility in the FST and attenuation of spontaneous motor activity in the OFT, but not increase in spontaneous alternation behaviour in the YMT by morin were found to be modulated by $GABA_A$ receptors because, flumazenil, $GABA_A$ receptor antagonist, was able to reverse the decrease in immobility time and spontaneous motor activity produced by morin. Consistent with this finding in relation to $GABA_A$ receptors, previous studies have reported that neuroactive flavonoids modulation of neurotransmission were related at least, in part, to this receptor (Marder *et al.,* 2002; Wang *et al.,* 2005).

Experimental and clinical studies indicate that the enhancement of 5-hydroxytryptaminergic system is also strongly implicated in the pathophysiology of schizophrenia, particularly in the negative and cognitive symptoms (Geyer and Moghaddam, 2002), and attenuation of 5-hydroxytryptaminergic pathways underlie the therapeutic effect of antipsychotic drugs (Chindo

et al., 2012; Chatterjee *et al.,* 2012a, 2015). Thus, antagonism of antipsychotic effect of a novel compound by metergoline (a non-selective 5-HT_1 and 5-HT_2 receptor antagonist), cyproheptadine (a non-selective 5-HT_2 receptor antagonist) and para-chlorophenylalanine (pCPA) (a 5-HT synthesis inhibitor) is an indication that the compounds may be mediating its action via interaction with 5-hydroxytryptaminergic system (Bubenikova-Valesova *et al.,* 2008; Chindo *et al.,* 2012). In this study, 5-HT receptor antagonists such as metergoline, cyproheptadine, and pCPA significantly reversed the effect of morin on locomotion, immobility time and cognitive alternation behaviour in the OFT, FST and YMT, respectively. Specifically, metergoline was found to block the effect of morin on locomotion in the OFT and immobility time in the FST, but did not reverse the effect of morin on spontaneous alternation behaviour in the YMT. Also, pretreatment with cyproheptadine and pCPA caused a blockade of morin-induced antipsychotic-like activity in all three tests in a significant manner, which further suggests the involvement of central 5-hydrotryptaminergic neurotransmission in morin activity, at least in part, due to 5-$HT_{1/2A/2C}$ receptor blocking activities.

In parallel with the 5-hydroxytryptaminergic and dopaminergic systems, noradrenergic system is also suggested to be implicated in the regulation of mood and psychiatric disorders (Yamamoto *et al.,* 2004: Maletic *et al.,* 2017). Noradrenergic heteroreceptors are also located on glutamate, GABA, dopamine and 5-HT neurons as well as in glial and immune cells. Therefore, in addition to being autoregulated by presynaptic alphal-2 (α_2) and beta-2 (β_2) adrenergic receptors (Hein, 2006), noradrenaline signaling is also regulated by other neurotransmitters, such as inhibitory GABA and excitatory glutamate systems (Jin *et al.,* 2016). Taken together, this suggests that noradrenergic receptors within these pathways play a prominent role in a broad range of brain functions such as mood and behaviour (Atzori *et al.,* 2016), as well as associated neuropsychiatric diseases (Maletic *et al.,* 2017). In fact, there is compelling evidence regarding the efficacy of antipsychotic drugs with noradrenergic effects in the treatment of schizophrenia (Onaolapo *et al.,* 2017; Maletic *et al.,* 2017). Moreover, high concentration of noradrenalin has also been shown to enhance spontaneous motor activity through actions at α_1 adrenergic receptors. Indeed, stress-induced hyperlocomotion has been shown to be blocked by infusion of an α_1 adrenergic receptor antagonist into the striatum of rats (Schmidt and shenker, 2014). In this study, the result showed that the noradrenergic system is also involved in the anti-immobility action and anti-spontaneous motor activity of morin, through an interaction study with

adrenergic α_1 receptors. This is because the pretreatment of animals with the adrenergic α_1 receptor antagonist, prazosine prevented the anti-immobility effect and decrement in spontaneous motor activity evoked by morin.

On the other hand, a_2-adrenoceptor also modulates the release of noradrenaline, 5-HT and several other neurotransmitters. Therefore, these receptors have been shown preclinically to underlie some of the responses of antipsychotic drugs in behavioural models of schizophrenia. In line with this, reduced filtering of sensory and sensorimotor information, demonstrated by both a reduced P50 suppression or reduced pre-pulse inhibition of the startle reflex, was corrected by activating a_2-adrenergic receptors with clonidine (Oranje *et al.,* 2013, 2014). Furthermore, other evidence from literature search showed that mirtazapine increased mental speed and attention control in schizophrenia. Although this action may be related to modulation of several 5-HT receptors, it is also most probably at least in part, due to modulation of a_2-adrenergic receptor (Terevnikov *et al.,* 2010). Moreover, risperidone has being reported as a potent α-adrenergic receptor antagonist (Cai *et al.,* 2012). In fact, in general, indirect noradrenergic agonists such as yohimbine (a_2-adrenergic receptor antagonist) are known to aggravate positive symptoms of schizophrenia, an effect which was also apparent in this study. However, this may be ameliorated by functional noradrenergic agonists such as clonidine, suggesting that increased α_2-adrenergic receptor density may also have detrimental effects in these patients (Maletic *et al.,* 2017). Thus, it is of considerable interest to understand the role that noradrenergic modulation may play in ameliorating schizophrenia-like symptoms. To this end, antagonism of the action of antipsychotic drugs by yohimbine therefore suggests interaction with noradrenergic system (Ayoka *et al.,* 2006). In this study, yohimbine, at a dose which does not promote a significant effect *per se* , was able to reverse the antipsychotic-like effect of morin in terms of spontaneous motor activity (positive symptom), although there was no significant effect on the anti-immobility and cognitive enhancement effects of morin. This result suggests that morin-induced behavioural changes observed in the OFT seems to partially involve interaction with a_2-adrenergic receptors for the treatment of positive symptoms.

The role of noradrenergic β receptors in the mechanism of action of antipsychotic drugs is still controversial. Several studies have also shown lower density of β_1 relative to β_2 expressions in the hippocampus of schizophrenia patients and their involvement in the mechanism of action of antipsychotics (Klimek *et al.,* 1999; Gazarini *et al.,* 2013). However, the results of this study are

in accordance with previous studies indicating that both β_1 and β_2 receptors might display significant role in the mechanism of action of various antipsychotic drugs (Klimek *et al.,* 1999). Accordingly, the decreased spontaneous motor activity in the OFT but not on the cognitive performance in the YMT and anti-immobility in the FST by morin was found to be mediated partially by β adrenergic receptors because, propranolol, a non-selective β receptor antagonist, was able to enhance the decrease in spontaneous motor activity produced by morin. Together, corroborating these findings in relation to adrenergic α_1, α_2 and β receptors, previous studies have reported that the anti-hyperlocomtive effects of the atypical antipsychotic, risperidone and others drugs were related, at least in part, to these receptors (Cai *et al.,* 2012; Maletic *et al.,* 2017).

The deficiency of cholinergic system is substantially implicated in the pathogenesis of schizophrenia, particularly in the cognitive symptoms (Chatterjee *et al.,* 2012a, 2012b), and enhancement of cholinergic pathways underlie the therapeutic effect of antipsychotic drugs (Chatterjee *et al.,* 2012b). There is evidence in literature for the role of aceylcholinesterase and α-7 nicotinic acetylcholine (α7nACh) receptor in the pathogenesis of schizophrenia. Blockade of these neurobiological substrates by atypical antipsychotic drugs have been found to increase the levels of acetylcholine and thus, enhancement of cholinergic neurotransmissions (Shirazi-Southall *et al.,* 2002; Chatterjee *et al.,* 2012a, 2012b). Also, the binding of muscarinic acetylcholine receptors within the cortical regions of the brain has been suggested as mechanism action of some antipsychotic drugs (Bymaster *et al.,* 2003). Therefore, depletion of cholinergic neurotransmission is also known to alter the effects of some antipsychotic drugs (Ayoka *et al.,* 2006; Chatterjee *et al.,* 2012a). In this way, atropine was used as a pharmacological tool in this study for elucidation of the involvement of cholinergic pathway in the action of drugs (Ayoka *et al.,* 2006; Aderibigbe *et al.,* 2010). The findings that the effects of morin was reversed by atropine in the OFT (spontaneous motor activity) and YMT (spontaneous alternation behaviour), suggests the involvement of central cholinergic mechanisms in its antipsychotic-like activity of morin in mice relatively to its effects against positive and cognitive symptoms.

