CHAPTER ONE

INTRODUCTION

1.1 MEDICINAL PLANTS

Medicinal plants are the most ancient sources of drugs for the treatment of both human and animal diseases (WHO, 1995).

Medicinal plant is defined as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or whose precursors may be used in the synthesis of useful drugs (WHO, 1995).

According to Le Strange (1977), one of the earliest records of the use of medicinal plants is that of *Hydnocrapus gaenn* from which *chamulmoogra* oil was obtained and found effective in the treatment of Leprosy (Le Strange, 1977).

Similarly, records confirm the use of the seed of Opium poppy (*Papaver somniferum L*) and castor oil seed (*Ricinus communis*) in Egypt for medicinal purposes even till date. Almost one-quarter of all medicines are derived from the over 250,000 flowering plants on the earth's surface and some of the secondary metabolites therein may be toxic to lower beings and/or man. Their use in the crude or refined form is of utmost interest in the efforts aimed at integrating herbal with orthodox medicine.

With the understanding that, only one in eight of the potential drugs were discovered in the rainforest worldwide (Cox and Balick 1996). The efficacy of plants extract is due to the presence of one or more biologically active principles. Pharmacological assays have shown that the activity is not always due to the main component of the plant, but sometimes minor ones, or even to the synergism of all or some of the active principles (Galeffi, 1980).

With modern advances in the techniques for isolation and structure determination of active principles even minute amounts of the principles can be isolated and their structures determined (Salemink, 1980).

Phytochemicals isolated from medicinal plants can be used as agents or starting materials in the synthesis of drugs. The use of herbs as medicine is very common in developing countries, particularly in the rural setting. Traditional medicine as a major African Socio-cultural heritage has been in existence for hundreds of years. It was once believed to be primitive and wrongly challenged by foreign religions dating back during the Colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners.

Traditional medicine has been the focus now for wider coverage of primary health care delivery in Africa and the rest of the world (Elujoba *et al.*, 2005).

WHO (1978) defined traditional medicine as the sum total of knowledge or practices neither explicable or inexplicable used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observation handed down from generation to generation, verbally or in writing.

It comprises of therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and it still in use today without much documented evidence of adverse effect. This traditional medicine comprised the use of plant, animal or mineral materials for healing (WHO, 1978), but the focus in this study is phytomedicine (plant medicine) or simply medicinal plants usage.

Iwu *et al.*, (1999) reported that the first generations of medicinal plants were simple botanical materials employed in more or less crude form. These medicines such as Chincona, Opium, Belladona and Aloe were selected based on empirical evidence as gathered by traditional practitioners.

The second-generation phytopharmaceutical agents were pure molecules whose compounds differ from the synthetic therapeutic agents only in their origin, for example, taxol from *Taxu spp*; quinine from *Chinchona* and reserpine from *Rauvolfia spp* (Iwu *et al.*, 1999).

In the development of third generation of plant medicine, the formulation is based on well-controlled, double blind, clinical and toxicological studies with phytomedicine to improve the quality, efficacy, stability and safety of preparation (Akerele, 1993).

Phytomedicine has played a key role in world health care (Calixto, 2000) with about 80% of Africans depending on it. Medicinal plant has demonstrated its contribution to the reduction of excessive mortality, morbidity and disability due to disease such as malaria, tuberculosis, sickle cell anaemia, diabetes and mental disorders (Elujoba *et al.*, 2005). It has reduced poverty by increasing the economic well-being of communities and developed health system by increasing health coverage to the people (Elujoba *et al.*, 2005).

Also, in veterinary practice, some medicinal plants are known to be specifically useful for the treatment of ailments of domestic animals. These plants include, *Achyranthes aspera*, which has been used for the treatment of cough in domestic animals; *Balanites aegyptiaea* for constipation; *Bombas ceiba* for bone dislocation; *Cleome gynanadra* for wounds*; Hyptis suaveslens* for conjuctivitis; *Plumbago zeylanica* for stomach troubles while *Salvadora oleoides* is used for the treatment of throat swellings in domestic animals (Sikawar, 1994).

Medicinal plants are now very popular in developing countries. Moreover, during the last decade, an increase in the use of plants has been observed in the metropolitan areas of developed countries (Harnack *et al.*, 2001). There is high necessity for knowledge about their biological activities, safety and efficacy and quality assurance of medicinal plants as reported by Calixto, (2000).

The study on the toxicity and medicinal activities of these plants is necessary because some of them although may be truly medicinal, they could also have some toxic potentials that may necessitate caution in their use and also quite a number of these medicinal plants may have other valuable medicinal capabilities that have not been explored or elucidated.

1.2 AIM AND SCOPE OF THE STUDY

Almost one-quarter of all medicine are derived from the over 250,000 flowering plants on the earths surface (Iwu, 1999). Their recent increased use in the treatment of all forms of ailments necessitates a more detailed study on the true medicinal value of these plants and also a thorough study of their toxicity potential.

This study evaluates the pharmacology as well as the toxic potential of the following five plants; Tithonia *diversifolia* (Hemsl.) A. Gray, *Acalypha wilkesiana* (Mull. Arg), *Lippia multiflora* (Moldenke), *Ocimum gratissimum* Linn and *Morinda morindoides* (Baker) Milne-Redh.

Recent studies have shown that most of these plants being regarded as medicinal herbs are toxic and can be carcinogenic to man and animals (Mereto *et al.*, 1996). Most of the traditional medical practitioners who use medicinal plants only deal with the efficacy of the herbs without taking into consideration the hazardous side effects that such herbs could cause. *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora*, *Ocimum gratissimum* and *Morinda morindoides* are plants which grow among pastures in Nigeria, grazing livestock are therefore exposed to them. Many of such plants serve as source of poison to livestock (Abatan, 1992).

The aims of this study are therefore to:

(a) Screen for

(i) toxic potential of the plants using haematology, serum biochemistry and histopathological parameters as indices of toxicosis

(ii) antifertility effect using sperm count and spermatozoa defects

(iii) antidiabetic potentials of the plants using alloxan induced hyperglycaemia in rats

(iv) analgesic activity using hot plate and acetic acid induced writhing movement methods and

(v) anti-inflammatory effects using carageenan induced right paw oedema method.

(b) Carry out bio-activity of chromatographic fractions, isolate bioactive agents from the plants and possibly determine the structure of these active compounds.

1.3 SIGNIFICANCE OF THE STUDY

The cost of orthodox health care delivery is on the increase daily, people in rural areas now find solace in patronizing the traditional physicians or use of herbs in treating their ailments and that of their animals due to their low cost and easy accessibility. Also, herbal medicine has become a useful compliment to the various modern drugs in the market and most, if not all medicines now contain medicinal plants constituents, which are considered generally essential and important aspect of our cultural heritage from which enormous economic and social benefits could be derived from local cultivation of medicinal plants (Adesina, 1998).

One major problem facing traditional medicine is the little or no knowledge of the toxicity of their products. Thus, some of these remedies have caused havoc that is even more than their life saving effect and this study will therefore determine the veracity of the pharmacologic effects of these plants, explore other likely medicinal potentials of the plants yet to be documented. The findings of this study will also reveal the likely toxic potentials of these herbs and their products.

CHAPTER TWO

LITERATURE REVIEW

2.1.1 *Tithonia diversifolia* (Hemsl.) A. Gray:

Tithonia diversifolia (Hemsl.) A. Gray (Plate1) is a plant that has been commonly used for diverse medicinal purposes in most parts of the world. The antiplasmodial activity of *T. diversifolia* was reported by Goffin *et al*., (2002), who isolated lactone targitin C showing activity against plasmodium organism and it was also observed to be cytotoxic. Madureira *et al., (*2002) reported the activity of *T. diversifolia* against chloroquine resistant *Plasmodium falciparum* and also reported the hepatic schizontocidal activity. Manobjyotic *et al.,* (1996) reported the artemisinic structure of the mature stem of *T. diversifolia*.

Bork *et al.,* (1996) observed that *T. diversifolia* has an anti-inflammatory and antibacterial activity and this was further confirmed by the work of Rungeler *et al., (*1998). Tona *et al.,* (1998, 1999; 2000) observed the antibacterial and antiamoebic properties of the aqueous extract of *T. diversifolia.*

Hamowia and Saffaf (1994), corroborated by the work of Jayawardena *et al.,* (2000) reported that the development and hatchability of gastrointestinal nematode eggs in goat faeces were reduced with increasing concentrations of *T. diversifolia* leaf extract.

Hamowia and Saffaf (1994) also reported on the antitumor activity of *T. diversifolia*, that sesquitepeniods from *T. diversifolia* have potential cancer chemopreventitive activity. Miura *et al.*, (2002) also reported that it exhibit antidiabetic effects as its aqueous extract improve the blood glucose level of KK-Ay mice by reducing insulin resistance.

I. *TITHONIA DIVERSIFOLIA* PLANT

Plate 1*:* *Tithonia diversifolia* (Hemsl.) A. Gray leaves

Names: *Tithonia diversifolia* (Hemsl.) A. Gray, sun flower

Family: Asteracea

Location: World wide

2.1.2 *Acalypha wilkesiana* (Mull. Arg)

This is another herbal plant whose leaves has been used traditionally as a diuretic in the management of hypertension (Kokko, 1984) and in the treatment of gastrointestinal and dermatological disorders (Oluyemi *et al.*, 1998). In Trinidad, for naso-pharyngeal infections and pain, the leaf-poultice is deemed good for headache, swellings and colds (Burkill, 1985).

As antibiotic agent, the leaf-extract is active against Gram +ve bacteria. The extracts were screened against *Salmonella enteritidis*, *Escherichia coli* and *Stapylococcus aureus* by standard methods. The results of antimicrobial activity showed that water and ethanol crude extracts were active on all the strains of pathogens tested at different concentrations, with ethanol extracts exerting more activity (Akinyemi *et al.*, 2006). Gallic acid, corilagin and gerenin compounds from the leaf extract have been said to be responsible for the observed antimicrobial activity (Adesina *et al.*, 2000). Its bacteriocidal effect was also found to vary among different bacteria species with Gram negative ones like *Escherichia coli, Klebsiella spp and Pseudomonas spp* being killed at lower concentration than the gram positive organisms like *Bacillus cereus, Bacillus subtilis and Staphylococcus spp.*

The release of sodium and potassium ions was found to be the mechanism of action of the extract and the amount leaked vary from one organism to the other (Oladunmoye *et al*., 2006).

As antifungal agent, the aqueous extract of *Acalypha wilkesiana* (Mull. Arg) (Plate 2) has been used for decades by the local inhabitants for treating skin problems. The water extract of *A. wilkesiana* was subjected to a clinico-laboratory study to assess its efficacy i.e. ability to clear standard organisms in agar plates and to stop symptoms and clear skin lesions of eczema cases in a 3-week study period. The extract showed significant antibacterial and antifungal properties in vitro and was found to be reasonably useful in the treatment of eczema. In a depressed economy as found in most developing countries of Africa, medicinal plants like *Acalypha wilkesiana* could be used as a cheap and readily available source of skin medication.

Jekayinfa *et al.*, (1997) reported that the leaves of this plant possess a broad spectrum of activity against fungi and bacteria and also a haemolytic activity in-vitro. It has been reported to contain some toxic agents like tannins, glycosides, anthroquinone (Oladunmoye *et al*., 2006) which can cause damages to some organs like the liver, kidney and the intestines.

II. *ACALYPHA WILKESIANA* PLANT

Plate 2: *Acalypha wilkesiana (*Mull. Arg*)* leaves

Names: *Acalypha wilkesiana* (Mull. Arg), Copperleaf, "Jinwini", "Eela"

Family: Euphorbiaceae

Location: World wide

2.1.3 *Lippia multiflora* (Moldenke)

The Verbenaceae is a large family of perennial herbaceous plants and is composed of 41genera with about 200 species. *Lippia multiflora* Moldenke (Plate 3) is a shrubby aromatic plant, growing up to 1.2 m with whitish flowers on cone-like heads in a terminal panicle, and nearly 12 mm long. It is widely distributed in West and Central Tropical Africa (Pascual *et al.*, 1996; Terblancche and Kornelius, 1996; Pooley, 1998). In Nigeria, it is found along forest, savannah, transitional and coastal savannah zones. Locally the plant is named 'Efinrin gogoro', 'Efinrin odan' or 'Efinrin Ajase' according to the some parts of South West Nigeria.

L. multiflora is used as traditional and herbal medicine to treat bronchial inflammation, malaria fever, conjunctivitis, gastrointestinal disturbance, enteritis, coughs and colds (Pascual *et al., 1996*), and possesses hypertensive, fatigue-relieving and diuretic properties (Kanco *et al.*, 2004). Some rural dwellers cook the herbs and use it to relieve stress and enhance sleep (Etou-Ossibi *et al.*, 2005). Traditionally, *L. multiflora* has been used as a substitute for tea and as a mouth disinfectant (Menut *et al.*, 1995). Scientifically, the oil from the plant has been reported to have insecticidal and pesticidal properties against body lice and has also shown marked antimicrobial activity (Avlessi *et al.*,

2005). It has been reported that the essential oil composition of *L. multiflora* from some locations were characterized by high terpenoid content, in particular: 1,8-cineole (Kanko *et al*., 2004; Avlessi *et al*., 2005), linalool, geranial and neral, ipsdienone and (*Z*)- and (*E*)-ocimenone, thymol and thymylacetate, *p*-cymene, sabinene, _-terpineol, _-phellandrene, myrcene and epoxymyrcene, myrtenol and limonene (Koumaglo *et al*.,1996; Kanko *et al*., 2004; Avlessi *et al*., 2005; Juliani *et al*., 2008).

III. *LIPPIA MULTIFLORA* PLANT

Plate 3: *Lippia multiflora* (Moldenke) Leaves

Names: *Lippia multiflora* (Moldenke); "Effirin odan", "Efirin Ajase"

Family: Verbenaceae

Location: Savannah and Coastal Savanna

2.1.4 *Ocimum gratissimum* Linn.

Ocimum gratissimum (Plate 4) belongs to the group of plants known as spices. The plant is an erect, small plumb with many barnacles usually not more than 1 m high (Vierra *et al*., 2001). It is of the family Labiatea, genus *Ocimum* and species *gratissimum* (Iwu, 1993). The plant is found throughout the tropics and subtropics and its greatest variability occurs in tropical Africa and India. In South East Asia, it is cultivated as a home garden crop and it is grown on a commercial scale in Vietnam. It is used for a variety of reasons. In culinary, it is used in salads, soups, pastas, vinegars and jellies in many parts of the world. The Thai people are popularly known to use it in food flavouring. In traditional medicine, the leaves have been used as a general tonic and anti-diarrhoea agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis, also the dried leaves are snuffed to alleviate headaches.