The pathological basis of schizophrenia is closely linked with increased brain levels of nitric oxide (NO) which suggests an important role in the mechanism of antipsychotic drugs (Sakurai *et al.,* 2004; Chatterjee *et al.,* 2012b, 2015). Most of the antipsychotic drugs promote a decrease in NO levels in the brains by inhibiting the activity of nitric oxide synthase (NOS) (Chatterjee *et al.,* 2012b). In fact, altered production of NO, as retrograde molecule, may contribute to a

dysfunction in the regulation of certain neurotransmitters and psychosis (Sakurai *et al.,* 2004; Chatterjee *et al.,* 2012a). For example, striatal NO release regulates the basal activity and responsiveness of dopamine neurons to cortical and striatal inputs (West and Grace, 2000). Of note, infusion of the NOS inhibitor, 7-nitroindazole sodium (7-NI) decreased the onset latency and extended the duration of the initial inhibitory phase induced by either orbital prefrontal cortex or striatal stimulation. On the other hand, in the same study, microdialysis experiment demonstrated that endogenous striatal NO production increases striatal extracellular dopamine levels (West and Grace, 2000). Thus, this ability of NO to regulate dopamine levels in the striatum may partially explain the role of increased nitrite levels in the brains of schizophrenia patients, although currently the role of NO in the pathophysiology of schizophrenia is ill-defined (Zhang *et al.*, 2012a). However, treatment with antipsychotic drugs has been shown to modulate NO levels in the brains of experimental animals (Sakurai *et al.,* 2004; Monte *et al.,* 2013; Vasconcelos *et al.,* 2015).

Thus, antagonism of antipsychotic effect of novel compounds by NO precursor (L-arginine) or NOS inhibitors such as methylene blue (a specific neuronal NOS inhibitor) and L-NAME (a non-specific NOS inhibitor) is an indication that the compounds may be mediating its action via interaction with nitrergic pathways (Hu *et al.,* 1993; Sakurai *et al.,* 2004). In this study, L-arginine, at a dose which does not promote significant effect, was found to reverse the anti-immobility effect, anti-spontaneous motor activity and cognitive enhancing behavioural effects of morin. Moreover, the combination of a sub-antipsychotic dose of L-NAME and the antipsychotic dose of morin used in the interaction study produced a synergistic antipsychotic effects, as evidenced by decreased spontaneous motor activity and immobility time, although the combination caused a significant decrease in spontaneous alternation in the YMT (cognitive impairment) relative to morin-treated mice. However, pretreatment with the combination of L-arginine and L-NAME prior to morin, significantly reversed the cognitive impairment by the combination of L-NAME and morin, suggesting synergistic inhibition of NO in the actions of L-NAME and morin. One possible explanation for the memory impairment observed with the combination of morin and L-NAME in this interaction study is the possible promiscuous signaling mechanism of endogenous NO in relation to learning and memory (Paul and Ekambaram, 2011). Inhibition of the anti-immobility effect decreased spontaneous motor activity and enhanced spontaneous alternation behaviour of morin in the OFT, YMT and FST by L-

arginine, suggest that morin-induced antipsychotic effects might be partially mediated via modulation of nitrergic system. Furthermore, pretreatment of animals with methylene blue (neuronal NOS inhibitor), was able to reverse the antipsychotic-like effect of morin in the OFT, YMT and FST. These results suggest that morin-induced behavioural changes observed in the OFT, YMT and FST seems to particularly involve, at least in part, an interaction with neuronal NO pathway.

Schizophrenia is a complex neuropsychiatric disorder with behavioural consequence of altered biochemical signatures and neurobiological substrates that are well described, both preclinically and clinically (Chatterjee *et al.,* 2012a, Monte *et al.,* 2013; Al-Asmari and Khan, 2014; Vasconcelos *et al.,* 2015). Accordingly, pharmacological evaluation of anti-schizophrenia drugs usually involves both behavioural and biochemical approaches. To corroborate the behavioural changes evoked in this study therefore involves the evaluation of the underlying neuropathologic biochemical substrates. Even though the neuropathological basis of schizophrenia remains to be elucidated, accumulating evidence indicates that hypofunction of NMDA receptors does occur in this disease (Vasconselos *et al.,* 2015). Although schizophrenia is a disease of multiple pathology, it is to be expected that altered NMDA function would imply significant changes in various neurotransmission systems (including dopamine, glutamate, GABA, 5-HT, acetylcholine) contributing to the complex patterns of behavioural alterations seen in schizophrenic patients (Chatterjee *et al.,* 2012a; da Silva *et al.,* 2017). For example, NMDA receptor stimulation has been shown to be involved in the tonic inhibition of mesolimbic dopamine release (inhibitory effect) while facilitating mesocortical dopamine release (stimulatory effect) (Coyle, 2006). In addition to dopamine, 5-HT and GABA have been suggested to be under the influence of NMDA receptor regulation. Thus, NMDA receptor antagonists increases extracellular levels of 5-HT and 5-hydroxyindoleacetic acid (5HIAA) in the prefrontal cortex of drug naïve animals (Chatterjee *et al.,* 2012a, 2012b). Furthermore, NMDA receptor stimulation by NMDA itself increases GABA release from cultured cortex neurons (Curley *et al.,* 2013), whereas NMDA receptor blockade decreases the levels of mRNA encoding the GABA synthesizing enzyme, glutamic acid decarboxylase 67 (GAD_{67}), especially in the parvalbumin (PV)-positive interneurons of the prefrontal cortex (Behrens *et al.,* 2007; Curley *et al.,* 2013), which suggests the involvement of endogenous dysfunction NMDA receptor-mediated transmission in the pathogenesis of schizophrenia (Chatterjee *et al.,* 2015;

Koh *et al.,* 2016). Accordingly, agent such as KET is becoming widely used in neuropsychiatric research to induce robust behavioural alterations in rodents accompanied by significant biochemical and neuropathological changes (Vasconcelos *et al.,* 2015; Onaolapo *et al.,* 2017). This agent has gained increased popularity as a suitable animal model that closely simulates the neuropathological substrates; hence it is used to elucidate the molecular features including altered neurotransmitter systems, neurotrophic factors, neuroimmune alteration as well as behavioural deficits associated with schizophrenia (Chatterjee *et al.,* 2012a; Monte *et al.,* 2013; Vasconcelos *et al.,* 2015; da Silva *et al.,* 2017).