IV. *OCIMUM GRATISSIMUM* PLANT

Plate 4: O*cimum gratissimum* Linn. plant

Names: *Ocimum gratissimum*, "Efirin"

Family: Lamiacaea

Location: Tropics and subtropics

2.1.5 *Morinda morindoides* (Baker) Milne-Redh

Plants of the *Morinda spp* (Family- Rubiaceae) (Plate 5) are important plants in traditional medicine. In Nigeria, *M. lucida* is one of the four most used traditional plants against fever (Burkill, 1997). The plant is also used as flavouring for food and as chewing stick. Other species of *Morinda* plant are *M. morindoides, M. citrifolia* (noni), *M. longiflora* and *M. germinate*. *M. morindoides* popularly called brim stone tree in English, "oju ologbo" in Yoruba (South west Nigeria),is a climbing, glabrous shrub that grows up to 10m high and is widely distributed in different parts of Africa such as Senegal, Cameroun and Nigeria (Burkill, 1997). *M. morindoides* has been used as decoctions among the natives against malaria (Awe *et al.*, 1998), diarrhoea, haemorrhoids, gonorrhoea, amoebiasis, rheumatism and other inflammatory diseases (Kambu, 1990). Biological studies have supported some of these traditional uses (Tona *et al.*, 2004). Previous phytochemical investigation on the leaves of *M. morindoides* led to the isolation of nine flavonoids and flavonoids o-glycosides (Cimanga *et al.*, 1995).

V. *MORINDA MORINDOIDES* (Baker) Milne-Redh PLANT

Plate 5: *Morinda morindoides* (Baker) Milne-Redh leaves

Plate 6: *Morinda morindoides* (Baker) Milne-Redh root
Names*: *Morinda morindoides
***Morinda morindoides* (Baker) Milne-Redh**
English: brimstone tree (Nigeria, Oliver).
Yoruba: ojú ológbó = cat's eyes;
***Family*: RUBIACEAE**
***Location*: West Africa**

2.2.0 MEDICINAL PLANTS

Suresh *et al.*, (1994) have investigated the phytochemical and pharmacological activities of 25 medicinal plants, commonly used in various experimental models such as CNS and analgesia. Plants, such as *Araucaria bidwill, Branchylepsis nervosa, Gongronema latifolium and Chasmathera dependens* have also been reported to have analgesic activities (Olugbenga *et al.*, 2002). According to Phillipson (1994), the importance of medicine of natural product molecule lies not only in their pharmacological or therapeutic effects but also in their role as template molecules for the production of new drug substance. Morphine from *Papaver somniferum* is used as an analgesic for the relief of terminal pain. It also serves as a template molecule for the development of analgesic drugs such as pethidine and pentazocine. The bisbenzylisoquinoline alkaloid, tubocurarine from *Chondrodendron tomentosum* is a potent muscle relaxant. In the developing countries, it is estimated that more than one billion people live in extreme poverty and huge number of them suffer and die for the want of safe water and medicines (WHO, 1995). In these environments, the populations rely on traditional medicine and local medicinal plants for primary health care (WHO, 1995). In addition, more than 80% of the World's population relies solely on indigenous systems of medicines which are primarily plant-based. The World Health Organization estimates that some 2000

species of higher plant are used medicinally throughout the world. In the compilation, Wagner and Farnsworth, (1985) described the uses of medicinal plants in countries such as China, Ghana, India, Japan, Mexico, Panama, Samoa and Thailand. All these countries use plant extracts and frequently several plants are combined in one prescription. It is believed that so many ingredients serve to provide the overall activity and that clinical activity cannot be attributed to a single active ingredient. This concept differs from the "Western" approach in which plants are extracted and fractionated in order to obtain a single active ingredient (Phillipson, 1994). An example to support this argument is artemisinin, the active principle isolated from *Artemisia annua*. An aqueous extract of the herb, which is used in traditional medicine, contains many other compounds. Some flavonoids have been isolated from *A. annua*, which showed synergistic effect on the activity of artemisinin against *Plasmodium falciparum* in vitro (Elford *et al.*, 1987; Liu *et al.*, 1992). Practitioners have over the years sharpened their skills so as to differentiate useful plants up to the species level. Thus Samoan people in the South Pacific use the aqueous extract of pulverized wood of *Homalathus notans* to treat yellow fever. The bark of *Erythrina variegate* is also applied to the skin to treat inflammation (Cox and Ballick, 1994).

By 1990, 25% of prescribed medicines were substances derived from plants (Fansworth and Bingel, 1977; Principe, 1989; Hamburger and

Hostettman, 1991). A non significant percentage of the plant derived chemical synthesis or semi-synthesis produces chemical compounds in the market. The rest like vincristine, vinblastine, and reserpine are extracted and purified directly from plants (Newal *et al.,* 1986; Farnsworth, 1990). The convention over the years in natural product research and drug development from natural sources is to isolate, identify and purify major compounds with pharmacological and or therapeutic effects. Early phytochemistry brought out the first isolated active principles from medicinal plants. Examples include the analgesic alkaloid, morphine, and the anti-malaria quinine (Haeburger and Hostettmann, 1999). Three main saponins named albizias saponins A, B, and C were isolated from the bark of *Albizia iebbeck* and their structures were established through spectral analyses. These may be responsible for the anti allergic properties of *Albizia iebbeck* (Pal *et al.,* 1996). Phytochemistry also revealed artemisinin as a most promising anti malaria drug. It is highly effective against *Plasmodium falciparum* strains resistant to other anti-malarial drugs like Chloroquine. A series of derivative have been synthesized and are available in the market. These are arthemether, arteether and sodium artesunate (Klayman, 1993).

In spite of the progress made in phytochemistry of natural products, chemically defined, isolated constituents of plant are not considered to be herbal medicines. Iwu (1996) suggested that the classical

approach of phytochemistry is a useful and classical academic endeavour, but it has contributed much to the discovery of new therapeutic entities or the development of modern herbal medicines from traditional remedies. This is the case in countries like China and India. He stated that a more feasible approach is the development of standardized plant products as phytomedicines. The international market for processed herbal products, such as phytomedicines is huge and it has been estimated at over US$26 billion per annum. In 1995, China alone generated about US$5 billion in the sale of medicined herbal products. In the USA, about US$8 billion was spent by consumers on plant derived drugs. With the world-wide increase in the acceptance by the medical profession of the "Holistic" philosophy of therapy which the traditional systems exemplify, there will be continuous growth in the demand for natural product drugs derived from various traditional medicines (Iwu, 1996).

2.2.1 PHYTOCHEMICAL CONSTITUENTS IN MEDICINAL PLANTS

The plants extracts may exhibit positive reactions to: alkaloids, flavonoids, tannins, saponins, anthraquinones and glycosides (Hirano *et al.*, 1989; Akinyemi *et al.*, 2006; Oladunmoye, 2006). Gallic acid, corilagin, gerenin, quercetin 3-0 rutinoside, keampferol 3-0 rutinoside,

sesquiterpenes, monoterpenes, triterpenoids, polyphenols may also be observed (Akinde, 1986; Adesina *et al.*, 2000).

2.2.2 SAPONINS

Saponins are found in many plants and animal kingdom. Saponins have a soapy character due to their surfactant properties. Saponins were found to possess antispasmodic activity in the isolated guinea pig's ileum; such an effect might contribute to explaining the traditional use of onion in the treatment of disturbances of the gastrointestinal tract (Ray Sahelian, 2005). Saponins have hemolytic, expectorative, anti-inflammatory and immune-stimulating activity. Beyond that, saponins demonstrate antimicrobial properties particularly against fungi and additionally against bacteria and protozoa (Chavali and Campbell, 1987). Saponins are complex compounds that are composed of a saccharide attached to a steroid or triterpene.

Demand for bean products is growing because of the presence of several health-promoting components in edible bean products such as saponins. Saponins are naturally occurring compounds that are widely distributed in all cells of legume plants. Saponins, which derive their name from their ability to form stable, soap like foams in aqueous solutions, constitute a complex and chemically diverse group of

compounds. In chemical terms, saponins contain a carbohydrate moiety attached to the triterpenoids or steroids (Price *et al.*, 1987).

Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. Clinical studies have suggested that these health-promoting components of saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones. They also cause haemolysis of red blood cells; their medicinal value is due to their expectorant effect (Shi *et al.*, 2004).

2.2.3 GLYCOSIDES

They are compounds formed by replacing the hydroxyl (-OH) group of a sugar by another group. If the sugar is glucose, the derivative is termed a glycoside. Some glycosides have important pharmacological properties. For example, digoxin is a heart stimulant. Cyanogenetic glycosides, which occur in various plants can liberate hydrogen cyanide when hydrolysed and are thus potentially toxic to animals (Drake *et al.*,

1982; Oladunmoye, 2006). A very large number of glycosides exist in nature, many of which possess important biological functions.

One class of naturally occurring glycosides is called the cardiac glycosides because they exhibit the ability to strengthen the contraction of heart muscles. These cardiotonic agents are found in both plants and animals and contain complex aglycons, which are responsible for most of the drug's action; however, the glycoside may modify the biological activity. The best-known cardiac glycosides come from digitalis and include digoxin and digitoxin. Glycosidic units are frequently found in antibiotics. For example, the important drug erythromycin A, possesses two glycosidically- linked sugar units. Glycolipids are a very large class of natural glycosides having a lipid aglycone. These complex glycosides are present in the cell membranes of microbes, plants, and animals (Reichstein, 2008).

2.2.4 ANTHRAQUINONES

A group of purgative drugs originally derived from plants (e.g. Aloes, Senna and Rhubaeb spp) but now produced synthetically have their active principles absorbed from the small intestine and metabolised to an anthraquinone, which is excreted into the large intestine, where it has an irritant effect (Xing and Soffer, 2001).

It is an organic compound that is available in a yellowish or light gray to gray-green crystal powder. The construction make up of anthraquinone provides the fundamental construction of many natural colours found in plants (as seen in *Acalypha wilkesiana*). Hence, the compound is effectively used in the manufacture of dyes as well as in wood pulp production. The substance is also nauseating for birds. The birds go through a digestive disorder after consuming grass doctored with anthraquinone and hence is considered to be an effective goose repellent (D'Arcy, 1991).

Anthraquinones are more likely to be present in the plants as glycosides owing to the variety of sugar contents and this enhances the range of the compound. Usually anthraquinones are found in the form of aglycone with one or more sugar molecules and the straight anthraquinone by-products include emodin from *Rhamnus spp* (Huangs and Wang, 1998).

Laxatives derived from anthraquinones basically intensify the bowel wall, aggravating augmented muscle contractions and peristaltic movements.

People using anthraquinone laxatives regularly need to be cautious as the wanton use of this herbal medicine has bitter side effects. Many

people who use these laxatives are of the firm belief that they cannot have their bowels cleared without the medicine. Little do they realize that the constant use of anthraquinones to stimulate their bowel muscles ultimately worsen their conditions as the tissues loose their stimulating tone. In fact, these people finally reach a situation which is irreversible and after some time find it difficult to give up the habit. Hence, the safest and the most effective manner to use anthraquinone laxatives are to take them for a limited period to solve the congestion of bowel. However, the best manner to use anthraquinone-based medicines is to add four parts of psyllium or linseed for a maximum period of six months and this combination is enough to clear any congested bowel and bring relief (Pahor, 1995).

2.2.5 TANNINS

It is one of a group of complex organic chemicals found in many plants. They can cause toxicity in animals. It is a yellowish complex organic compound that is used as a constipating agent in the treatment of diarrhoea and dysentery. They can be used as an antidote in alkaloid poisoning. Its toxicity may cause necrosis of the gastrointestine and intestinal mucosa. Renal toxicity and hepatic necrosis may result from its toxicity (Beesley *et al.*, 2002).

The tannins are natural polyphenols, able to precipitate water-soluble alkaloids and possess an inhibitory action on the angiotensin converting enzyme (ACE). The ACE inhibitory effect of these tannins may explain the hypotensive effects of some traditional herbs (Lui Ju-Chi *et al.*, 2003).

2.3.0 SOME MEDICINAL PLANTS WITH PHARMACOLOGICAL ACTIVITIES

2.3.1 Plants Used as Anti- Pyretics

A number of plants originally studied for their antipyretic effects have ultimately been shown to act on the cause of the fever and have antimicrobial, antimalarial, trypanocidal or anti-inflammatory activities (Oliver-Bever, 1986).

The ethanolic extracts of *Allantus excelsa* (Mahanimba), *Toddalia asiatica* (Kanehana) and *Araucaria bidwilli* (Monkey-puzzle) showed moderate to significant degree of antipyretic activity in experimental rat model of 20% yeast suspension induced hyperthermia (Soresh *et al.*, 1995). The anti-pyretic activity of TBR-002, an herbal formulation was found to be almost equal to paracetamol in a rat model of pyrexia induced by subcutaneous injection of 15% yeast suspension (Subramoniam *et al.*, 1995). The stem bark extract of *Magnifera indica* exhibited anti-pyretic activity in mice with yeast induced pyrexia (Awe *et al*., 1998). *Fateorhiza macrantha* and *Tinospora bakis* are reported to depress the CNS and to

have antipyretic and hypotensive activities (Oliver-Bever, 1986). Studies have shown that *Morinda lucida* and *Khaya grandiofoliola* demonstrated antipyretic effects (Olajide, 1998). Also, plants with anti-malarial activities demonstrated antipyretic activity (Awe *et al.*, 1991a; Awe and Kamide, 1991b). *Morinda lucida* was shown to posses both in vitro and in vivo antimalarial activity against *Plasmoduim falciparum* and *Plasmodium berghei* respectively (Makinde *et al.*, 1985; Obih *et al.*, 1985; Makinde *et al.*, 1994). Also some plant such as *Spathodia campulata* (both leaf and stem bark extracts), *Solanum erriathum, Khaya grandiofoliola* have been reported to posses anti malarial activity (Makinde *et al.*, 1987; 1988; 1990). Ethanolic and Petroleum extracts of *Artenisla japonica, A. maritenia* and *A. nifegeriea* have been reported with anti malaria activity. In vitro study of the three compounds inhibited schizont maturation in Chloroquine sensitive strains of *Plasmodium falciparum* (Valecha *et al.*, 1994).

Several studies have also shown that many anti-malaria and anti-bacteria drugs possess anti-fertility effect in the male animal. For instance chloroquine, quinine and quinacrine have been reported to inhibit Leydig cell steroidogenesis and fertility in male (Saitan, 1978). Furthermore, chloroquine was reported to reduce sperm motility and hence fertility by a reduction in the average number of fetuses of cohabited female rats (Adeeko and Dada, 1998). Osinubi *et al.*, (2005) reported toxicity of

quinine to testicular gonocytes and seminiferous tubules of rats. Pyrimethamine, a prophylactic antimalaria drug has also been shown to cause spermatogenic arrest and male infertility in mice (Consentino *et al.*, 1990). Raji *et al.*, (2005b) also reported significant reduction in sperm motility, viability and count and testosterone levels in the rat treated with artemether, an antimalaria drug derived from *Artemisia annua*, a medicinal plant.

Similarly antibiotics such as nitrofurans (Paul *et al.*, 1954), erythromycin and tylosin (Lastikka *et al.*, 1976), gentamycin, neomycin and penicillin (Timmermans, 1974) and tetracycline (Kushniruk, 1976) have been reported to cause varying degrees of spermatogenic inhibition in man and animal models. Interestingly, antimalaria and antibacterial medicinal plant extracts that have also been reported to be highly potent against chloroquine resistant *Plasmodium falciparum* (Trager and Polomsky, 1981), produced significant reduction in epididymal sperm counts, serum levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in male rat (Njar *et al.*, 1995; Raji and Bolarinwa, 1997; Parveen *et al.*, 2003). *Alstonia boonei* a tropical plant, reputed in traditional medicine to have antimalarial, antipyretic, analgesic and anti-inflammatory properties (Ojewole, 1984; Olajide *et al.*, 2000) was reported to cause duration and dose dependent changes in the body

weights, organ weights and sperm characteristics. Sperm viability, motility and counts were significantly reduced in rats treated with *Alstonia boonei* (Raji *et al.*, 2005c).

Azadirachta indica, a medicinal plant with antiplasmodium activities in mice (Gbile, 1986) was reported to cause mass atrophy of spermatogenic elements and Leydig cells. *Azadirachta indica* was also reported to affect the structure and function of the testis and spermatozoa in male rats (Shaikh *et al.*, 1993; Raji *et al.*, 2003).

Cnestis ferruginea a plant that is in the family Connaraceaeis found in the Southern part of Nigeria is reputed to have antiplasmodial activity. Obeleagu and Wright (2005) observed that the crude extract of *Cnestis ferruginea* has antiplasmodial activity against *Plasmodium falciparum* (3D7 strain).

Artemisinin, a peroxide-containing sesquiterpene lactone isolated from the herb *Artemisia annua*, has been found to possess potent antimalaria activity and low toxicity both in animals and humans (China Cooperative Research Group on Quinghaosu and its derivatives as Antimalarias, 1982).

Artemisinin or Qinghaosu is the active principle of the Chinese medicinal herb *Artemisia annua*. It has been used as treatment of fevers in China for more than 1000 years. The antimalarial value of *Artemisia*

annua was first documented in Zhou Hou bei ji Fang (Handbook of prescription for emergency treatments) written as early as 340 AD by Gettong of the Eastern Jin Dynasty. The active antimalaria constituent of this plant was isolated in 1971 and it was named **artemisinin.**

Artemisinin was originally developed in 1972 in China (Chinese Institute of Material Medicine) from the plant *Artemisia annua* (sweetworm wood), a sesquiterpene lactone (empirical formula $C_{15}H_{22}O_5$). Artemisinin is the active ingredient in qinghao, a Chinese herb tea that have been used for 150 years to treat malaria and haemorrhoids. It grows in the wild in China and has been found to grow in other parts of the world too, though the species may vary a bit. Locally, it is prepared as an infusion of the dried leaves (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalaria, 1982).

Artemisinin, a natural product, found in leafy portions of *Artemisia annua* (qinghao), is a plant used by Chinese herbalists since 168 B.C. Initially a treatment for haemorroids, 1 litre of water steeped with a handful of qinghao leaves was later found to be effective in reducing fevers (Klayman, 1985).

Crude ether extracts yielded compounds which were effective in mice infected with the murine malaria parasite, *Plasmodium berghei*. Chinese scientists then purified and determine the chemical structure of this sesquiterpene lactone in 1972, naming it qinghaosu (China

Cooperative Research Group, 1982) although it is now generally called artemisinin.

Artemisinin derivatives are active against other haemoparasites like *Schistosoma mansoni* and *Schistosoma japonicum* in vitro and in experimental animals (Xiao and Catto, 1989; Sano *et al.*, 1993). This is of mechanistic interest since schistosomia like malaria parasites degrade hemoglobin and produce hemozoin (Homewood *et al.*, 1972).

From the result of the study done by Akande, (2009) it can be concluded that artemisinin group especially artemether, a methylether of dihydroatemisinin is effective in the treatment of trypanosomosis.

2.3.2 Plants with CNS Activities

Valdya, (1997) has illustrated the scope of CNS active medicinal plants in therapeutics. Various extracts derived from *Pangomia pinnata* decreased pentobarbitone sleeping time, probably by stimulation of the hepatic microsomal enzyme system (Singh *et al.*, 1996). Similar properties were exhibited by its roots. However, the petroleum ether extract (PEE) of the roots enhanced pentobarbitone sleeping time probably due to CNS depression (Singh *et al.*, 1997). The PEE of *Pangonia pinnata* also reverses Alzeimer disease induced by dyribotenic acid (Kumar *et al.*, 1996). The acetone solute petroleum ether extract of *Lawsonia inermis* potentiated clonidine induced hypothermia but no effect on haloperidol induced catalepsy, thus showing no effect on

dopamine mediated behaviour (Lyer *et al.*, 1998). Caffeine is mostly used as CNS stimulant, while theobromine and theophylline are used more often for their effects on the myocardium. The *Cola spp* have, like coffee or tea, a stimulant action due to the presence of xanthine derivatives. *Centella asiatica* (Unbelliferae) is reported to improve the mental ability and behaviour of mentally retarded children through triterpenic glycosides (Burgen and Mitchell, 1972).

The methanolic extract of *Casle fistula* has been reported for its CNS activities. The extract significantly was potentiated by sedative actions of sodium pentobarbitone, diazepam, meprobamate and chlorpromazine (Mazumder *et al.*, 1998). Similarly, the aqueous leaf extract of *Pyrenacanthia staundtii* was also reported for CNS activities by potentiating the effect of hexobarbitone (Awe *et al.*, 2005). Investigation of the neurochemical effects of different fusarial toxins elaborated from *Fusarium moniliform* and *Fusarium orysporum* showed that *Fusarium moniliform* had irreversible and non-specific MAO inhibitory activity comparable to nialamide (Ganguli *et al.*, 1996). Jaiwal *et al.*, (1994) reported anxiolytic effect of leaf extract of *Azadirachta indica* (Neem). The leaf extract exhibited anxiolytic effect comparable to diazepam at low doses (100-200 mg/kg) when tested in rats. However, higher doses (>400 mg/kg) did not show anxiolytic activity. Ethanol extract and cold

aqueous infusion of *Vitex leucoxylon* (Ninorchi in Tamil) leaf depressed spontaneous motor activity, antagonized d-amphetamine induced stereotype and oxotremorine induced tremors and shortened the duration of immobility. Extract of rhizomes of *Nelumbo nucifera* (Karnal) was found to cause significant reduction in spontaneous activity, decrease in the exploratory behavioural pattern by the head Hp and Y maze tests, muscle relaxant activity and potentiating of pentobarbitone induced-sleeping time (Mukherjee *et al.*, 1996). Mitra *et al.*, (1996) has shown that the root powder of *Panax ginseng* did not affect pentobarbitone induced – sleeping time or spontaneous motor activity. Although it potentiated amphetamine-induced increase in motility, it attenuated other amphetamine effects such as stereotype and lethality in aggressive mice. Haloperidol catalepsy was potentiated while the behavioural responses of 5-hydrox-trytophan and L-dopa were both attenuated. It exhibited significant aggression inhibiting effect in doses that had no effect on spontaneous movements. The results have been discussed on the basis of interaction of Panax ginseng with the functioning of various neuro-transmitters. All extracts of petroleum ether (PEE), n benzene (BE), Chloroform (CE), acetone (AE) and Ethanol (EE) of the leaf of *Abies pindrow* Boyle (Silver fir) showed potentiation of pentobarbitone sleeping time i.e CNS depressant effect. The PEE, BE, CE and EE (highest efficacy) showed significant anti-depressant activity. On the

other hand, EE was found to potentiate immobility, suggesting that this fraction is devoid of anti-depressant effect (Single *et al.*, 1998). Chronic treatment with the root extract of *Withania somnifera* (Ashwagandha) attenuated the development of dependence to morphine in mice. By itself *Withania somnifera* showed no analgesic effect (Kulharni *et al.*, 1997).

The reputation of *Strychnos spp*. as bitter tonics may be due to a bitter taste with stimulation of the taste papillae and, as a reflex action, hypersecretion of saliva and gastric juices. Strychnine is also a strong convulsant leading to death from spasmodic contraction of the thorax and diaphragm. The use of strychnine has now been mainly limited to investigations on the mode of action of anticonvulsant drugs (Burgen and Mitchell, 1972).

Anticonvulsant and sedative actions are in *Aformosia laxiflora* (Fabaceae). The plant is however very toxic (Oliver-Bever, 1986). Plants with CNS depressant action often have a simultaneous activity in several sections. This applies particularly to those having analgesic, narcotic, sedative, hypnotic and antipyretic activity and each effect may be the predominating consequence of a general action on the cerebral cortex (Turner and Richens, 1978).

In a study carried out by Manocha *et al.*, (1996), *Ginkgo biloba* decreased the protective effect of sodium valproate and carbamazepine against picrotoxin as well as strychnine induced convulsion in mice.

Further studies showed that pre-treatment with *Ginkgo biloba* extract potentiated the convulsions produced by picrotoxin and strychnine, indicating the involvement of GABAnergic system, and chloride channels (for picrotoxin) and modulation of action of glycine neurotransmitter (for strychnine) by *Ginkgo biloba* (Manocha *et al.*, 1997). The anti-convulsant profile *Withania somnifera* (obtained from commercial source in India) was determined, in a lithium-pilocrine model of status epilepticus (SE) in rats. Acute treatment with the root extract prolonged the latency to forelimb clonus but failed to protect against mortality. Acute pre-treatment with the extract enhanced the antiepileptic effect of diazepam and clonazepam chronic treatment with extract (100 or 200 mg/kg p.o. for 7 days) reduced mortality. Electrophysiological data further supported the behavioural findings. The protective effect of the root extract appears to involve GABA mediation (Kulkarni, 1998). Different extracts, fractions and subfractions of seeds of *Syzygium Cuminii*. (Collected from Brazil) were evaluated for behavioural effects in mice, particularly in relation to their sedative and anticonvulsant actions. Oral treatment with the hydro-alcoholic extract showed anticonvulsant activity in pentylenetetrazol (PTZ) and maximum electroshock-induced convulsions; suggesting that *S. cumunii* has some active principles with central depressant properties and some of them also present an anticonvulsant action (Lima *et al.*, 1998). Extract of ginseng (*Panax ginseng*) has been shown to have

neuroprotective effect. The probable mechanism for neuroprotective effect of triterpene glycosides components was suggested (Popov *et al.*, 1998). *Pyrenacanthia staundtic* has been reported to be protective against Strychnine-induce convulsion (Awe *et al.*, 2005).

2.3.3 Plants with Analgesic Effects

Many varieties of plants containing triterpenoid were reported to exhibit analgesic activity (Recio *et al*., 1995). Antinociceptive effects of 20 triterpenes of lupine type from cacti have been reported by acetic acid-induced writing test (Kaoru *et al.*, 1998).

Gossypin, a bioflavonoid from the yellow petals of *Hibiscus vitifolius* (Bhasadwaji) has been shown to have antinociceptive activity, similar to morphine and involving multineurotransmitter systems, mainly the cholinergic and GABAnergic neurotransmitter pathways. Gossypin pre-treatment significantly decreased the development of acute tolerance to morphine induced antinociception (acetic acid writhing assay). Thus it has a potential as an analgesic with the advantage of lack of tolerance and development liability (Vaz, 1998). Suppression of acetic acid writhing was reported with both the ethanol extract and cold aqueous infusion of *Vitex leucoxylon* (Mitra *et al.*, 1996).

Azadirachta indica showed analgesic properties in mice. Pre-treatment with the opioid antagonist, naloxone and central noradrenaline depletory, DSP-4 attenuated the analgesia whereas the serotonin synthesis

inhibitor, PCPA potentiated the same, suggesting that both central and peripheral mechanisms and complex neural pathways (opoid and non-opoid i.e monoaminergic) may be involved in this effect (not involving the opoid pathway) against acetic acid induced writhing in mice (Sukumar *et al.,* 1995). Using the same model in rats, significant analgesic activity was detected in leaf and seed of *Vernonia lasiopus* and *Vernonia galamensis* (Johri *et al.,* 1995) and alcoholic extract of *Ochna obtusata* (Kana Kchampalc) stem bark (Sivaprakasam, *et al.,* 1996). *Panax ginseng* exhibited anti-nociceptive activity and potentiated the anti-nociceptive activity of both pentazocine and aspirin (Mitra *et al.,* 1996).

The PEE, BE and EE of the roots of *Pongamia pinnate* showed significant analgesic effect in the tail flick test. The PEE and direct EE of the seeds also showed significant analgesic activity at doses higher than 100mg/kg (Mumar *et al.,* 1996). Different extract of *Abies pindow* Royle leaf (PEE, BE, CE, AE and EE) showed significant analgesic effect in the hot wire induced tail flick response in rats. Possible mechanism of action could be its inhibitory effect on PAF and prostaglandins as this contains phyto-constitutents such as flavonoids and triterpenes (Single *et al.,* 1998).

Alcoholic extract of the roots of *Clerodendron seratum* (Bharanji) showed significant analgesic activity in mice (Narayanan *et al.,* 1998).

Methanolic extract of *Epidendrum mosenii* stems showed pronounced analgesic properties in the acetic acid induced abdominal constriction test in mice (Floriani *et al.*, 1998). This activity appeared to be related to the presence of two triterpenes, pholidotinanol 24-methylene cycloartanol, which were identified using special and chemical analysis. Also Olajide *et al.*, (2000) reported analgesic activity of net oil. *Rhigcarya racemifera* and *Kolobo petalum auriculatum* (Manispermaceae) are reported to have analgesic effects attributed to O-methyl flavianthine which has a structure similar to that of morphine (Oliver-Bever, 1986).

2.3.4 Plants with Anti-Inflammatory actions

Makwana *et al.*, (1994) reported the significant inhibition of carrageenan induced paw oedema and granulation tissue formation by ethanolic extract of the leaf of *Vitex leucoxylon* in rats. The aqueous suspension of dried latex of *Calotreopis procera* (Arka) showed anti-inflammatory activity when tested in the carrageenan and formalin induced rat paw oedema models (Kumar and Basu, 1994). Alcoholic extracts of the root of *Pongamia pinnate* showed significant anti-inflammatory activity compared to phenylbutazone in carrageenan and PGE, induce oedema models (Singh and Pandey, 1996). Possible mechanism of action could be prostaglandin inhibition especially by EE and AE.

All extracts of *Abies pindow* Royle leaf showed anti-inflammatory effect in various animal models of inflammation such as carrageenan induced paw oedema granuloma pouch and Feud's adjuvant arthritis (Singh and Pandey, 1997). Chemical analysis indicated the presence of glycosides and steroids in the PEE and BE and terpenoids and flavonoids in the AE and EE. Flavonoids and terpenoids are polar substances effective in acute inflammation whereas glycosides and steroids are non-polar substances effective in chronic inflammation (Singh and Pandey, 1997). The methanolic extract of the aerial part of *Sidarhom bifolia* (Atibala) showed significant oedema suppressant activity in the carrageenan induced paw oedema model in rats. Probable mechanism of action may be due to its inhibitory effects on the release of mediators of inflammation such as histamine, 5-hydroxytryptamine, bradykinin etc (Rao and Mishra, 1997). Gmelina (Gopobhadra) root powder was effective in reducing the oedema in the carrageenan induced rat paw oedema model of acute inflammation but when tested against the cotton pellet granuloma model of chronic inflammation, it not only reduced the weight of the granuloma but also the lipid peroxide content of granuloma exudate and liver and gama-glutamyltranspeptides in the granuloma. It also normalised serum albumin, serum acid and alkaline phosphatase levels (Ismail *et al.*, 1997). Probable mechanism of its anti-inflammatory effect may be its anti-proliferative, anti-oxidative and lysosomal

membrane stabilization effects. Studies have shown that, the methanol extract of *Nelumbo nucifera* rhizome as well as the steroidal triterpenoid isolated from it (betulinic acid), possessed significant anti-inflammatory activity when evaluated in the carrageenan and 5-hydroxytryptamine induced rat paw oedema models (Mukherjee *et al.*, 1997). The effects produced were comparable to that of phenylbutazone.