Ketamine is a non-competitive NMDA antagonist, which has served as a reliable animal model to induce behavioural alterations and to test for compounds capable attenuating behavioural hyperactivity of neuropsychiatric disease such as schizophrenia (Monte *et al.,* 2013; Onaolapo *et al.,* 2017). The pathologic mechanism that underlies KET-induced schizophrenia-like behaviours has been shown to be mediated through blockade of NMDA receptors (Chatterjee *et al.,* 2012a; Hołuj *et al.,* 2015; Onaolapo *et al.,* 2017). In this context, chronic KET injection has been found to induce alterations in central dopaminergic, glutamatergic, 5-hydroxytryptaminergic, GABAergic and cholinergic transmissions that correlates to the corresponding behavioural (positive, negative and cognitive symptoms) phenotypes (Chatterjee *et al.,* 2012a). Previous investigations have shown that the dopaminergic pathways are critical for the control of locomotor activities (Salamone *et al.,* 2005; Benturquia *et al.,* 2008), and that increased locomotor activity and stereotypy behaviour in both acute and chronic injections of KET are as a result of sub-cortical hyperdopaminergic activity (Chatterjee *et al.,* 2012a). As shown in preventive and reversal study on the dopamine concentration in the brain regions examined, particularly in the striatum, tend to support KET-induced sub-cortical hyperdopaminergic state. This data was further supported by enhanced locomotor activity regarded as hyperlocomotion, which represents positive symptoms. Furthermore, dopaminergic transmission in the prefrontal cortex has been suggested to be hypofunctional in schizophrenic patients (Ihalainen *et al.,* 2016). This is because blockade of NMDA receptors in the ventral tegmental area (VTA) promotes a decrease in cortical dopamine release, which may be partially responsible for the negative and cognitive symptoms, since dopamine receptors modulate motivational and working memory functions (Neill *et al.,* 2010; Chatterjee *et al.,* 2012a), which are affected in schizophrenia (Neill *et al.,* 2010; Ihalainen *et al.,* 2016). Therefore, schizophrenia is, at least, associated with

interconnected abnormalities of glutamatergic and dopaminergic transmissions (Javitt *et al.,* 2007) that can be reproduced by the chronic administration of KET in mice (Chatterjee *et al.,* 2012a).

In this study, repeated administration of KET was found to produce increased concentrations of dopamine in the striatum in both preventive and reversal treatment protocols relative to vehicle-treated mice, which was prevented and reversed by morin, haloperidol and risperidone. However, in the reversal protocol, KET significantly decreased dopamine concentration in the prefrontal cortex as expected when compared with vehicle-treated mice, suggesting mesocortical hypodopaminergia. A significant increased prefrontal cortical dopamine concentration was observed in the preventive treatment, suggesting a possible transient sensitization of dopaminergic transmission, which indicates altered cortical dopamine to glutamate ratio (Lindefors *et al.,* 1997). On the other hand, although there was no significant change in hippocampal dopamine levels in the preventive treatment, but a significant increase in dopamine concentration in the hippocampus of KET-treated mice was observed in the reversal treatment. The observations from this study confirmed earlier studies which showed that acute or sub-acute administrations of KET potentiates prefrontal cortical dopamine release in drug naïve animals, whereas chronic administration decreases dopamine release in the prefrontal cortex (Lindefors *et al.,* 1997), which is a characteristics of the disease pathology (Chatterjee *et al.,* 2012a). The increase in dopamine release following sub-acute exposure to KET in this study is in agreement with the dopamine hypothesis of schizophrenia, which states that the the symptoms of schizophrenia are in part a manifestation of functional hyperactivity of mesocortical dopamine transmission (Stahl, 2007). The influence of cortical NMDA receptors on the dynamics of dopaminergic transmission in the prefrontal cortex is of particular interest since dopamine receptors modulate working memory functions (Ihalainen *et al.,* 2016). An excess of dopaminergic transmission in the cortex may interferes with such cognitive functions (Williams and Goldman-Racik, 1995). Studies have shown that repeated administration of KET to rats produces a postsynaptic dopamine receptor sensitization, due to decreased endogenous ligand (Lindefors *et al.,* 1997). Thus, the increased and attenuated dopaminergic transmission in the prefrontal cortex following repeated exposure to KET in the preventive and reversal protocols in this study was accompanied with corresponding spatial and non-spatial memory impairments. The ability of morin to significantly prevent and reverse the alterations in prefrontal cortical and

hippocampal dopaminergic transmissions in the preventive and reversal treatments, further confirms its atypical antipsychotic-like property in mice.

Experimental and clinical studies have shown that enhancement of 5-hydroxytryptaminergic system is also strongly implicated in the severity of positive, negative and cognitive symptoms of schizophrenia (Geyer and Moghaddam, 2002). It is relevant to this discussion that NMDA blockade has been shown to cause a 5-$HT_{2A/2C}$ dependent increased 5-HTergic transmission, leading to alteration in neurotransmitter systems in different parts of the brain accompanied by complex pattern of behavioural deficits (Chatterjee *et al.,* 2012a, 2015). For example, in the striatum, the domain representing positive symptoms, reports have shown that regulation of striatal dopamine and glutamate release might be mediated through enhanced 5-HT_{2A} receptor activity (Chatterjee *et al.,* 2012a). Also, literature data has shown that 5-HT enhances glutamate-mediated excitation of motoneurons particularly of the substantial nigra through 5-HT_{2A} and 5-HT_4 dopaminergic neuron based receptors (Hsiao *et al.,* 2002) and selective inhibition of metabotropic glutamate receptor (mGluR)-mediated component of glutamate response. This is beased on the observation that mGluR consists of a slow inhibitory inward current, and enhanced 5-HT activity shifts striatal neuronal response of glutamate towards excitation (Schreiber and Newman-Tancredi, 2014). Furthermore, in pyramidal neurons of the prefrontal cortex, where majority of negative and cognitive symptoms are simulated (Monte *et al.,* 2013), both 5-HT_2 and 5-HT_4 receptors modulate post-synaptically $GABA_A$-mediated effect (Feng *et al.,* 2001). In particular, enhanced 5-HT_2 receptors promote a phosphorylation of $GABA_A$ receptors via activation of protein kinase C (PKC) and of its anchoring protein RACK1 (receptor for activated C kinase), which ultimately reduces $GABA_A$–mediated chloride-currents and cortical inhibitory post-synaptic potential (IPSP) (Johnston *et al.,* 2014). Also, in the hippocampus, 5-HT modulates GABA release from specific subset of interneurons. Specifically, in the cornu ammonis-3 (CA3) pyramidal neurons, activation of 5-HT via $5HT_{A1}$ receptors selectively depresses the $GABA_B$ component of GABA-mediated IPSP (Winterer *et al.,* 2011). Moreover, 5-HT (via $5HT_{A1}$) and $GABA_B$ receptors interaction has been shown to increase G-protein activated inward rectifying K (GIRK) current, leading to suppression of hippocampal gamma oscillations on CA3 neurons thus, disrupting the balance of cortical inhibition and excitation (Winterer *et al.,* 2011; Johnston *et al.,* 2014). This is a cellular mechanism that has been proposed, at least in part, a cause of

cognitive symptoms of schizophrenia, and new target for novel antipsychotic drugs (Schreiber and Newman-Tancredi, 2014).

Indeed, it has been shown that some atypical antipsychotics (clozapine, risperidone, aripriprazole) act as 5-HT_{1A} or 5-$HT_{2A/C}$ receptor antagonists and/or inverse agonists (Meltzer *et al.,* 2011). Accordingly, 5-hydrxytryptaminergic modulation is thought to be crucial for the purported advantages of these medications over typical antipsychotic agents (chlorpromazine, haloperidol) (Linck *et al.,* 2012; Onaolapo *et al.,* 2016). Moreover, agonists of these receptors may also present a desirable antipsychotic-profile, since activation of 5-HT_{2A} receptors enhances dopamine release, whereas 5-HT_{2C} activation inhibits it (Linck *et al.,* 2012; Chindo *et al.,* 2012). For example, these drugs increase release of dopamine in the prefrontal cortex, which may explain their reduction of negative symptoms in schizophrenic patients (Johnston *et al.,* 2014). Additionally, it has been hypothesized that adult neurogeneration in the dentate gyrus may be stimulated with 5-HT_{1A} agonism (Schreiber and Newman-Tancredi, 2014), suggesting possible relief of cognitive deficits in psychiatric disorders (Johnston *et al.,* 2014). Finally, atypical antipsychotics with 5-HT_{1A} agonism reduce some side effects associated with typical antipsychotics, such as catalepsy, possibly by regulating excitation in cortical networks (Johnston *et al.,* 2014). Taken together, in this study, chronic injection of KET in the preventive and reversal treatments caused significant increases in 5-HT concentrations in the three brain regions (striatum, prefrontal cortex and hippocampus) suggesting a possible sensitization of 5-hydroxytryptaminergic transmission to repeated administration of KET via NMDA hypofunction, a disruption of 5-HT-mediated control over dopamine in the striatum and prefrontal cortex, as well as 5-HT-mediated control over glutamate- and GABA-facilitated transmission in the prefrontal cortex and hippocampus (Nazar *et al.,* 1999; Dean, 2001), which characterizes the positive, negative and cognitive symptom apparently observed in this study. These findings further support previous studies, which showed that KET induced alteration in 5-HT transmissions in the striatum, prefrontal cortex and hippocampus, and causes behavioural deficits due to altered sensorimotor gating mechanisms (Chatterjee *et al.,* 2012a, 2012b; Johnston *et al.,* 2014). The ability of morin to significantly prevent and reverse these alterations in striatal, prefrontal cortical and hippocampal 5-hydroxytryptaminergic transmissions similar to risperidone, may further suggests its atypical related antipsychotic-like activity particularly

against negative and cognitive symptoms in mice (Meltzer *et al.,* 2011; Linck *et al.,* 2012; Chatterjee *et al.,* 2012a, 2015).

Studies have documented altered glutamatergic transmissions in the pathophysiology of schizophrenia (Herrmann *et al.,* 2012; Lisek *et al.,* 2017). On the basis of clinical findings, it is also thought that the expression of the symptoms of schizophrenia is mediated by disruption of cortical glutamatergic neurotransmission and dysfunction in afferents and efferents of the nucleus accumbens, the main part of the striatum (Lisek *et al.,* 2017). It is known that it is the glutamate transport by astrocyte that determines the availability of released glutamate to pre- and post-synaptic receptors, as well as its spillover to extra-synaptic sites (Herrmann *et al.,* 2012). Remarkably, several studies have documented altered expression and function of astrocytic glutamate transporters in schizophrenia (Featherstone *et al.,* 2012; Herrmann *et al.,* 2012; Lisek *et al.,* 2017), and it has been suggested that an increased glutamate uptake by astrocytes may considerably contribute to its purported glutamatergic hypofunction (Herrmann *et al.,* 2012). Also, abnormal secretion of glutamate does not always reflect enhanced glutamatergic neurotransmission, as the excess of this neurotransmitter in the synaptic cleft can be efficiently taken up by the system of excitatory amino acid transporters (EAATs) (Lisek *et al.,* 2017). The EAAT2 is the principal mechanism of glutamate clearance from the synapse (Lisek *et al.,* 2017). Interestingly, KET-induced glutamatergic dysfunction have been found to partially cause over expression of mesolimbic membrane reuptake glutamate pumps and downregulation of cortical EAAT2 (Featherstone *et al.,* 2012; Lisek *et al.,* 2017); which is consistent with striatal glutamatergic (NMDA receptor) hypofunction to control dopamine release and cortical excitotoxicity due to over activation of non-NMDA receptors (Lisek *et al.,* 2017). Furthermore, another critical cellular functions affected by KET is calcium-dependent signaling (Sleigh *et al.,* 2014). In line with this, KET-mediated glutamatergic dysfunction has recently been partially attributed to plasma membrane calcium ATPase inhibition thereby, decreasing total calcium clearing potency; which raises the possibility of excessive glutamate release (Sleigh *et al.,* 2014; Lisek *et al.,* 2017).

Therefore, it is plausible to speculate that differential expression of glutamate transporters as well as cytosolic calcium overload can be novel mechanisms of psychogenic actions of KET, and modulating glutamate uptake can, thus, be regarded as a viable strategy to improve glutamatergic function. Notably, this mechanism may be compatible with the alleged advantages of atypical

over typical antipsychotics, which lie on; the ability of atypical antipsychotic drugs such clozapine, risperidone to counteract NMDA receptor induced behavioural effects (Chatterjee *et al.,* 2012b). Accordingly, typical antipsychotic agents such as haloperidol have been shown to be devoid of effect on glutamate uptake (Herrmann *et al.,* 2012). On the contrary, it has been previously reported that clozapine reduced both the expression of the glutamate transporter-1 (GLT-1) and glutamate uptake in astrocyte cultures (Herrmann *et al.,* 2012), as well as glutamate transport in rat prefrontal cortex (Lisek *et al.,* 2017). Moreover, the atypical antipsychotic agent, aripiprazole decreased gene expression of glial and neuronal glutamate transporters in cortical and hippocampal regions, suggesting a transcriptional mechanism to strengthen glutamatergic transmission (Herrmann *et al.,* 2012). However, in this study, repeated administration of KET decreased mesolimb glutamate concentrations in the striatum, which suggests striatal NMDA receptor hypofunction. In contrast, KET induced a significant increase in mesocortical glutamate concentrations in the prefrontal cortex, although markedly decreased glutamate concentration in the hippocampus, suggesting dysfunctional cortical glutamatergic neurotransmissions. The ability of morin to prevent and reverse the regional alterations of glutamate concentrations in the striatum, hippocampus, and prefrontal cortex, suggests a transcriptional mechanism to strengthen glutamatergic transmissions in a region-dependent manner.

The role of altered GABAergic neurotransmission via interplay between hypofunctional glutamatergic transmission and NMDA receptor in the pathophysiology of schizophrenia has been widely reported (Stahl, 2007; Behrens *et al.*, 2008; Chatterjee *et al.*, 2012a). Hence, estimation of changes in the 67 kilodalton isoform of GAD, the enzyme in charge of GABA synthesis from glutamate becomes imperactive. Remarkably, NMDA receptor antagonist (e.g., KET)-induced psychosis (positive symptoms - hyerlocomotion and stereotypy) has been partially attributed to the blockade of NMDA receptors located on the inhibitory GABAergic neurons in the mesolimbic brain regions (Chatterjee *et al.,* 2012a), resulting to disinhibition and increase in neuronal excitations, through a decreased GABAergic inhibitory regulation of NMDA-induced limbic-striatal dopamine and glutamate release (Curley *et al.,* 2013; Brown *et al.,* 2015); which suggests that altered GABAergic transmissions in the striatum could at least in part, contribute to exacerbation of positive symptoms (Chatterjee *et al.,* 2012a; Brown *et al.,* 2015). Furthermore, activity of GABAergic neurons particularly in the prefrontal cortex and hippocampus is essential for normal working memory function (Curley *et al.,* 2013), which suggests that altered GABA

neurotransmission in the prefrontal cortex and hippocampus could contribute to the cognitive impairments in schizophrenia patients. Consistent with this hypothesis, postmortem studies have repeatedly found reduced levels of mRNA for GAD_{67} and GABA plasma membrane transporter 1 (GAT-1) in the prefrontal cortex of individuals with schizophrenia (Curley *et al.,* 2013; Koh *et al.,* 2016). However, the affected GABA neurons include those that express the calcium binding protein parvalbumin (Behrens *et al.,* 2008). Parvalbumin-positive neurons include the chandelier subclass; reduced GABA signaling from chandelier cells to pyramidal neurons could contribute to working memory dysfunction via a pathophysiological mechanism involving decrease protein for GAD_{67} and parvalbumin-positive GABA neurons (Fujihara *et al.,* 2015; Koh *et al.,* 2016).