Treatment with Ease, a poly-herbal formulation significantly reduced Freud's adjuvant induced non-established and established arthritis in rats. In vitro, it provided significant protection against protein denaturation and RBC membrane damage and exhibited significant proteinase inhibitory action thus indicating its possible use as an anti-arthritic (Chalterjee and Das, 1996). Jigrine, another poly-herbal formulation, exhibited anti-inflammatory activity against carageenan induced acute inflammation but not against cotton pellet granuloma (Sub acute inflammation). Effect on biochemical parameters suggested that the mechanism of its anti-inflammatory effect could be in its anti-oxidant and membrane stabilizing effect (Karunakar *et al.*, 1997). Methanol extract of *Chasmanthera dependens* and aqueous leaf extract of *Gongronema latifoluim* have been reported to possess anti-inflammatory properties when tested in carrageenan induced-paw oedema and cotton pellets granuloma in rat (Olugbenga *et al.*, 2001; 2002).

Various triterpenoids obtained from plants or prepared by chemical modification of natural isolates were reported to exhibit some physiological activities such as anti-inflammatory effect (Takgi *et al.*, 1980; Recio *et al.*, 1995). Also *Morinda lucida* Benth (Rubiacae) called the Brimstone tree and 'Oruwo' in the Yoruba Language is a plant popularly known for its efficacy in inflammations (Watt and Breyer-Bran dwijjk, 1963; Oliver-Bever, 1986; Iwu 1993). *Khaya grandiofolia*, *Bridelia ferruginea* stem bark, and *Combretum micranthum* (Combretaceae) have been reported for their anti-inflammatory activities (Olajide *et al.*, 1998; 1999c; 2000; 2003).

2.3.5 Plants with Antimicrobial Properties

Bacterial infections are very common and could result in diarrhoea and inflammation. Mostly, medicinal plants that have anti-inflammatory properties may also possess anti-bacterial properties (Rungeller *et al.*, 1998). *Tithonia diversifolia* has been found to have anti-inflammatory and anti-bacterial properties. Plants that contain triterpenes and related compounds have been found to possess anti-microbial properties (Devi *et al.*, 1995; Mazzanti *et al.*, 1998, Navarro *et al.*, 1998; Sharma *et al.*, 1998). Triterpenes and glycosides isolated from extracts of the leaves of *Betula* species demonstrated anti-bacterial activity against Gram positive bacterium *Staphylococcus*. Phenolic compounds and acids are generally known for their anti-microbial activity. For example, betulinic acid has

some inhibitory activity against HIV (Yoshiki and Hashimoto, 1996; Montagnac *et al.*, 1997).

Clausanol, a carbazole alkaloid, isolated from alcoholic extract of the stem bark of *Clausena anisata* was found to be active against gram positive and gram negative bacterial and fungi (Chakraborly *et al.*, 1995). Substantial anti-microbial, anti-fungal and moderate insecticidal, sporicidal and cytotoxic activities were observed with hexane extract of the stem bark of *Amona glabra*. Chromatographic fractionation of the stem led to the isolation of Kaur-16-en-19-0ci acid, which was found to be largely responsible for the biological activities (Padmaja *et al*, 1995). The alcoholic extract of dry nuts of *Semecarpus anacarditum* showed bactericidal activity in vitro against gram negative (*Escherichia coli, Salmonella typhi* and *Proteus vulgaris*) and gram positive *Staphylococcus aureus* and *Corybebacterium diphtheriae* (Nair and Bhide, 1996). The acetone and alcoholic extracts of the leaves of *Casia alata* showed significant in-vitro anti-bacterial acitivity against *Staphylococcus aureus, Bacilus subtilis, Eschericha coli, Salmonella typhi* and *Salmonella dysentrae*. In addition, the extract also inhibited the growth of *Klebsiella pnueumoniae* (Sakhrkar *et al.*, 1998).

The enthanol extract of *Azardirachta indica* leaves demonstrated much more significant anti-dermatophytic activity as compared with

aqueous extract when tested in-vitro against 88 clinical isolates of dermatophytes using agar dilution technique (Dahanukar *et al.,* 1997).

The MIC 90 of ethanol extract was 100ug/ml whereas that of aqueous was 500ug/ml (Venugopal and Vengopal, 1994). Ral, (1996) screened 17 medicinal plants against pathogen *Pestalotiopsis magniferae* and the results revealed that 14 plants had anti-mycotic activity of which *Azardirachta indica* gave 84.66% anti fungal activity. The root of *Withania somifera* was found to be effective in prolonging the survival of mice infected intravenously with *Aspergillus fumigatus* (Dhuley, 1998). This activity observed was probably attributed to increase phagocytosis and intracellular killing capacity of macrophages induced by treatment with *Withania somifera*. Essential oils extracted from *Cananaga adorata, Bowellia thurifera and Ocimum basilicum* have been reported to have activity against *Aspergillus niger*. The antifungal activity exhibited by these plants was as a result of their antioxidant properties (Barrata *et al.,* 1998). Also *Baeconia arborea and Rhus cortaria* exhibited antimycotic activity against *Candida albicans* (Navarro *et al,* 1998; Lauk *et al.,* 1998).

Glyceyrrhiza, a triterpenoid glycoside obtained from *Glyceyrrihza glabra* was tested against RNA viruses like the Measles virus; Polio vaccine viruses' type 1, 2 and 3; Polio wild type viruses 1, 2 and 3 as well as DNA viruses like the Herpes type 1 and 2 viruses in vitro. It inhibited

the DNA virus plaque formation at lower concentration (0.608mM) while the RNA viruses were inhibited at higher concentration (1.216mM) (Badam, 1994). Pandarinnathan *et al., (*1998) carried out in-vitro screening of margrove plant extract and detected anti-immuno-deficiency virus activity using tetra zoluim-based colorimetric assay. Seven extracts were found to be effective, with bark of *Rhizophora apiculata and Rhizophora lamarchi* completely inhibiting the virus adsorption to the cells. Ursolic acid and related triterpenes isolated from *Cynomorium songarium h*as been shown to have inhibitory effect on immuno-deficiency virus (HIV-1) Protease (Machanomei *et al.,* 1997). Oleanolic acid, promolic acid and structurally related triterpenoids isolated from several plants including *Rosa woodsto* leaves, *Prosopus glandulosa* (leaves and twigs*), Phoradenoron, juni peruim* (whole plant) have been demonstrated to have inhibitory effect on HIV-1 (human immuno-deficiency virus) (Kashiwada *et al.,* 1998). *Rhus javanica* has been shown to exhibit anti-herpes simplex virus (HSV) activity and potentiate the anti-HSV activity of acyclovir in vitro and in vivo (Nakano *et al.,* 1998). The anti-HIV activity of aromatic herbs within the Labiatae (Lamiaceae) was evaluated in vitro. Of 51 extracts (aqueous or 70% ethanol) obtained from 46 herb species, 45 showed significant inhibitory effect against HIV-1-induced cytopathogenicity in MT-4 cells (Yamasaki *et al.,* 1998). Phenolic compound and acids are known for their anti-viral activities. For

example, betylinic acid has some inhibitory activity against HIV (Yoshiki and Hashimoto 1996; Montagnae *et al.*, 1997).

2.3.6 Plants with Anti-cancer Activities

The potential role of various plants in cancer therapy as a direct anti-cancer agent, chemopreventive agent and radiosensitizer or immunity enhancer has been reviewed (Dahanuka and Kulkarni, 2000).

Evaluation of the in vitro anti-cancer effects of bioflavonoid, such as quercetin, catechin, leteolin and vutin against human carcinoma of larynx (Hep-2) and sarcoma 180 (180-S) cell lines showed that only luteolin caused depeletion of glutathione in the cells and a decline in DNA synthesis as seen by thymidine uptake studies, thus demonstrating its anticancer potential (Elengovan *et al.*, 1994). The anti-tumor effects of the crude extract of *Centella asiatica* as well as its partially purified fractions were demonstrated using both in-vitro short and long term chemosensitivity test system and in-vivo models. The purified fraction inhibited the proliferation of the transformed cell lines of *Ehrilich ascites* (Babu *et al.*, 1995). Banerjee *et al.*, (1998) studied the modulatory influence of the alcoholic extract of leaves of *Ocimum sanctum* on various enzymes in the liver, lung and stomach of mouse. Oral treatment with the extract significantly elevated the activities of Cytochrome P450, Cytochrome b5, arylhydrocarbon hydroxylase and glutathione of

carcinogens as well as mutagens. Plants found containing triterpenoid component have been reported to possess anti-tumor promoter activity (Ohigashi, 1986), and anti-HIV activity (Fujioka *et al.*, 1998).

2.3.7 Immune Active Plants

Modulation of the immune response through stimulation or suppression may help in maintaining a disease free state (Dahanukar *et al.*, 2000). Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Upadhyay, 1997). Katyar *et al.*, (1997), evaluated some medicinal plants for immuno-modulatory activity. These plants which include *Withania sominifera, Alluim sativum, Azadirachta indica, Piper longum, Asparagus racemesus, Glycurrhiza glabra, Aloe vera, Cimelia arborea* and *Tinospora cordifolia. Tinospora cordifolia* and *Withania somnifera* protected animals against infections in normal and immuno-suppressed states induced by hemisplenectomy and surgery (Dahanukar and Thalte, 1997). It has been found to activate the mononuclear cells to release cytokines like GMCSF (Thalte *et al.*, 1994) and in a dose-dependent manner (Dahanukar *et al.*, 1997).

2.3.8 Trace Elements in Medicinal Plants

The attention for the investigation of inorganic constituents was drawn by Hakion Abdul Hamid who is the originator of the discipline

"Elementology" (Arora *et al.*, 1984). It was believed that health depends upon the organized state of elements in the body and their imbalance causes disease (Golden, 1988). Therefore, restoration of balance by drugs can cure disease. Medicinal properties of some plants have been attributed to the presence of inorganic elements in variable quantity (Gaunch, 1972). According to Vohora (1986), disturbance of element contents has been accountable for the development of more than 135 diseases. Also, supplementations of diet with magnesium (mg) have been found to induce remission of early symptoms of leukemia among experiemental animals (Aikawa *et al.*, 1971). It is also found that changing Ca and Mg index is very symptomatic in cancer disease (Dobrowoski, 1987). It is a well known fact that inorganic trace elements are very active in very low concentration (Kazi *et al.*, 1999; Sahito *et al.*, 2001). Analysis of different parts of medicinal plants have shown that the presence of many essential and important elements such as Ca, Mg, Zn, Fe, Co, Mn and Zinc are very effective in killing virus (Randal, 1984).

2.3.9 Elemental Analysis of Medicinal Plants

Various medicinal plants, *Ocimum sanctum, Tinospora cordifolia, Azadirachta indica, Nerium andicum* and *Acorus calamus* etc were analysed for the presence of minor and trace elements by Instrumental Neutron Activation Analysis (INAA). Concentrations of 13 elements

were determined. Zinc, Manganese, and sodium were significantly higher in *Ocimum sanctum* leaves while Zinc was higher in *Azadirachta indica* leaves (Samudralwar and Garg, 1996). Specific parts of several plants (fruits, leaves, stembark and roots) often used as medicines have been analysed (Single and Garg, 1997) for 20 elements; Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, K, Mn, Ml, Na, P, Rb, Sb, So, Se, Sr, and Za using INAA, similarly elemental analysis of some herbal plants used in the control of diabetes has been done by the techniques of Neutron Activation Analysis (NAA) and Atomic Absorption Specroscopy (AAS) (Augusti *et al.*, 1995). These elements, Mg, Na, Cl, Al, Cu, Pb, Cr, Cd, Fe, Ca, Zn and Hg were found to be present in various proportions.

2.4.1 The Physiology of Pain

Pain is a complex phenomenon involving both neurophysiological and psychological components (IASP, 1979). Pathophysiological mechanisms involve neural pathways, and a variety of pain-producing substances and modulating mechanisms. These include peptide, noradrenaline and endogenous opioid peptides (Iggo, 1982).

The perception of pain involves two processes, the first is the detection of pain, or nociception; the second is the conscious experience associated with perception of the pain (IASP, 1979). Special sensory nerve or nociceptive nerve endings are stimulated as a result of painful

stimuli. These nerve afferents are unmyelinated or small myelinated fibres, which run to the spinal cord. They synapse in the dorsal horn of the spinal cord on to a neuron, which projects in the controlateral side in the lateral spinothalamic tract. Pain elicited by stimulation of nociceptive afferent pain fibres is known as neurogenic pain (Goodman and Gliman, 1996).

2.4.2 Pathophysiology of pain

The sensory detection of nociception may involve stimuli of diverse kinds, including mechanical, thermal and chemical. When these receptors are activated, the impulses generated are transmitted along peripheral nerve fibres to the central nervous system. Pain is complex for many reasons, including the fact that there are many pain-producing substances and many ways of modulating impulses and pathways. Some of the chemicals and natural substances known to produce pain are: acetylcholine, potassium chloride, serotonin, histamine, bradykinin, certain prostagladins, somatostatin, vasoactive intestinal polypeptide and noradrenaline. Spinal and sympathetic reflexes may contribute to nociception. Reflex sympathetic dystrophies, for example can be very painful and may be accompanied by inappropriate sweating and changes in the caliber of blood vessels (Melzack and Wall, 1965).

2.4.3 Mechanisms involving Pain Inhibition

The first mechanism is a blood circuit which can limit the access of the nociceptors to the contralateral spinothalamic tract: this is stimulated on low threshold thermal stimulation. An example of this mechanism in operation is the effect of gentle rubbing of a painful area to ease the pain (Yoxall, 1978; Iggo, 1982).

The second mechanism which can inhibit the neural traffic from nociceptors to the contralateral spinothalamic tracts and from there to the higher centers and consciousness is the descending inhibitory system which originates from the raphe nucleus of the midbrain. The neurotransmitter 5-hydroxy-tryptamine (5-HT) is important in the spinal inhibition of nociceptive transmission. Activation of the descending serotonergic bulbospinal system inhibits behavioural and dorsal horn neuronal responses to noxious stimuli (Mayer *et al.,* 1971; Zemlan *et al.,* 1980). Direct microintophoretic application of 5-HT to dorsal horn neurons mimics the inhibitory effects of activation of the descending serotonergic pathway (Belcher *et al.,* 1978). The models in which an antinociceptive effect of intrathecally administered 5-HT has been demonstrated in rats include tail flick test (Schmauss *et al.,* 1983; Xu *et al.,* 1994), the paw pressure test and the formalin test (Bardin *et al.,* 1997a; 1997b, 2000).

2.4.4 Methods of Assessing Pain

Animal studies on pain employ behavioural measures that are of two types; simple withdrawal reflexes and more complex voluntary and intentional behaviours that are unlearned or learned (Chapman *et al.*, 1985).

Two general types of research studies result in pain in animals. First, pain may be a consequence of techniques and methodology, the purpose of which is totally unrelated to the nature and magnitude of pain. Pain is therefore a side-effect or unwanted outcome associated with the experiment. Secondly, there are studies, which explore the phenomenon of pain itself, utilizing behavioural, physiological, anatomical and pharmacological approaches. The major concern being examined here is the assessment of this type of experimental pain in awake animals. These studies provide knowledge that can ultimately be applied to the management of acute and chronic pain conditions in human and animals (Dubner *et al.*, 1975; Addison, 1984).

i) Simple Reflex Measures

These include the tail-flick test, then limb withdrawal reflex, and the jaw-opening reflex. In most cases, latency measure is used to assess reflex responses. In the tail-flick reflex, a radiant heat stimulus is focused on the blackened area of the tail and the animal flicks it tails

to escape the stimulus. The technique was to demonstrate analgesia and the effectiveness of analgesic agents in this model is highly correlated with their effectiveness in relieving pain in humans (Grumbach, 1966). It has been used to assess pain produced by brain stimulation stress, or the micro-injection of opoiods (Dubner and Bennett, 1983). In the limb-withdrawal test, thermal or electrical stimuli are typically employed and the latency of a brisk motor response is used as the behavoiural end point (Bonnett and Peterson, 1985). The jaw-opening reflex (Mitchell, 1964) is elicited by electrical stimulation of a tooth and electromyo-graphic recordings from jaw muscles are used to assess the behaviour. These simple reflex measures permit the animals to have control over stimulus magnitude and thus ensure that the animal can control the level of pain to which it is exposed.

ii) Organised Unlearned Behaviour

More complex behaviours are often used as measures of pain because they involve a voluntary purposeful act requiring supra-spinal sensory processing (Chapman *et al.*, 1985). Commonly used methods is the rodents hot-plate test in which a rat or mouse is placed on a plate preheated to 50-55^{o}C. The time required to elicit a paw licking response, usually of the hind paws, is measured. A variant of the hot-

plate reaction is the face-rubbing escape responses in which the rats are exposed to facial heat stimuli (Ronsenfield *et al.,* 1983). The writhing response is produced in rodents by injecting pain producing chemical substances intra-pertitoreally. The acute peritonitis resulting from the injection produces a response characterized by internal rotation of one foot, arching by the back, rolling on one side, and accompanying abdominal contractions. The writhing response is considered a model of visceral pain (Vyklicky, 1979).

2.4.5 Drugs in Treatment of Pain and Their Mechanisms

The non-narcotic analgesics such as indomethacin, aspirin, acetaminophen, dipyrone and the newer non-steroidal anti-inflammatory drugs (NSAIDS) such as rofecoxib and celecoxib constitute heterogenous group of compounds differing in chemical structure and sharing certain pharmacological action in the periphery where pain originates. They are distinct from narcotic analgesics in that they do not bind to the opiate receptor site. Unlike narcotic analgesics, tolerance or physical dependence does not develop with use. Non-narcotic analgesics have a ceiling effect, in that increasing the dose beyond a certain level does not produce additional analgesic effect, although it may increase the duration of effect. Since many of the peripherally acting analgesics act as potent prostaglandin

synthetase (Cyclooxygenase) inhibitors, they possess analgesic, anti-pyretic, antiplatelet and anti-inflammatory properties (Goodman and Gilman 1996).

Clinically, opioids are classified commonly as weak or strong. These terms are necessarily imprecise but refer principally to relative efficacy rather than potency (Hanks and Hoskin, 1987). Weak opiods exhibit a ceiling to their analgesic effect because of an increased incidence of unwanted effects. In contrast, strong opiods have much wider range of efficacy, and can relieve more severe pain than weak opioids (Goodman and Gilman, 1996).

Opioids may also be classified in terms of receptors, which are found in several areas of the brains, particularly in the periaqueductal grey mater, and also throughout the spinal cord. Morphine is the prototype agonist for the miu receptor, ketocyclazocine for the kappa receptor, and a compound called SHF-100047 for the sigma receptor (Martin *et al.*, 1976). In addition, leu-enkephalin is known to bind on the delta receptor (Holaday 1985). The miu and Kappa receptor are concerned with analgesia; buprenorphine is a partial miu agonist, whereas butrophanol and nalbuphine is partial kappa agonist (Goodman and Gilman, 1996).

All of the NSAIDs are thought to exert their analgesic effect by inhibiting prostaglandin biosynthesis through inhibition of the enzyme

cyclo-oxygenase (Vane, 1973) that leads to conversion of arachidonic acid to prostagladin F, D, and E as well as prostracycin and thromoboxane A2. Tissue damage initiates a complex set of events leading to activation of primary afferent nociceptors in periphearal nerves to the pain-producing effects of substances such as bradykinin (Keele, 1969). Bradykinin which stimulates prostaglandin synthesis is inhibited by NSAID. The analgesic efficacy of NSAIDs is limited because they have no effect on nerve conduction by other chemical mediators such as serotonin and leukotrenes (Clarke, 1990). Some NSAIDs exert their analgesic effects through both peripheral and central action (Kaylaap, 1998). Peripheral activity is related to their inhibitory action on released prostagladins (Goodman and Gilman 1977). Dipyrone known to possess stronger analgesic and antipyretic and weaker anti-inflammatory activities than other NSAIDs exert its activity by activating inhibitory pain pathways descending from periquaducetal gray mater to meduall spinals (Carlsson *et al.*, 1986). It therefore means that dipyrone action as analgesic is mediated through serotonergic pathway (McCormack and Brune, 1991). Morphine an opoid plays an important role in antinociception through serotonergic pathway. It is known that an increase in 5-HT transmission augments the antinociceptive effect of morphine, whereas a decrease in 5-HT transmission attenuates the same effect (Pini *et al*,. 1997). It has also

been shown that 5-HT, reduced morphine-induced nociception (Millian and Colpaert, 1991).

2.5.1 Inflammation

Inflammation is generally defined as the response of living tissues to an injurious stimulus and is recognized as primary defense mechanisms **(**Willoughby, 1978). It is a process of vital response to injury, infections, trauma and any other insults. Inflammatory response brings about response recruitments of blood leucocytes, activation of tissue macrophages and mediators (Ward and Lentsh, 1999).

The first recorded description of inflammation is attributed to Cornelius Celsius in the first century AD, who noted redness, swelling, heat and pain as the four basic symptoms (Movat, 1985). The event of inflammation that underlie these manifestation are induced and regulated by a large number of chemical mediators including eicosaniods, kinins, complement proteins, histamines and monokines (Metcalfe *et al.*, 1981, Vane and Botting, 1998).

Eicosaniods function to mediate various physiological responses such as inflammation, blood clotting, vascular dilation and immunity (Linder, 1991; Kapil *et al.*, 1992). Eicosaniods can be divided into four classes; prostaglandins, leukotrienes, thromboxane

and prostacylin. Large part of the inflammatory process is regulated specifically by the prostaglandins and leukotrienes (Ammon 1991; 1993; 1996; Safayhi *et al*, 1996).

2.5.2 Prostaglandins and Cyclooxygenases

Among the many mediators of inflammation, the prostaglandins are one of the most important (Flymn *et al.*, 1986). The key enzyme in their synthesis is prostaglandins endoperoxide synthesis (PGHS) or Cyclooxygenase that possess the catalytic sites. The cyclooxygenase active site converts arachidonic acid to the endoperoxide PGG2. The second, a peroxidase active site, then converts the PGG2 to other endoperoxide PGH2 prostaglandins H2 is further processed by specific isomerases to form prostaglandins, prostacyclin and thromboxane A2. Cyclooxygenase (Cox) activity has been studied in preparations from sheep seminal vesicles and a purified enzmatically active Cox was isolated in 1978 by Hender and his colleagues. It is now known that cox exists in at least two isoform, Cox-1 and Cox-2 (Vane and Botting 1998).

The constitutive isoforms, Cox-1 has clear physiological functions. Its activation leads, for instance, to the production of prostacyclin which when released by endothelium is antithrombogenic (Moncade *et al.*, 1976), and when released by the gastric mucosa is cytoprotective (Whittle *et al.*, 1980). It is also Cox-1 in platelet that

leads to thromboxane production, causing platelet aggregation to prevent in appropriate bleeding (Vane and Botting 1998).

The existence of the inducing isoform, Cox-2 was first suspected when Needleman and his group showed that cytokines induced the expression of Cox protein (Raz *et al.*, 1989) and that bacterial polysaccharide increased the synthesis of prostaglandins in human monocytes in vitro, and in mouse peritoneal macrophages in vivo.

This increase was inhibited by dexamethasone and associated with de novo synthesis of new cox protein. A year or so later, Cox-2 was identified as a distinct isoform encoded by different gene from Cox-1.

Cox-2 is induced by inflammatory stimuli and by cytokines in migratory and other cells, suggesting that the anti-inflammatory action of NSAIDs are due to inhibition of Cox-2 whereas the unwanted side effect such as irritation of the stomach lining and toxic effect on the kidney are due to inhibition of the constitutive enzyme, cox-1 (Vane and Botting, 1998).

2.5.3 *Leukotrenes*

Leukotrenes (LTC4) and its products, LTD4 and LTE4, make up the complex previously known as the slow-reacting substance of anaphylaxis (SRS-A). Leukotrenes are generated by most cell types that participate in inflammatory reactions including mast cells,

basophils, eosinophils, neutrophils and monocytes (Naclero *et al.*, 1991). Studies of the effects of H1-receptor antagonists on leukotrenes release suggest that the mechanism may involve blocking the activity of the receptor-coupled G protein (Rihoux *et al.*, 1997).

Leukotrenes are derived from arachidonic acid which is made available from cells membrane phospholipids by the actions of phospholipase A2 (PLA).

2.5.4 *Free radicals (Oxidant) and Inflammation*

Most oxidants generated during the inflammatory response are derived from phagocytic cells (neutrophils, macrophages and monocytes). These are released into the extracellular environments, in part because one of the oxidants (nicotinamide adenine dinucleotide phosphate oxidase) or NAOPH oxidase is assemble in enzymatically active form on the surface of phagocytic cells (Ward and Lenstch, 1999). As shown in Fig. B, the principal oxidant-generating pathways include NADPH oxidase and inducible nitric oxide (Rossen *et al.*, 1995; Royal *et al.*, 1995; Ward and lentsch, 1999). NADPH exists as inactive subunits that are located both on the cell membrane and in the cytosolic. Cell activation translocation of cytosolic subunits to the cell membrane, result in a multimeric complex that exhibit oxidase activity. The pathway of oxidant generation by NADPH oxidase is

characterized by a series of single (instead of double) additions of electrons interactions with molecular oxygen to cause reduction, to form superoxides anion ($O^{-}_{2)}$. These are produced in inflammation which may further cause cell injury in inflammatory process.

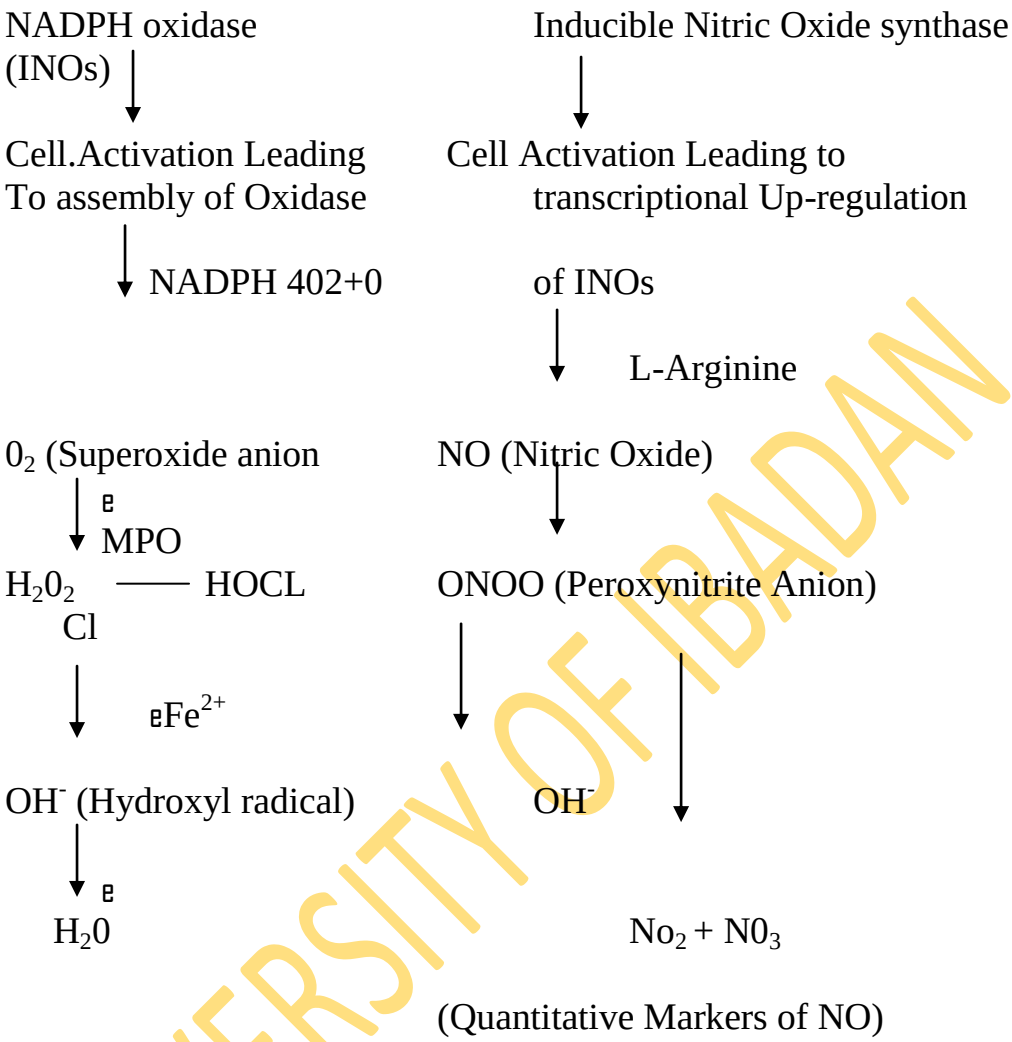


Fig B. Mechanism of Oxidant Production by activated phagocytic cells (Source: Lentsch and Ward, 1999).

2.5.5 Drug Treatment of Inflammation and their Mechanisms

Aspirin is the oldest and best known anti-inflammatory agent and has remained the most commonly used drug for relieving inflammatory symptoms (Kenneth, 2000). Other older anti inflammatory drugs such as phenylbutazone and indomethacin are commonly known as non-steriodal anti-inflammatory agents and inhibit the synthesis of pro-inflammatory prostaglandin E2 (Vane, 1971). Later, it was demonstrated that aspirin inhibits cyclooxygenase-1 (Cox-1) activity by acetylating serine 530, which is located close to the active site (tyrosine 358 of cox-1) which hinders the access of arachdonic acid to the active site (Roth and Majerus, 1975; Lecomte *et al.*, 1994). The principal pharmacological effect of aspirin and related non-steroidal anti-inflammatory agent (NSAIDs) are due to their ability to inhibit synthesis by blocking the cyclooxygenase activity by both Cox-1 and Cox -2 (Vane and Botting, 1998).

Indomethacin introduced in 1963 is an indole derivative. It is a potent non-selective Cox inhibitor and may also inhibit phospholipase A and C, reduced polynuclearneutrophil (PMN) migration, and decrease T-cell and B-cell proliferation (Douidar *et al.*, 1988). Indomethacin inhibit cox enzyme by binding competitively with the enzyme at the active site to form a reversible enzyme inhibitor complexes (Kurumbali *et al.*, 1996).