More specifically, parvalbumin-positive GABA neurons regulate the synchronized oscillatory activity of cortical pyramidal neurons in the gamma band range (30–80 Hz) (Lewis *et al.,* 2008). Gamma band oscillations in the human dorsolateral prefrontal cortex and hippocampal CA1 region increase in proportion to working memory load (Fujihara *et al.,* 2015). Consequently, altogether, a deficit in the synchronization of pyramidal cell activity, resulting from altered regulation by parvalbumin-positive GABA neurons due to decreased GAD_{67} immunoreactive, is hypothesized to contribute to both reduced gamma band oscillations and impaired dorsolateral prefrontal cortical- and hippocampal-dependent cognitive performance seen in schizophrenia patients (Curley *et al.,* 2013; Fujihara *et al.,* 2015; Koh *et al.,* 2016). In this study, repeated administration of KET in the preventive and reversal study, also significantly decreased the immunoreactivity of GAD_{67} expressions in the striatum, prefrontal cortex and hippocampus, which indicates decreases in GABA-mediated (synthesis and release) transmissions in the striatum, prefrontal cortex and hippocampus, suggesting prefrontal- and hippocampal-dependent cognitive control functions regulating working memory and response inhibition impairments (Lewis *et al.,* 2008; Koh *et al.,* 2016). This finding is congruent with previous investigations which showed that KET induced decrease in GABA-mediated transmissions via decreases in the GAD_{67} expressions (Behrens *et al.,* 2007, 2008; Fujihara *et al.,* 2015; Koh *et al.,* 2016). However, the result of this study shows that morin and risperidone significantly increased the expressions of GAD_{67} in all three brain regions examined, suggesting brain regional increase in GABAergic transmissions; which also might be compatible with the actions of neuroactive flavonoids on GABAergic enhancement in the CNS (Wang *et al.,* 2005).

Supportive evidence has shown that psychiatric disorders are associated with changes in signal transduction or alterations in growth factors in the nervous system (Fraga *et al.,* 2013). Brain derived neurotrophic factor (BDNF) is one of the major neurotrophic factors that primarily support the development, regeneration, survival and maintenance of neuronal functions (Janardhanan *et al.,* 2016). BDNF is synthesized by neurons in the striatum, frontal cortex and hippocampus of rodents (Fraga *et al.,* 2013). Specifically, BDNF facilitates synaptic transmissions via modulation of neurotansmistter release (Jovanovic *et al.,* 2000), formation and maintenance of axonal elongation (Dong *et al.,* 2012), and regulates synaptic density and cognition such as learning and memory (Baydyuk and Xu, 2014); which are the two cognitive parameters significantly affected in patients with schizophrenia (Janardhanan *et al.,* 2016). Remarkably, BDNF has been reported to promote the development of GABAergic neurons in the striatum, prefrontal cortex and hippocampus (Mizuno *et al.,* 1994) via induction of GABA-related proteins such as GAD_{67} (Mizuno *et al.,* 1994; Buckley *et al.,* 2011). Also, BDNF has been shown to prevent glutamate-induced neuronal death (Lindholm *et al.,* 1993). Moreover, decreased brain regional expressions of BDNF has been indicated in animal models of schizophrenia (Fraga *et al.,* 2013; Vasconcelos *et al.,* 2015), and in the brain/serum of schizophrenic patients (Buckley *et al.,* 2011; Zhang *et al.,* 2012b). Despite the fact that the mechanism of NMDA receptor hypofunction-associated decrease in BDNF levels is yet unknown, increasing body of evidence suggests possible implication of increased levels of cytokines (Roussos *et al.,* 2013) and pro-oxidants (Vasconcelos *et al.,* 2015); since neuroinflammation and oxidative stress have been shown to compromise synaptic plasticity and cell survival through decrease in cyclic response binding elements and activated proteins (Baydyuk and Xu, 2014). However, the beneficial effects of antipsychotic drugs particularly of atypical antipsychotics, at least in part, have been attributed to elevation of BDNF levels (Fernandes *et al.,* 2014). In this study, KET produced a decrease in the expressions of immunoreactive proteins of BDNF in a regional (striatum, prefrontal cortex and hippocampus), suggesting neuronal and neurotransmitter dysfunctions. This finding further supports previous investigation which showed that KET decreased BDNF expressions in the striatum, prefrontal cortex and hippocampus (Fraga *et al.,* 2013; Vasconcelos *et al.,* 2015). Thus, increment in BDNF expressions in the striatum, prefrontal cortex and hippocampus in KET-treated mice by morin, indicates that its neurotransmitter modulating and schizophrenia-like behaviour attenuating effects might be mediated in part, through the enhancement of neurotrophic factor; which might

also be compatible with the increased expressions of GAD_{67} observed in this study (Mizuno *et al.,* 1994).

Recently, cholinergic alterations have been recognized as major mediators to the pathological changes that characterized cognitive impairment seen in schizophrenia patients, particularly as it concerns outcome and prognosis (Tsai *et al.,* 2013; Chatterjee *et al.,* 2015; Vasconcelos *et al.,* 2015). Indeed, chronic administration of KET has increasingly become popular as suitable animal model that has been described to closely simulate pathological resemblance with that seen in schizophrenia patients; including cholinergic alteration (Chatterjee *et al.,* 2012a). KET induced memory impairment have been attributed to increase in the activity of AChE enzyme in the brain particularly in the prefrontal cortex and hippocampus; an action that may be responsible for its ability to reduce the concentration of acetylcholine in the brain and thus, cognitive impairment (Chatterjee *et al.,* 2012a, 2012b). Also, KET has been shown to alter glutamatergic neurotransmission and affect memory performance via the blockade of 7-alpha nicotinic cholinergic receptor (Chatterjee *et al.,* 2012a). The finding that morin decreased the enhanced AChE enzyme activity alone (whole brain) or in combination with KET, and/or LPS plus KET in the prefrontal cortex and hippocampus suggest its potential beneficial effect against certain schizophrenia symptoms associated with cognitive impairment. Moreover, repeated administration of morin alone was found to increase memory performance via decrease AChE enzyme activity in mice whole brains.

Overall, the extent of severity of schizophrenia-like behaviours upon KET treatments may not be entirely due to alterations in neurotransmitter systems and decreased expressions of brain neurotrophins. Oxidative and nitrergic stress as well as neuroinflammation have also been implicated in the pathological changes that characterized the behavioural perturbations seen in patients with schizophrenia (Pazvantoglu *et al.,* 2009; Vasconcelos *et al.,* 2015; da Silva *et al.,* 2017). Compelling lines of evidence has shown that oxidative stress, which is a state of an imbalance in the production of reactive oxygen species (ROS) and antioxidant defense systems (Wood *et al.,* 2009), serve as an indicator in the measurement of severity of schizophrenia-like behaviours and effectiveness of therapy; and levels of these antioxidants are modulated by antipsychotic therapy (Tsai et al. 2013; Onaolopa *et al.,* 2017). Although schizophrenia is a complex neuropsychiatric disease of multiple pathologies, functional deficits in central GABAergic neurotransmission have been postulated to be partially due to increased levels of the

superoxide-producing enzyme, nicotinamide adenine denucleotide phosphate (NADPH) oxidase-2 (Nox-2; gp91 Phox) (Behrens *et al.*, 2007; Koh *et al.*, 2016). This was based on the observation that enduring loss of pyramidal cells containing parvalbumin-positive GABAergic interneurons, particularly neuronal loss within the prefrontal cortex and hippocampus, correlated with cognitive deficits as well as impairments in behavioural response inhibitions seen in schizophrenia following cortical oxidative aberrations (Koh *et al.*, 2016).