Other drugs that act similarly include Flurbiproten, Medofenamic acid and Diclofenac.

Glucocorticoids otherwise known as steroidal anti-inflammatory drugs are known to inhibit phospholipase A2, the enzyme responsible for the liberation of arachidonic acids from membrane lipids (Barnes and Adecock, 1993). Glucocorticoids have been shown to selectively inhibit the expression of cox-2 (Kujubu *et al*., 1992; *Winter et al*., 1993).

2.6 Diabetes mellitus

Diabetes mellitus is a chronic disorder of glucose intolerance; it is characterized by high blood glucose level and glucosuria, which results from dysfunction of pancreatic β-cell and insulin resistance and is associated with hyperlipidaemia and other problems such as obesity and hypertension. The defective β-cells result in total lack or partial synthesis of insulin. The insulin resistance is caused by the membrane where glucose is not transported to the cells for oxidation. As glucose is not metabolized, high amount of glucose is left circulating in the blood (hyperglycemia). To keep the normal level of glucose in the blood the kidney removes the extra sugar from the blood and excretes it in the urine (glycosuria). Because glucose is not utilized by the body cells, the body is under constant impression of hunger and that is why diabetics feel

increased appetite (polyphagia) and eat more frequently (Robinson *et al.*, 1986). In humans, diabetes is most common in elderly population and can be controlled with diet and drug therapy; hypoglycemic drugs and insulin (Khan *et al.*, 1983; Khan and Ahmed, 1994). Recently spices and natural products therapy have been used for the treatment of diabetes mellitus. The primary objectives of the treatment of all diabetes include alleviation of symptoms of hyperglycemia, prevention and treatment of associated complications and disorders, improvement of the quality of life and hence reduction in mortality caused by the disease (Shera, 1999).

The disease states underlying the diagnosis of diabetes mellitus are now classified into four categories: type 1; 'insulin-dependent diabetes" type 2; "non insulin-dependent diabetes", type 3; "others" type 4, "gestational diabetes mellitus" (Expert Committee 2002; Mayfield, 1998).

For type 1 diabetes which is as a result of selective β-cell destruction and severe or absolute insulin deficiency, only insulin therapy and oral hypoglycemic agents are used while for type 2 diabetes which is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion, drug therapy (both hypoglycemic agents and insulin), dietary, spices and natural products therapy are used. Juice from the fruit of jaman (*Syzygium cumini*) has been shown to be antidabetic (Kirtikar *et al.*, 1990; Noomrio and Dahot, 1996). The type 3

diabetes refers to multiple, other specific causes of elevated blood glucoses; non pancreatic disease, drug therapy and so on. Gestational diabetes, which is the type 4, is defined as any abnormality in glucose levels noted for the first time during pregnancy.

2.6.5 Drugs used in the Management of Diabetes mellitus

Four groups of oral antidiabetic agents are now available, these are; insulin secretagogues, (sulfonylureas, meglitinide, and D-phenylalanine derivatives), biguanides, thiazolidinediones, and α-glucosidase inhibitors. The sulfonylureas and biguanides are the traditional initial treatment of choice for type 2 diabetes. Novel classes of rapidly acting insulin secretagogues, the meglitinides and D-phenylalanine derivatives, are alternatives to the short-acting sulfonylurea (tolbutamide). The thiazolidinediones, under development since 1980s, are very effective agents that reduce insulin resistance. α-Glucosidase inhibitors have a relatively weak antidiabetic effect and significant adverse effects and they are used primarily as adjunctive therapy in individuals who cannot achieve their glycemic goals with other medications (Bertram, 2004).

INSULIN SECRETAGOGUES

Sulfonylureas

Glibenclamide (Glyburide) is an oral antidiabetic agent and is in the category of insulin secretagogues. It is a sulfonylurea. The major action of sulfonylurea is to increase insulin secretion from the pancreas. It

also reduces the serum glucagons levels and closes potassium channel in extra pancreatic tissue. Sulfonylurea binds to a 140 kda high affinity sulfonylurea receptor that is associated with a β-cell inward rectifier ATP sensitive potassium channel. Binding of sulfonylurea inhibits the efflux of potassium ions through the channel and results in depolarization. Depolarization, in turn opens a voltage-gated calcium channel and results in calcium influx and the release of preformed insulin (Groop *et al*, 1987; Gribble *et al.*, 1996; Bertram, 2004). Other sulfonylurea include; tolbutamide, tolazamide, chlorpropamide, glipizide and glimepiride which are divided into first-generation sulfonylureas (tolbutamide, chlorpropamide and tolazamide) and second-generation sulfonylureas (glyburide, glipizide and glimepiride). The second-generation sulfonylureas have fewer adverse effects and drug interactions, but should be used with caution in patient with cardiovascular disease or in elderly patients in whom hypoglycemia would be especially dangerous (Bertram, 2004).

Meglitinides

Meglitinides are relatively new class of insulin secretagogue. Repaglinide, the first member of the group was approved for clinical use in 1998 (Bertram, 2004). These drugs modulate β-cell insulin release by regulating potassium efflux through the potassium channels.

D-Phenylalanine

Nateglinide, a D-phenylalanine derivative is the latest insulin secretagogue to become clinically available. It stimulates very rapid and transient release of insulin from Beta-cells through closure of the ATP sensitive K^+ channel. It also partially restores initial insulin release in response to an intravenous glucose tolerance test. This may be a significant advantage of the drug because type 2 diabetes is associated with loss of this initial insulin response (Bertram, 2004).

Biguanides

The blood glucose-lowering action of biguanides does not depend on the presence of functioning pancreatic B-cells. Patient with type 3diabetes have considerably less fasting hyperglycemia as well as lower postprandial hyperglycemia after biguanides, however, hypoglycemia during biguanide therapy is essentially unknown. Currently proposed mechanism of action include: (1) direct stimulation of glycolysis in tissues, with increase glucose removal from blood (2) reduced hepatic and renal gluconeogenesis (3) slowing of glucose absorption from the gastrointestinal tract with increased glucose to lactate conversion by enterocytes, and (4) reduction of plasma glucagons levels (Bertram 2004).

Thiazolidinediones

The primary action of thiazolodinediones is the nuclear regulation of genes involved in glucose and lipid metabolism and adipocyte differentiation. The drug therefore acts to decrease insulin resistance. Thiazolidinediones are ligands of peroxisome proliferators-activated receptor-gamma (PPAR-y), part of the steroid and thyroid super family of nuclear receptor. These PPAR-y receptors are found in muscle, fat, and liver. PPAR-y receptors are complex and modulate the expression of the genes involved in lipid and glucose metabolism, insulin signal transduction, and adipocyte glucose and other tissue, differentiation. In persons with diabetes, the drug promotes glucose uptake and utilization and modulates synthesis of lipid hormones or cytokines and other protein involved in energy regulation in adipose tissue. It also regulates adipocyte apoptosis and differentiation. Thiazolinediones, in addition to targeting adipocytes, myocytes, and hepatocytes, they also have significant effects on tesicular endothelium, the immune system, the ovaries and tumor cells. Two thiazolidinediones currently available are poiglitazone and rosiglitazone.

Alpha glucosidase inhibitors

Only monosaccharides such as glucose and fructose can be transported out of the intestinal lumen and into the bloodstream. Complex

starches, oligosaccharides, and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase, and α-glucosidase that are attached to the brush border of the intestinal cells. Acarabose and miglitol are competitive inhibitors of the intestinal α-glucosidases and reduce the postprandial digestion and absorption of starch and disaccharides. Miglitol differ structurally from acarbose and is six times more potent in inhibiting sucrase. Acarbose and miglitol both target of the α-glucosidases; sucrose, maltase, glycoamylase, dextranase. Miglitol alone has effects on isomaltase and β-glucosidase, which split β-linked sugars such as lactose. Acarbose alone has a small effect on α-amylase. The consequences of enzyme inhibition is to minimize upper intestinal digestion and defer digestion (and thus absorption) of the ingested starch and disaccharides to the distal small intestine, thereby lowering postmeal glycemic excursions, and creating an insulin-sprint effect (Bertram, 2004).

Alloxan

Alloxan, a beta cytotoxin is a mesoxyalylurea, an agent which selectively destroys the β-cells of the pancrease and thereby induces hypoinsulinemic diabetes mellitus (Omamota *et al.*, 1981). This chemical attained great importance in research because its administration is a

convenient means for the production of insulin-deficiency diabetes in otherwise normal experimental animals. Mechanistic studies suggest that alloxan and D-glucose are structurally similar and therefore may interact with a common receptor on the β-cell (Weaver *et al.*, 1978).

2.7.1 Serum Glucose and Lipid Profile

Lipids are water insoluble and are transported in the body in an aqueous medium in combination with various specific protein and this result in lipid-protein complex called lipoprotein. These lipoproteins consist of triglycerides, cholesterol, esters and the central core surrounded by a host of unesterified cholesterol, phospholipids and proteins (Malhotra, 1987). Plasma lipoproteins occur in four major forms; High or heavy density lipoprotein (HDL), Low-density lipoproteins (LDL), Very low-density lipoproteins (VLDL) and Chylomicrons. The highest proportion of cholesterol is found in the low-density lipoprotein fractions. Cholesterol level in the blood is increased in diabetes mellitus, nephritic syndrome, obstructive jaundice, hypoparathyroidism, myxodema and xanthomatosus. Low levels of cholesterol are found in hyperthyroidism, pernicious anaemia, hemolytic jaundice, malabsorption syndrome, severe wasting in acute infection (Malhotra, 1987). Cholesterol cannot be catabolized to straight chain molecule or to acetyl CoA; therefore, it cannot be used as an energy source by the cells. High Density

Lipoprotein (HDL) is one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the periportal cells to the liver where the cholesterols is converted to bile acids and excreted into the intestine (NHCDCS, 1992). Accurate measurement of HDL is of vital importance when assessing patient risk from coronary heart disease. This method involves direct measurement of HDL without sample compared to precipitation method.

The combined risk factor of coronary heart disease in human can be determined following the estimation of serum cholesterol and HDL. The ratio of serum cholesterol to HDL has predictive value in determined risk of coronary heart disease more accurately. For normal males, the ratio of 5:1 and for normal females, the ratio of 4.5:1 are considered as average risk. Lower ratios significantly reduce the risk, whereas ratios of 9.5:1 and 7:1 for males and females respectively are believed to double the risk of chronic heart disease. An inverse relationship has been observed between the risk of chronic heart disease and the concentration of HDL (Malhotra; 1987). HDL represents approximately 20-25 percent of the total cholesterol in serum of humans. It may work as a scavenger of cholesterol from the tissue ridding the body of excess cholesterol. Low

HDL may be predictive of coronary heart disease risk whereas high level of HDL confers protection.

In human, people with high HDL levels are resistant to the development of arterosclerosis. HDL removes cholesterol from peripheral tissues and the cardiovascular system. People with high LDL levels on the other hand are prone to development of arteosclerosis. LDL is sometimes called bad cholesterol because it may serve as a source for the cholesterol that accumulated in arterisclerotic plagues. Serum glucose, triglycerides, LDL has been shown to be on increase in diabetes while there is a decrease in HDL levels (Bierman, 1992; Bopanna *et al*., 1997; Ugochukwu *et al*., 2003). This is so because apart from the regulation of carbohydrate metabolism, insulin also plays an important role in a number of ways. Insulin is a potent inhibitor of lipolysis; since it inhibits the activity of the hormone sensitive lipase production thereby suppresses the release of free fatty acids (Loci *et al.*, 1994). During diabetes, enhanced activity of this enzyme increase and thus releases more free fatty acids into the circulation (Agerdh *et al.*, 1990). Increased fatty acids concentration also increases the metabolism of fatty acids, producing more acetyl CoA and cholesterol during diabetes. In normal condition, insulin increases the receptor-mediate removal of LDL and decreased insulin during diabetes causes hypercholesterolemia.

Hypercholesterolemia and hyperglyceridemia have been recorded in diabetic rat (Bopanna *et al.*, 1997).

2.8 *Serum Biochemical Parameters of Diagnostic Importance*.

The serum biochemical parameters have been used for the diagnosis of disease and for the determination (Biomarker) of the toxic effect of some toxins in animals. This is possible because, the emergence of such a disease or toxicosis raised the levels of these serum parameters above normal. The presence of toxins in the extra cellular fluid around the cell disrupt the cellular integrity and various enzymes present in these cells escapes into the surrounding fluid compartment and into the serum or cerebro spinal fluid, where their activity can be measured as a useful index of that cell's integrity (Arthur, 1981; Abatan 1992).

2.8.1 Alanine aminotransferase (ALT/SGPT)

The enzyme ALT otherwise known as glutamate-pyruvate transaminase (GPT) is of diagnostic importance in hepatic necrosis; it is used in small animals and primates. The liver of large animals like horses, cattle and sheep contain only small amount of this enzyme and thus it cannot be used in such animals. Although SGPT is not liver specific since it is also elevated in myocardial and skeletal disease but despite that, the enzyme remains a test of choice to measure necrosis in small animals (Cornelius, 1989).

2.8.2 Aspartate aminotransferase (AST/SGOT)

The enzyme AST otherwise known as glutamate-oxaloacetate transaminase (GOT) is present in high concentration in a number of tissues, including, skeletal muscle, cardiac muscle, the erythrocytes, kidneys and the liver (Carlson, 1996). This enzyme is a non-specific indicator for tissue necrosis and tends to be less sentitive to mild insults than the tissue specific enzymes creatine phosphokinase or sorbitol dehydrogenase. Elevation of creatine phosphokinase and SGOT indicates muscle damage whereas elevation of sobitol dehydrogenase and SGOT indicates liver damage SGOT level increases in aflatoxicosis-induced liver failure (Cheeke and Shull, 1985).

2.8.3 Gamma-glutamyl transferase (GGT)

Gamma-glutamyl transferase is an important biomarker of hepatobiliary disorders and cholestasis in large animals. The enzyme is stable and reliable results can be obtained from samples submitted several days after blood samples have been collected, provided the serum sample is kept in a refrigerator. The activity of this enzyme is highest in the cells of the periportal region of the liver, in the pancreas and in the renal tubular cells. Damages to the renal tubular cells lead to a release of GGT into the tubular lumen and the urine. Increase in GGT relative to creatinine in the urine has been used as an index of acute renal tubular damage.

In large animals, elevation in serum GGT is one of the more reliable indicators of damage to the liver and biliary obstruction. Disease processes such as pyrolizidine alkaloid intoxication, chronic active hepatitis, and cholangeohepatitis produce liver damage primarily in the periportal region, leading to marked and persistent elevation of GGT activity in the serum. In these instances, elevations in serum alkaline phosphatase activity are generally associated with the increase in GGT.

2.8.4 Alkaline phosphatase (ALP)

Alkaline phosphatase is used in most animals as biomarkers for intra-hepatic or extra-hepatic obstruction of the biliary system. The enzyme is also released by osteoblast from metabolically active bones. ALP has been useful in evaluation of liver disease in large animals, particularly in horses with pyrolizidine alkaloid intoxication, chronic active hepatitis and cholangiohepatitis and in some patients with cholelithiasis. Since this enzyme is not organ specific in large animals, it is necessary to interpret elevations in ALP activity in relation to more organ-specific enzymes such as sorbitol dehydrogenase (SDH) and gamma-glutamyl transferase GGT (Carlson, 1996). In aflatoxicosis-induced liver failure, ALP level has been shown to increase (Coppock *et al.*, 1989).