Repeated administration of KET or LPS, bacterial endotoxin found in the outer membrane of Gram-negative bacteria, has been shown to induce persistent increase in superoxide by a calcium dependent-mechanism of up regulation of the pro-inflammatory cytokine, IL-6 and increased activation Nox-2 (gp91 phox subunit) (Qin *et al.*, 2004; Behrens *et al.*, 2007; Koh *et al.*, 2016). Notably, pyramidal cells containing parvalbumin-positive GABAergic neurons are highly vulnerable to ROS overload (Lisek *et al.*, 2017). Several studies have also shown that ROS initiate increased lipid peroxidation, which triggers degeneration of several neuronal populations including pyramidal neuron (Behrens *et al.*, 2008; Koh *et al.*, 2016). Accordingly, ROS-producing NADPH oxidase Nox-2 enzyme and ROS acts as a cellular signaling mechanism that initiates robust changes in cortical GABAergic interneurons (Behrens *et al.*, 2008), as well as dopamine D_2 and glutamatergic NMDA receptors (Sorce *et al.*, 2010); which are major molecular substrates implicated in the pathophysiology of schizophrenia and drug targets for the treatment of the disease (Chatterjee *et al.*, 2012a). Thus, this supportive evidence further suggests the role of oxidative stress in the pathogenesis of schizophrenia (Monte *et al.*, 2013; Vasconcelos *et al.*, 2015; da Silva *et al.*, 2017). Moreover, KET and LPS-induce schizophrenia has been linked to increase oxidative stress in the whole brain and in specific brain regions relevant for schizophrenia via regional activation of Nox-2 in the striatum, prefrontal cortex and hippocampus (Behrens *et al.*, 2008; Vasconcelos *et al.*, 2015). In this study, morin suppressed the activation of Nox-2 in the brain (striatum, prefrontal cortex and hippocampus) of mice treated with KET in the preventive and reversal study. Therefore, the attenuation of schizophrenia-like behavioural effect of morin observed in this study, at least in part, may be related to reduction of oxidative stress through suppression of Nox-2 (gp91 Phox) expressions.

Also, KET or/and LPS plus KET have been reported to deplete antioxidant enzymes such as GSH, SOD and CAT, thereby increasing the vulnerability of the brain cells to increased lipid peroxidation and nitrergic alteration, as evidenced by increased levels of MDA and NO

respectively. NO is a beneficial retrograde messenger or modulator, but in conditions such as oxidative stress, it is potentially toxic (Monte *et al.,* 2013). NO generation by activated microglia has been shown to cause excitotoxicity through induction of excessive glutamate release and inhibition of neuronal respiration (Chatterjee *et al.,* 2012a). However, reduced levels of GSH, leading to potential NMDA receptor dysfunction has been consistently reported to play a crucial role in the pathogenesis of schizophrenia (Herrmann *et al.,* 2012). Accordingly, the results of this study confirmed that the schizophrenia-like behaviours induced by KET or/and LPS plus KET respectively were accompanied by increased oxidative/nitrergic stress in the whole brain and in specific regions of the brain (Monte *et al.,* 2013; da Silva *et al.,* 2017), as indicated by elevated whole brain as well as striatal, prefrontal cortical and hippocampal levels of MDA, NO and decreased antioxidant systems (GSH, SOD and CAT). Thus, the ability of morin to prevent and reverse both KET or/and LPS plus KET induced schizophrenia-like behaviours in mice suggests mechanisms involving attenuation of oxidative and nitrergic stress in the brain. Moreover, administration of morin alone demonstrated significant decreases in MDA and NO concentrations, suggesting decrease in lipid peroxidation and nitrosation. Also, morin alone significantly increased GSH concentrations as well as enhanced SOD and CAT activities in mice whole brains thus, further suggesting antioxidant property; which is akin with previous studies (Gottlieb *et al.,* 2006; Selvakumar *et al.,* 2012; Chen *et al.,* 2017). GSH is a tripeptide consisting of glycine, cysteine and glutamate, which participates in essential aspects of cellular homeostasis (Herrman *et al.,* 2012). The cellular ability to synthesize GSH is an important factor in the management of oxidative stress-induced neurotoxicity (Herrman *et al.,* 2012). Therefore, the increase in GSH in the whole brain, specifically in the striatum, prefrontal cortex and hippocampus in this study, suggests a transcriptional mechanism to strengthen glutamatergic transmissions in favour other neurotransmissions.

Intraperitoneal injection of LPS (Zhu *et al.,* 2014), or KET (da Silva *et al.,* 2017) or both (Abdel-Salam *et al.,* 2015; Réus *et al.,* 2017) provides an excellent aetiological construct for the study of schizophrenia-like behaviour associated with neuroimmune activation. Systemic administration of LPS has gained evidence particularly as a suitable animal model that closely mimics the neurodevelopmental pathologic pathways including altered neuroimmunotransmitter-mediated psychobehavioural changes (Zhu *et al.,* 2014). This is based on the hypothesis that neuroimmune alterations such as maternal infection and increased microglia activation are consistently observed in

the brains of schizophrenic patients (Monji *et al.,* 2013; Bloomfield *et al.,* 2016), and in developmental animal models of schizophrenia in association with increased release of inflammatory enzymes and expressions of inflammatory proteins (Ribeiro *et al.,* 2013; MacDowell *et al.,* 2013; Zhu *et al.,* 2014). Myeloperoxidase (MPO) for example, has been established as a potent inflammatory enzyme stored in the azurophilic granules of polymorphonuclear neutrophils and macrophages, but released into extracellular fluid during inflammatory conditions (Al-Asmari and Khan, 2014). Moreover, evidence has shown that monocytes, leukocytes and microglia from the brains of schizophrenic patients have been reported positive for MPO activity (Al-Asmari and Khan, 2014). Consequently, increased MPO activity has been reported to be involved in enhanced generation of brain cytokines and oxidant-induced blood brain barrier (BBB) dysfunction (Üllen *et al.,* 2013), and bacterial meningitis-induced hippocampal neuronal damage (Liechti *et al.,* 2015). Remarkably, LPS- and KET-induced neuroimmune alterations have been found to be connected with increased brain levels of MPO respectively (Eduviere *et al.,* 2016; da Silva *et al.,* 2017). In line with this evidence, the study evaluated the effect of morin on LPS plus KET-induced neuroimmune alteration, based on MPO enzyme activity in specific regions of mice brains. Pretreatment of LPS prior to KET increased brain concentrations of MPO activity in the striatum, prefrontal cortex and hippocampus, suggesting anomalous neuroimmune influence. Thus, the beneficial effects of morin against LPS-enhanced KET-induced schizophrenia-like behaviours in this study may, in part, be attributed to suppression of neuroinflammatory processes through inhibition MPO enzyme activity.

Furthermore, neuroinflammation by LPS has been reported to occur via increased activation of microglia cells in a brain region-specific manner (Zhu *et al.,* 2014). This inturn has been shown to cause the release of pro-inflammatory cytokines (IL-6, TNF-α) and its associated psychobehavioural changes (Monji *et al.,* 2013). For example, TNF-α has been noted as one of the important early mediators of inflammatory diseases. It possesses potential toxic effects that results in hypersensitivity reactions with chronic inflammation leading to increased BBB permeability of other pro-inflammatory cytokines such as IL-6 and behavioural deficits (van Heesch *et al.,* 2013; da Silva *et al.,* 2017). Seemingly, IL-6 signaling is involved in several neuroprogressive actions in the brains of schizophrenia patients (da Silva *et al.,* 2017), including: severity of symptoms (Monji *et al.,* 2013), treatment resistance (Lin *et al.,* 1998) as well as influences on neurotransmitters (Zalcman *et al.,* 1994). Indeed, peripheral administration of IL-6 in animals has been reported to cause significant aterations in dopaminergic and 5-hydroxytryptaminergic turnover in the prefrontal cortex and hippocampus (Zalcman *et al.,* 1994). Remarkably, prenatal immune activation with LPS has been

reported to alter the development of mesolimbic and mesocortical dopaminergic system via increased release of TNF-α and IL-6, and decreased release of neurtrophic factors such as BDNF (Baharnoori *et al.,* 2013). In addition, LPS has been shown to trigger the upregulation of the neuroactive tryptophan catabolite (TRYCAT), kynurenic acid (KYNA) via increased production of the pro-inflammatory cytokine, TNF-α and IL-6, and oxidative stress (Schwarcz *et al.,* 2012; Schwieler *et al.,* 2015). Consequently, kynurenine is a pathologic endogenous NMDA receptor antagonist and a negative modulator of α7nACh receptor that regulates midbrain dopamine firing, 5-HT synthesis and cortical cholinergic neurotransmissions thus, causing cognitive impairments (Nilsson *et al.,* 2006; Schwarcz *et al.,* 2012; Linderholm et al. 2015). Moreover, increased levels of kynurenic acid have been consistently reported in brains (particularly prefrontal cortex and hippocampus) and cerebrospinal fluid of schizophrenic patients (Schwarcz *et al.,* 2012). Indeed, previous studies have also reported elevated brain levels of TNF-α and IL-6 in patients with schizophrenia and their positive correlations with increased severity of symptoms of the disease, especially of negative and cognitive symptoms (Monji *et al.,* 2013). However, it has been suggested that the relative capacity of antipsychotic drugs to normalize the release of pro-inflammatory cytokines may be an important contributing factor underpinning the clinical efficacy of antipsychotic drugs. In this study, it was observed that the schizophrenia-like behaviour produced by the co-administration of LPS and KET was accompanied by increased concentrations of TNF-α and IL-6 in the striatum, prefrontal cortex and hippocampus, suggesting psychopathological changes due to neuroinflammation (Zhu *et al.,* 2014; Abdel-Salam *et al.,* 2015; da Silva *et al.,* 2017). Thus, the ability of morin to attenuate elevated levels of TNF-α and IL-6 in the striatum, prefrontal cortex and hippocampus in LPS-KET-induced neuroimmune alteration in mice may, at least in part, be playing a role in its antipsychotic effects.

In addition to proinflammatory cytokines, resistant schizophrenic patients have also showed increased expressions of key inflammatory proteins such as COX-2, iNOS and NF-κB, which may play a prominent role in the disease neuroprogression through their communications with proinflammatory cytokines (MacDowell *et al.,* 2013). Indeed, the negative and cognitive symptoms induced by LPS have also been linked to up-regulation of different inflammatory proteins including COX-2, iNOS and NF-κB (MacDowell *et al.,* 2013; Ribeiro *et al.,* 2013). COX-2 for example, has been shown to be involved in neurotransmitter modulation and cortical activity-dependent synaptic remodeling via increased production of prostaglandin-E_2 (PGE_2) (Müller *et al.,* 2010b). PGE_2 is a molecule of the pro-inflammatory cascade, already described to be overactive in this disease as an

immunotransmitter and may stimulate the production of pro-inflammatory cytokines, e.g., IL-6 (Müller *et al.,* 2010b). However, suppression of COX-2 expressions has been reported to attenuate schizophrenia-like behaviour in LPS-treated animals (MacDowell *et al.,* 2013). Deserves mentioning that, adjunctive therapies using anti-inflammatory drugs such as minocycline (Monte *et al.,* 2013) and non-steroidal anti-inflammatory drugs such as celecoxib (Müller *et al.,* 2010b) have been hypothesized and favorable results have been demonstrated (Levkovitz *et al.,* 2010). Thus, it might be speculated that the antipsychotic-like effect of morin observed in the LPS plus KET study, may also be related to suppression of neuroimmune activation via inhibition of the up-regulation of COX-2 expression in the striatum, prefrontal cortex and hippocampus of mice brains. Also, neuroimmune activation is also accompanied by increased expression of iNOS in different brain regions and is responsible for the augmented production of NO (Ribeiro *et al.,* 2013). Previous studies have shown that increased iNOS expressions causes nitrergic stress-induced excessive glutamate release and excitotoxicity, and increased generation of free radicals (Chatterjee *et al.,* 2012a, 2015; Ribeiro *et al.,* 2013). Thus, the ability of morin to suppress LPS plus KET-induced expressions of iNOS in the striatum, prefrontal cortex and hippocampus, suggests that its antipsychotic effect might be partially mediated via inhibition of NO production.

Furthermore, previous studies have shown the existence of a positive correlation between elevated pro-inflammatory cytokines (e.g., TNF-α and IL-6) and NF-κB activation in neuroinflammation (Song et al., 2009; Roussos *et al.,* 2013; Jung *et al.,* 2017). Moreover, pathway analysis using schizophrenic patient genes underpins NF-κB as a hub, where different, diverse signal transcription factors and cytokines, critical for schizophrenic genetic vulnerability factors, converge (Song *et al.,* 2009; Roussos *et al.,* 2013). Accordingly, LPS has been shown to cause increased expressions of NF-κB (Zhang *et al.,* 2016), and its associated inflammatory responses via altered transcriptional machinery, which in turn induces continuous neuroinflammation, dysfunctional neurotransmitter systems and synaptic plasticity consistent with the neuropathology of schizophrenia (Zhu *et al.,* 2014). Chronic neuroinflammation in the brain of schizophrenic patients increases the levels of cytokines, oxidative/nitrergic stress and decreases in neurotrophic factors like BDNF due to regional-brain imbalance of volume neurotransmissions, particularly of dopamine and glutamate; which leads to progressive neurodegeneration and severity of the schizophrenia-like behaviours (Song *et al.,* 2009; Buckley *et al.,* 2011; Monji *et al.,* 2013). The results of this study confirmed that schizophrenia-like behaviours induced by co-administration of LPS and KET was accompanied by increased

neuroinflammation, as evidenced by elevated regional brain (striatum, prefrontal cortex and hippocampus) translocational expressions of NF-κB protein (Abdel-Salam *et al.,* 2015; Jung *et al.,* 2017). Thus, the ability of morin to suppress KET plus LPS-induced regional neuroinflammation critical to schizophrenia pathology, suggests that its antipsychotic effect might, at least in part, be mediated via down regulation of NF-κB expressions. Accordingly, the result presented here about the preventive effect of morin on LPS-enriched expressions of inflammatory molecules and proteins in KET-induced schizophrenia-like behaviours further support a multi-target antipsychotic property of morin.

Increasing body of evidence has shown that neuronal loss is a typical characteristic of many neuropsychiatric diseases. Studies have shown that reduction in viable neuronal cells might alter physiological activity such as neuronal receptor expression, dendritic arborization, synaptic plasticity as well as neurotransmissions, which may eventually result in impaired neuronal function (Vutskits *et al.,* 2006). However, there are also mounting evidences in literature that oxidative stress from excessive generation of ROS is strongly implicated in reduction of neuronal cells in specific brain regions in patients with neuropsychiatric disease such as schizophrenia (Sorce *et al.,* 2010). Accordingly, previous studies have also reported that repeated administration of KET has been shown to induce neuronal loss particularly, of pyramidal cells containing parvalbumin-positive GABAergic interneurons via increased cytokine release and activation of ROS-producing NADPH oxidase Nox-2 enzyme as previously described (Behrens *et al.,* 2008; Koh *et al.,* 2016).