2.8.5 Bile Acids and Bilirubin

Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form a soluble compound. The conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. All unconjugated, albumin bound form is also present in the circulation. Its soluble end does not normally pass through the kidneys into urine. An increase in bilirubin concentration in the serum or tissue is called jaundice; Jaundice occurs in toxic or infectious disease of the liver e.g hepatitis B or obstruction of the bile duct and in rhesus incompatible babies.

Useful information may be obtained by determining which form of bilirubin is elevated; therefore when an obstruction is present in the duct or gall bladder, unconjugated or indirect bilirubin results. A high level of unconjugated bilirubin indicates that too much haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving.

Measurement of total serum bile acid concentration has shown to be a sensitive and specific indicator of hepatic injury and disease, as well as of disorders of the enterohepatic circulation in various species (Iga and Klassen, 1982; Hardison *et al.*, 1983). Intravascular haemolysis increases level of bilirubin, associated with free haemoglobin in plasma, most of

the bilirubin will be unconjugated. Failure of conjugation or excretion occurs often transiently in severe acute hepatitis and as one of the latter events in progressive generalized liver failure; this type of hyperbilirubinaemia is accompanied with an increase in liver enzyme. However, in some cases of terminal cirrhosis or neoplastic situation of liver, there may be no hyperbilirubinaemia. Obstructive biliary disease otherwise known as cholestasis leads to obstruction of the intrahepatic or post hepatic bile duct in the liver. In complete bile duct obstruction, very high plasma bilirubin concentration can be observed while the faeces could be pale due to absence of sterco-bilinogen or urobilinogen. In the early stages of bile duct obstruction, the bilirubin will be almost all conjugated and plasma activities of liver parenchyma enzymes are usually normal but ALP will be markedly increased. In latter stages, accumulation of bile will cause actual liver damage and therefore an increase in unconjugation bilirubin and plasma level of the other enzymes (Loeb, 1989).

2.8.6 Blood Urea Nitrogen

The decrease in glomerular filtration rate, result in increase blood urea nitrogen (BUN). The causes may be prerenal azotaemia such as dehydration, shock, cardiovascular disease, and increase protein catabolism, disease such as small intestinal haemorrhages in which blood

is broken down to amino acid, necrosis, starvation, fever and infection. The cause may also be due to renal azotaemia in which the nephrons are not functional when the kidneys have been compromised. The other cause is post renal azotaemia when there is obstruction or leakages in the urinary tract. The measurement of serum urea nitrogen or serum creatinine constitutes an extremely insensitive indicator of impaired renal functions in rodents and rabbits. Serum creatinine is higher in young male rats than in young females, but this difference is abolished by 8th months of age. The use of the endogenous creatinine clearance test is a more sensitive and specific test of impaired glomerular filtration than the measurement of urea nitrogen or creatinine. It requires a timed urine specimen and a serum sample (Ringler and Dabich, 1979).

2.8.7 Cholesterol and Triglycerides

Cholesterol is a steroid with a secondary hydroxyl group in the carbon-3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atheroselerotic risk and the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders (diabetes). Blood cholesterol level is of diagnostic importance in small

animals. Hypercholesterolaemia may occur with liver failure. It may also occur in nephritic syndrome and diabetes mellitus. Very high levels of serum cholesterol usually of >50um/l is pathognomonic to hypothyroidism.

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases (Stein and Myers, 1995). Hypertriglyceaemia may be due to renal failure, acute necrotizing pancreatitis. Equine hyperlipidaemia in horse is due to prolonged dietary carbohydrate deficiency caused by inadequate grazing, or anorexia secondary to other disease like polio (Loeb, 1989).

2.8.8 Proteins

The constituents of serum total proteins are albumin and globulin; increase in serum total protein may be due to hyperalbuminaemia. Abnormality in the x-globulin fraction is known as gammopathy, it can be polyclonal or monoclonal. It is polyclonal when several antibody producing cells are secreting various types of immunoglobulin (Ig) in increased amount whereas monoclonal is when only a single type of Ig is

being produced because it is derived from one of Ig producing cells. Hypoproteinaemia may be as as result of hypoalbuminaemia due to protein synthesis such as dietary protein deficiency, maldigestion and malabsorption, which may be due to exocrine pancreatic insufficiency (EPI) or protein loosing enteropathy (PLE) and bacteria over growth. Other causes include liver failure due to hepatic atrophy of fibrosis in advanced liver disease. It may also be due to loss of albumin in renal disease with chronic protein, especially, with the glomerulonephropathy in which albumin is lost due to its low molecular weight.

2.9 Testicular Degeneration

Testicular degeneration involves retrogressive changes in the germinal epithelium of the seminiferous tubules. It is the most frequent cause of reduced fertility in male animals. The severity of this condition ranges from mild defect of spermatogenesis to gross testicular atrophy in which only the spermatogonia and sertoli cells remain, and this can be easily observed during sperm count.

The cause of testicular degeneration is mainly due to the sensitivity of the seminiferous tubule epithelium to harmful influences. One of the important causal agents is poisoning which may be due to toxic plants or chemicals such as cadmium, chlorinated naphthalein and arsenicals. All these cause vascular injury in the testes, leading to testicular necrosis.

The affected testis is smaller and softer than normal; it is firm and shrunken due to fibrosis and calcification in advanced stages. The morphological defect in the spermatozoa may be primary or secondary depending on whether the defect is caused by testicular degeneration or due to storage problem.

CHAPTER THREE

MATERIALS AND METHODS

3.1 *Experimental Animals*

The animals used for this study were adult albino rats weighing between 150 - 250g and of both sexes. The animals were housed in College of Veterinary Medicine, University of Agriculture, Abeokuta Experimental Animal House Unit, under standard environmental conditions, maintained on a natural light and dark cycle and had free access to food and water. They were fed with feeds manufactured commercially by Top Feeds Nigeria Limited. All experiments were carried out between 09:00am and 09:00pm.

3.2 Preparation of the aqueous extracts of the plants

Plants for this study i.e, *Tithonia diversifolia (Hemsl.) A. Gray, Acalypha wilkesiana (*Mull. Arg*), Lippia* m*ultiflora, Ocimum gratissimum* Linn *and Morinda morindoides* (Baker) Milne-Redh were harvested from Abeokuta town in Ogun State, except for *Morinda morindoides* (Baker) Milne-Redh which was harvested from Odeda village in Odeda Local Government of Ogun State, Nigeria, where they grow as weeds. The confirmatory identification of these plants was done by Mr Wale Ekundayo at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria.

Weighed amounts of air dried leaves/root were pulverized into powder using pestle and mortar and dissolved in known amounts of distilled water. The measured concentration of the extracts was then administered orally with the aid of cannula to the test groups of rats. The fresh aqueous extract of these plants were produced every other day in order to ensure that their active principles are still potent and whatever remains of extract of the first day were always kept refrigerated for use the next day.

3.3 *Pilot Toxicity Study*

The acute toxicity study were carried out on the animals to determine if these plants are toxic using the "up and down" procedure recommended by (WHO) in which 3000 mg/kg dose of each extract was givens to 10 rats per plant extract orally at once, the animals were then observed for seventy-two hours (three days) for any physical signs of toxicity or death. The faeces were examined for symptom of diarrhoea, or blood in faeces and the rate and amount of voided urine were also examined for any signs of abnormality, but basically mortality was the major focus of this study.

3.4 *Sub-Acute Toxicity Study*

Graded Doses Calculation and Administration

The graded doses of the crude plant extracts were administered to the animals based on the formula below.

$Y_2 = Y_1 R^{N-1}$

Y_1= an arbitrary dose/ the 1st chosen dose

Y_2 = 2nd dose

R = a constant = (2)

N = No. of doses considered

Y_1 = 100 mg/kg, so Y_2 = 400 mg/kg, the initial chosen dose of 100 mg/kg was discarded and the 400 mg/kg dose adopted based on usual practice by traditional medical practioners and the next 2 multiples of the 400 mg/kg were adopted as the 2nd and 3rd dose i.e, 800 mg/kg and 1600 mg/kg respectively.

Each plant was assigned 3 groups of animal representing 3 different concentrations of the plant extract with the fourth administered with equal volumes of distilled water representing control and each group consisted of 5 animals per group. The aqueous extracts were administered to the animals orally using a stomach canula on a daily basis for 28 days. The control group rats were given water equivalent to the maximum volume of extract given.

3.5 Blood Sample Collection

Two groups of blood samples were collected with the aid of capillary tubes from the retro orbital plexus of diethyl ether anesthetized

rats, Group 1 into heparinised bottle for analysis of haematological parameters, while the other group was collected into plain bottles (ideal sample bottles for serum chemistry). The rats were then sacrificed through cervical dislocation and organs such as liver, kidneys, pancreas, spleen, heart, testes and epididymis were collected into Bouins solution in sample bottles for histopathology. Sperm were also collected for sperm count and sperm cell morphological study.

3.6 Determination of haematological parameters

The blood samples collected into the plain bottles (non heparinised bottles) were used in the determination of blood parameters such as packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), and total and differential white blood cells (WBC) (lymphocytes, neutrophils, eosinophils, basophils, monocytes) and platelets counts. Erythrocyte indices were also calculated.

3.6.1 Erythrocyte count

This was determined by the haemocytometer method as described by Jain, (1986). Red blood cell diluting pipette marked 101 above the bulb was filled with blood sample to exactly 0.5 mark. The tip of the pipette was inserted into the erythrocyte diluting fluid (Growers solution) and through a steady suction the pipette was filled with the fluid to the mark above the bulb. The pipette was then shaken for three minutes by a

simple wrist movement with the pipette held horizontally between the thumb and the middle finger. The haemocytometer was then filled with the diluted blood in the pipette and allowed a few minutes for the cells to settle. The erythrocytes were then counted. The erythrocytes counted was determined from the sum of all cells in the five small square multiplied by 10,000 and this gives the total erythrocyte per cubic millimeter.

3.6.2 Estimation of Haemoglobin concentration

The cyanomethaemoglobin method was used (Jain, 1986). 0.02ml of the blood sample was added to 4ml of drabkins diluent. The mixture was allowed to stand for 10 minutes after thorough mixing and the absorbance of the resulting solution was then read in a colorimeter at 540nm wavelength. The haemoglobin value of the blood sample was calculated as follows:

Photometer reading of unknown x gm% Hb of standard x Dilution factor = Hb conc. (g/dl).

3.6.3 Determination of the Packed Cell Volume

The plain capillary tubes were filled with blood samples to about two-third of the tubes and sealed at one end with plastersene and then centrifuged for 5-6 minutes at 3,500 rpm in the haematocrit centrifuge. The packed cell volume in percent was then read directly from a graphic reader.

3.6.4 Total Leucocyte count

The white blood cell diluting pipette marked 11 above the bulb was filled with blood samples to the mark 0.5 and this was then filled with leucocyte diluting fluid to the mark 11 above the bulb. The mixture was shaken for 3 minutes until well mixed (Leucocyte diluting fluid is made up of glacial acetic acid and 2 ml gentian violet). 2-3 drops were discharged from pipette before filling the counting chamber of the haemocytometer. The cells were allowed one minute to settle and the number of the leucocytes in the four large corners square of the counting chamber was counted under low power microscope lens (x16). The sum of the cells counted in the four corner squares multiplied by 50 gives the total leucocytes per cubic millimeter.

3.6.5 Leucocytes differential count

The white blood cell differential count was done by using blood smears, fixed with alcohol and stained with Giemsa stain. The different white blood cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils) could be identified under oil immersion microscope and counted per ten fields. Identification of the different types of leucocytes was enhanced using colour plates of Diggs Dorothy, and Ann Bell, Abott Laboratories.

3.6.6 Determination of Erythrocyte Indices

The erythrocyte indices define the size and haemoglobin content of the erythrocytes from the values obtained from RBC count, haemoglobin concentration and PCV. The indices include: mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH).

Mean corpuscular volume (MCV), expresses the concentration of haemoglobin in the average erythrocyte.

$$MCV = \frac{PCV \times 10}{RBC \text{ in millions/mm}^3}$$

MCV is expressed in flow rates (fl).

Mean corpuscular haemoglobin concentration (MCHC), expresses the concentration of haemoglobin in the average erythrocyte.

$$MCHC = \frac{Haemoglobin \times 10}{PCV}$$

MCHC is expressed in percentage.

Means corpuscular haemoglobin (MCH) is the amount of haemoglobin by weight in the average erythrocyte.

$$MCH = \frac{Haemoglobin \times 10}{RBC \text{ counts}}$$

MCH is expressed in picograms (pg).

3.7 *Determination of serum biochemical parameters*

3.7.1 *Measurement of serum sodium*

The serum sodium level is measured in order to estimate the electrolyte balance in the body and to assess the electrolyte therapy to animals.

PROCEDURE:

The serum was diluted as 1:100 with distilled water. To 0.1ml serum in a test tube was added 9.9ml distilled water.

The stock was prepared by dissolving 5.85gm of sodium chloride in 1 liter distilled water. The working standards can be prepared by measuring 10ml, 11ml, 12ml, 13ml, 14ml, and 15ml of stock in 100ml volumetric flasks and diluting this up to 100ml with distilled water. This gives 100ml, 110ml, 120ml, 130ml, 140ml and 150ml mEq/liter sodium.

A standard curve is prepared by putting the readings of flame photometer and mEq/liter standard on either side of the graph paper. Put the readings of test serum and read the values of sodium from standard curve in mEq/liter (Chauhan and Agarwal, 2006).

3.7.2 *Measurement of serum potassium*

PROCEDURE:

The serum was diluted as 1:100 with distilled water. To 0.1ml serum in a test tube was added 9.9ml distilled water.

The stock was prepared by dissolving 0.746gram of potassium chloride (KCl) in 1 liter distilled water. The working standards can be prepared by measuring 3ml, 4ml, 5ml, 6ml, and 7ml of stock in 100ml volumetric flasks and diluting this up to 100ml with distilled water. This gives 3ml to 7 mEq/liter potassium.

A standard curve is prepared by putting the readings of flame photometer and mEq/liter standard on either side of the graph paper. Put the readings of test serum and read the values of sodium from standard curve in mEq/liter (Chauhan and Agarwal, 2006).

3.7.3 *Measurement of serum total bilirubin*

PROCEDURE:

This is a colorimetric method based on the diazo reaction for bilirubin (Jendrassik and Grof. 1938; Nosslin, 1960; Michaelson 1961). Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

The reagents include:

Reagent 1-29mmol.l sulphanilic acid and 0.17N Hydrochloric acid

Reagent 2-25mmol/l sodium Nitrite

Reagent 3-0.36mol/l caffeine and 0.52mol/l sodium benzoate

Reagent 4-0.93mol/l tartrateand 1.9N sodium hydroxide

Procedure:

Total Bilirubin:

Pipette into cuvette the following:

	Sample Blank (ml)	Sample (ml)
Reagent 1	0.20	0.20
Reagent 2	-	0.50
Reagent 3	1.00	1.00
Reagent 4	0.20	0.20

Mix, and allow to stand for 10 minutes at 20-25^{o}C and then add reagent 4

Reagent 4	1.00	1.00

Mix allow to stand for 5-30 minutes at 25^{o}C and then read the absorbance of the sample against the sample blank (ATB).