Although reducing oxidative and nitrergic stress, as well as glutamatergic hypofunction induced by KET can be advantageous in alleviating schizophrenia-like behaviours (Monte *et al.,* 2013), convincing evidence in literature has confirmed the ability of compounds with antioxidant and anti-inflammatory potentials to prevent neuronal degeneration by oxidative stress, improve dendritic development and survival of GABAergic neurons (Behrens *et al.,* 2008; Koh *et al.,* 2016). Moreover, test compounds with antioxidant property have been shown to alleviate oxidative- and neuroinflammation-induced schizophrenic behaviour (Zhu *et al.,* 2014). Thus, ability of morin to prevent and reverse neuronal alterations in all the brain regions examined in all brain histological and histomorphological studies, suggests neuroprotection. Notably, administration of morin alone significantly increased viable neuronal cells relative to vehicle-treated mice. Furthermore, the results from this study confirmed that oxidative- and

neuroinflammation-induced schizophrenic behaviour by KET and LPS are accompanied by loss of prefrontal cortical pyramidal neuron and dendritic remodeling (Wischhof *et al.,* 2015; Koh *et al.,* 2016); which are morphological subfields implicated in cognitive dysfunctions (Koh *et al.,* 2016). An *in vitro* study showed that inflammatory cytokines such as TNF-α, IL-1β, and IL-6 inhibit development of dendrites in embryonic cortical neurons, consistently with the neuropathology of schizophrenia (Romero *et al.,* 2007). Previous studies have also consistently reported decreased soma size and dendritic arbors of the prefrontal cortical pyramidal neurons in patients with schizophrenia, which is compatible with the severity of the cognitive impairments that characterize the disease (Glantz and Lewis, 2000; Hu *et al.,* 2015). Thus, ability of morin to increase the cortical somata size and dendritic arborazation of pyramidal neuron, further suggests neuronal protection particularly of GABAergic interneurons in the prefrontal cortex, normalization of cortical inhibition and excitation and prefrontal cortical-dependent higher-order CNS functions such as cognitive performances (Yuchio *et al.*, 2015; Koh *et al.*, 2016).

However, whether morin exert atypical-like antipsychotic activity similar to risperidone, as a potential agent for the treatments of schizophrenia-like behaviours, need further investigation, particularly against the negative and cognitive symptoms. Of note, increasing lines of evidences indicates that dietary polyphenolic compounds can cross the blood brain barrier to exert beneficial neurological activity (Santos *et al.,* 1998; Youdim *et al.,* 2004; Eduviere *et al.,* 2016; Annafi *et al.,* 2017). However, in the context extrapyramidal side effects, the test for catalepsy e.g., extrapyramidal symptom revealed that morin is devoid of cataleptic behaviour in mice in a similar manner to risperidone, but not haloperidol. Furthermore, rota rod test showed that the antipsychotic effect of morin was not due to weakness in neuromuscular strength. Indeed, it is worthy to state here that morin is generally regarded as safe and a non-toxic naturally occurring polyphenol with established health benefits and widely consumed as dietary supplements in the form of fruits and vegetables (Caselli *et al.,* 2016). Previous toxicological studies revealed that morin exhibits very low toxicity profile, and its chronic administration is well tolerated and safe as a natural bioflavonoid: following 5% (w/w) dietary administration of morin for 13 weeks (Cho *et al.,* 2006), and/or oral administration of morin at 200 mg/kg body weight for 14 days (Jonnalagadda *et al.,* 2013). Moreover, morin shows has been reported to elicit systemic protective actions, reducing negative side effects of several drugs, without interfering with their functions (Caselli *et al.,* 2016). Also, the use of morin to increase absorption and effectiveness of

drugs as ‘bioenhancer’ has been proposed and beneficial outcomes have been demonstrated (Suzuki and Sugiyama, 2000; Singh et al., 2010; Caselli et al., 2016). All these safety profiles suggest that morin could be a promising natural drug (Caselli *et al.,* 2016) that could be used as a potential agent in the management of debilitating neuropsychiatric disorder like schizophrenia requiring almost a life time medication.

CHAPTER SIX

6.0. SUMMARY AND CONCLUSION

The results of this study revealed that morin significantly reduced spontaneous motor activity and attenuated KET-induced hyperlocomotion in mice. Also, morin suppressed APO- and KET-induced stereotypes. The increase in immobility in forced swim test and social withdrawal due to KET or/and LPS plus KET administrations were reduced by morin in a significant manner. The results of this study also revealed that morin significantly enhanced cognitive functions when administered alone and improved memory performances in mice treated with intraperitoneal injection of KET or/and LPS plus KET comparably to risperidone but not haloperidol. Moreover, the decreased social recognition memory due to administration of LPS in combination with KET was significantly increased by morin. Regarding catatonic behaviour, morin demonstrated significant antipsychotic effects devoid of extrapyramidal side effects, as evidenced by lack of alteration of descent latency and time of performance of mice in the wood block catalepsy and rota rod tests, respectively.

One significant finding in this study is the observations that morin similarly to risperidone, but not haloperidol modulates biogenic amines, cholinergic, neurotrophic, inflammatory, oxidative and nitrergic pathways in different part of mice brains (striatum, prefrontal cortex and hippocampus) to mediate its antipsychotic-like activity. More specifically, morin significantly modulated the levels and/or transmissions of dopamine, glutamate and 5-HT as well as enhanced GABAergic transmissions via increase in GAD_{67} expressions in brains of mice treated with KET in a brain region-dependent manner. The increase in acetylcholinesterase activity in mice brains following repeated administration of KET, or/and LPS plus KET were significantly reversed by morin. Also, morin enhanced neurotrophic activity via increase in BDNF expressions in the striatum, prefrontal cortex and hippocampus. Importantly, morin prevented inflammatory responses via decreaments in the levels of MPO, TNF-α, IL-6 as well as inhibition of COX-2, iNOS and NF-κB immno-expressions in the striatum, prefrontal cortex and hippocampus of mice brains treated with LPS plus KET. Furthermore, morin signficiantly increased the activities of superoxide dismutase and catalase, enhanced glutathione concentrations in the brains of mice subjected to repeated administration of morin alone or in combination with KET, or/and LPS plus KET; as compensatory mechanisms of action. Moreover, morin decreased malonyladehyde

and nitrite concentrations alone or in combination with KET, or/and LPS plus KET, which are key biomarkers of oxidative and nitrergic stress in mice brains. Morin also demonstrated antioxidant activity and reduced oxidative stress by suppressing the expressions of Nox-2 (gp91 Phox subunit) in the striatum, prefrontal cortex and hippocampus of mice brains. On the other hand, morin demonstrated significant neuronal protection against KET or/and LPS plus KET-induced neuronal alteration in mice in a region-dependent manner; specifically by decreasing neuronal death in a region-specific manner, and increasing dendritic spine arborizations and and soma size of pyramidal neurons of the prefrontal cortex of mice brains.

In conclusion, this study showed that morin attenuated schizophrenia-like behaviours (positive, negative and cognitive symptoms) in experimental animal models, and devoid of cataleptogenic effects relevant to extrapyramidal side effects. These effects may be via mechanisms related to modulations of psychotropic biogenic amines, cholinergic transmissions through inhibition of acetylcholinesterase activity and enhancement of neurotrophic factor; inhibition of inflammatory mediators, oxidative and nitrergic pathways, as well as neuronal protections of different parts of mice brains (striatum, prefrontal cortex and hippocampus). Taken together, these findings suggest that morin may be relevant in the management of schizophrenia-like behaviours, thus supporting its development as an antipsychotic agent.

6.1. Contribution to knowledge

The study provides comphrensive evidences for the antipsychotic-like effects of morin in experimental animal models in mice.

Through this study, a compactable animal model combining LPS-induced neuroinflammation with ketamine-induced schizophrenia (LPS enhanced KET-induced schizophrenia), a ‘two-hit’ animal model of schizophrenia showing the involvement of inflammatory response in relation to schizophrenia-like behaviour was developed.

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