Direct Bilirubin:

Pipette into cuvette the following:

	Sample Blank (ml)	Sample (ml)
Reagent q	0.20	0.20
Reagent 1	-	0.05
Sodium chloride (9g/l)	2.00	2.00
Sample	0.20	0.20

Mix and allow to stand for exactly 5 minutes at 20-25oC read the absorbance of the sample against the sample blank (ADB).

Calculation:

Total bilirubin (mg/dl) = 10.8 x ATB

Direct bilirubin (mg/dl) = 14.4 x ADB

Indirect bilirubin = Total - Direct.

3.7.4 *Measurement of serum albumin*

PROCEDURE:

Colorimetric estimation of serum albumin (Chauhan and Agarwal, 2006) was done using the sigma diagnostic albumin reagent (Sigma Diagnostic, U.K) which contained bromocresol green (BCG). 0.025ml of serum was added to BCG and the level of albumin read at 628nm using a spectrophotometer.

Calculation:

Concentration of albumin in sample (gm/dl)

$$= \frac{\text{optical density of test sample}}{\text{optical density of standard}} \times \text{Concentration of standard}$$

3.7.5 *Measurement of glucose*

PROCEDURE:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD) (Chauhan and Agarwal, 2006). The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red violet quinoneimine dye as indicator. Twenty microlitre (20µl) of the sample was pipetted into test tube containing 2000 µl of the reagent, also 20µl of the standard was pipetted

into test tubes containing 2000 µl of the reagents and 2000 µl of the reagent was pipetted into the test tube of the reagent blank. These were mixed and incubated for 25mins at 15-25^{o}C or for 10mins at 37^{o}C. The absorbance of the standard (A of standard) and that of the sample (A of sample) was measured against the reagent within 60mins.

3.7.6 *Determination of serum cholesterol*

PROCEDURE:

Cholesterol was determined enzymatically by colorimetric method using cholesterol esterase and cholesterol oxidase (Siedel *et al*., 1983; Wiebe and Bernert, 1984; Greiling and Gressner, 1995).

$$\text{Cholesterol esters} + H_20 \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{RCOOH}$$

Cholesterol ester is elevated by the action of cholesterol esterase to yield free cholesterol and fatty acids.

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one hydrogen peroxide.

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone monoimino)-phenazone} + 4H_2O$$

The hydrogen peroxide created forms a red dye stuff by reacting with 4-aminophenazone and phenol under the catalytic action of

peroxidase. The colour intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

3.7.7 *Determination of Serum Triglycerides*

PROCEDURE:

This is a colorimetric test, based on the method of using a lipoprotein lipase from microorganisms for the rapid and complete hydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dye stuff (Eggstein and Kreutz, 1966; Bucolo and David, 1973; Greiling and Gressner, 1995), which is then measured.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + 3\text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosohate} + O_2 \xrightarrow{\text{Glycerol phosphate Oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenzone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone–monoimino) - phenazone} + 2H_2O + HCL$$

3.7.8 *Determination of Serum High Density Lipoproteins (HDL) and Low Density Lipoprotein (LDL)*

In this method, Low Density Lipoprotein fraction is precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ion. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

Procedure:

Pipette into centrifuge tubes:

	Macro	Semi Micro
Sample/Standard	500µl	200µl
Precipitant	1000µl	-
Diluted Precipitant	-	500µl

Mix and allow standing for 10 minutes at room temperature. Then centrifuge for 10 minutes at 400 rpm. Separate the clear supernatant within two hours to determine the cholesterol content by the CHOD-PAP method.

Cholesterol CHOF-PAP Assay (Friedewald *et al*, 1972):

Wavelength	546nmHg
Cuvette	1cm light path
Temperature	37^{o}C
Measurement	against reagent blank

Pipette into test tubes:

	Reagent Blank	Standard	Sample
Distilled water	100ul	-	-
Supernatant	-	-	100ul
Standard Supernatant	-	100ul	-
Reagent	100ul	100ul	100ul

Mix and incubate for 5 minutes at 37^{o}C. Measure the absorbance of the sample (Asample) and standard (Astandard) against the reagent blank within 60 minutes.

Calculation:

HDL Cholesterol

When using a factor:

	Macro		Semi Micro	
Wavelength	mmol/l	mg/dl	mmol/l	mg/dl
Hg 546 nm	7.06	274	8.27	320

When using a standard:

Concentration of HDL Cholesterol in supernatant

$$= \frac{\text{AA sample}}{\text{AAsample}} \text{ X Conc. Of standard}$$

LDL Cholesterol

In mmol/l

$$= \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.2} - \text{HDL Cholesterol}$$

In mg/dl:

$$= \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

3.7.9 *Measurement of Total Protein*

The method used is based on Biuret reaction in which copper in Biuret reagent reacts with the peptide bonds of serum protein to form purple coloured complex (Gornall, 1949).

$$\text{Copper} + \text{serum protein} \xrightarrow{\text{Alkaline phosphate}} \text{copper– protein complexes – (purple)}$$

Reagents:

Biuret reagent- copper sulfate 0.15% (W/V), sodium hydroxide 3% (W/V) with tartrate and iodine added.

Protein standard solution albumin (5g/dl) and globin (3g/dl)

Procedure:

	Reagent blank	Standard solution	serum sample
Water	0.1ml	-	-
Protein	-	0.1ml	-

Sample	-	-	0.1ml
Biuret reagent	5ml	5ml	5ml

These were mixed thoroughly and allowed to stand for 15 minutes at room temperature. The test sample was then read against the blank at 540nm in spectrophotometer.

Calculation:

$$\text{Serum total protein (g/dl)} = \frac{\text{A test x 8}}{\text{A standard}}$$

Where A test and A standard represent the absorbance of test and standard solutions respectively and 8g/dl is the total protein in the standard solution.

3.7.10 *Measurement of blood urea nitrogen (BUN)*

This method is based on a direct interaction of urea with diacetylmonoxide (Fearon reaction) (Crocker 1967).

Diacetylmonoxide + urea $\longrightarrow$ chromogen + hydroxylamine

Urea concentration is directly proportional to the intensity of the colour produced.

Reagents:

BUN acid reagent contains ferric chloride in phosphoric acid and sulfuric acid.

BUN colour reagent contain diacetylomoxide 0.18 (W/V) and thiosemicarbazide, while urea nitrogen standard solution contains urea at a urea nitrogen level of 30 mg/dl with benzoic acid as preservative.

Procedure:

	Blank	Standard	Test
Bun acid reagent	3ml	3ml	3ml
BUN color reagent	2ml	2ml	3ml
Urea nitrogen std sol.	-	0.2ml	-
Serum sample	-	-	0.2ml

Shake thoroughly and place in boiling water for 10 minutes. Read the absorbance at 540nm with the blank as reference. BUN level in mg/dl was read from a prepared calibration curve.

3.7.11 *Measurement of alkaline phosphatase (ALP)*

The method used is colorimetric method (Rec Gsse (DGKC) 1972). This involves the reaction of p-nitrophenyl phosphate in water in the presence of alkaline phosphatase to produce phosphate and p-nitrophenol.

$$\text{P-nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{P-nitrophenol}$$

The reagents used include buffer solution of diethanolamine in 1mol/l at pH 9.8 and 0.5mmol/l of magnesium chloride and the substrate, which is 10mmol/l of p-nitrophenyl phosphate.

Procedure:

Pipette into cuvette	Macro	Semi Micro	Micro
Sample	0.05ml	0.02ml	0.01ml
Reagent (25, 30, 37^{o}C)	3.00ml	1.00ml	0.50ml

Mix and read initial absorbance and start timer simultaneously. Read again after 1, 2, and 3 minutes.

Calculation:

U/l = 3.300 x AA 405 nm/min Macro

U/l = 2760 x AA 405 nm/min Semi Micro

U/l = 2760 x AA 405 nm/min Micro

3.7.12 *Measurement of alanine aminotrasferase (ALT)*

Alanine aminotransferase is also known as glutamic pyruvate transaminase (GPT) and is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyhydrazine (Rictmans and Frankel, 1957).

GPT

α-Oxoglutarate + L-alanine L-glutamate + pyruvate.

Reagents:

Solution 1: phosphate buffer 100mmol/l, pH 7.4

L-alanine 200mmol/l and α-Oxoglutarate 2.0mmol/l

Solution 2: 2.0mmol/l of 2, 4-dinitrophenylhydrazine

0.4 mol/l of sodium hydroxide.

Procedure:

Pipette into test tubes:

	Reagent Blank	Sample
Sample	-	0.1ml
Solution 1	0.5ml	0.5ml
Distilled Water	0.1ml	-

Mix and incubate for exactly 30 minutes at 37^{o}C, then add solution 2

Solution 2	0.5ml	0.5ml

Mix allow to stand for exactly 20 minutes at 20-25^{o}C and add

Sodium hydroxide	5.0ml	5.0ml

Mix and read the absorbance of sample (Asample) against the reagent blank after 5 minutes. The activity of ALT in the serum can be obtained from the prepared table.

3.7.13. *Measurement of aspartate aminotransferase (AST)*

Aspartate aminotranferase otherwise known as glutamate oxaloacetatic transminase (GOT) can be measured by monitoring the concentration of oxalocate hydrazone formed with 2, 4-dinitropheylhydrazine (Reitman and Pranket, 1957).

GOT

α-oxoglutarate+L-aspartate L-glutmate + oxaloacetate

Reagents:

Solution 1: phosphate buffer 100mmol/l, pH 7.4, L-aspartate 100mmol/l, α-Oxoglutarate 2mmol/l.

Solution 2: 2, 4-dinitrophenylhydrzine 2mmol/l

Procedure:

Pipette into test tubes:

	Reagent Blank	Sample
Sample 1	-	0.1ml
Solution 1	0.5ml	0.5ml
Distrilled water	0.1ml	-

Mix and incubate for exactly 30 minutes at 37^{o}C and add

Solution 2	0.5ml	0.5ml

Mix and allow standing for exactly 20 minutes at 20-25^{o}C then add

Sodium hydroxide	5.0ml	5.0ml

Mix and read the absorbance of sample (A sample) against the reagent blank after 5 minutes**.**

3.8 *Histopathological Observation*

The liver, kidney, pancreas, lungs, spleen, heart and testes were harvested into sample bottles containing Bouin solution for histopathology.

The tissues were dehydrated in 70%, 95%, absolute ethanol and another absolute ethanol for duration of two hours in each solution. The clearing of the tissue was done in three changes of xylene for the same time duration (2hrs in each). The impregnation of the tissues was done in two changes of molten paraffin wax and this was completed in the wax oven. They were then embedded in molten paraffin wax, blocked after solidifaction, and sectioned on the microtome at four-microns thickness. The sections were stained with haematoxylin and eosin (H and E) and mounted on permanent slides and then observed under high power (x 40) microscope lens.

3.9 *Determination of the sperm count and morphology*

Two studies were made on the sperm, (the sperm concentration and morphology). The sperm concentration was determined by harvesting epididymis into a graduated test tube containing 5.0ml of distilled water to obtain a final volume (volume of distilled water + epididymis). The epididymis in distilled water was properly crushed with the pair of mortar and pestle. A drop of the solution formed was placed into haemocytometer with a cover slip. The haemocytometer is made up of 25

counting chambers and sperm cells were counted in the 5 diagonal and inner chambers. Sperm concentration = A x 1000 x 5 sperm cells/ml where A is the number of sperm cell counted.

The sperm morphology involved counting the abnormalities present in the sperm cells (spermatozoa). A drop of sperm from the epididymis was mixed on a slide with a drop of Wells and Awa stain. The smear of the mixture was then made on the slide and observed under high power (x 40) microscope lens. The sperm abnormalities considered were tailless head, headless tail, rudimentary tail, bent tail, curved tail, curved midpiece and bent midpiece.

3.10 EXPERIMENT 2A: ANTI-DIABETIC STUDY

***Determination of the Antidiabetogenic Effect of the Extracts*.**

3.10.1 *Induction of diabetes mellitus in the rats.*

Diabetes mellitus was induced by single injection of 5% alloxan monohydrate (5g/100ml of normal saline) at a dose rate of 125mg/kg intraperitoneally (Katsumata *et al.,* 1999; Trivedi *et al.,* 2004; Rajesh *et al.,* 2004). Diabetes mellitus was confirmed after 48 hours (on the third day). Only those rats with blood glucose level of 150mg/dl or greater were included in the study (day 0). The control group was not administered with alloxan but given normal saline intraperitoneally. Third day to the alloxan injection was taken as day zero and the fasting blood glucose of all the groups were measured using electronic glucometer (ACCU chek Advantage, Roche diagnostic GmbH) (Nagappa *et al.,* 2003), from the blood taken from the tail clip of the rats.

3.10.2 *Experimental Design*

Forty-eight rats weighing between 150g -200g and of both sexes were used for this study. They were subdivided to 8 groups (A-H) of 6 rats per group. Groups A-E were administered with 400 mg/kg aqueous plant extracts of *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum gratissimum* and *Morinda morindoides* respectively for twenty-one days orally, group F was given (10mg/kg) glibenclamide an oral hypoglycemic daily for twenty one days. Group G is the untreated

diabetic control group and was given distilled water orally for twenty-one days while the group H animals serves as the control group and were not pretreated with alloxan but administered with distilled water orally for twenty-one days.

The fasting blood glucose level and the serum lipid profile were measured on the twenty-first day (day 21) of the experiment from the blood collected from the retro orbital plexus of the rats. Another groups of rats had their blood glucose level monitored at 0min, 30min, 1hr, 2hrs, 4hrs, 6hrs, 8hrs and 24hrs.

3.10.3 Collection of blood samples from rats

Three millilitres (3 mls) of blood was collected from retro orbital plexus of dietyl ether anaesthesized rats, into non-heparinized sample bottles for the glucose assay and lipid profile analysis. Also the following tissues; pancreas, liver and kidney of the rats were harvested and preserved in Bouin solution for histopathology study.

3.10.4 *Determination of fasting blood glucose*

The rats were fasted for 18 hours before their blood samples were taken but they were given water ad - libitum. Electronic glucometer (ACCU check Advantage, Roche diagnostic GmbH), (Nagappa *et al*, 2003) was used to take readings of blood glucose; readings for each rat were repeated thrice and the average reading was used.

3.10.5 Determination of serum cholesterol

Cholesterol was determined enzymatically by colorimetric method using cholesterol esterase and cholesterol oxidase (Siedel *et al*, 1983; Wiebe and Bernert, 1984; Greiling and Gressner, 1995).

$$\text{Cholesterol esters} + H_20 \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{RCOOH}$$

Cholesterol esters are elevated by the action of cholesterol esterase to yield free cholesterol and fatty acids.

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one hydrogen peroxide.

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone monoimino)-phenazone} + 4H_2O$$

The hydrogen peroxide created forms a red dye stuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The colour intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

3.10.6 Determination of Serum Triglycerides

This is a colorimetric test, based on the method of using a lipoprotein lipase from microorganisms for the rapid and complete hydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-

chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Eggstein and Kreutz, 1966; Bucolo and David, 1973; Greiling and Gressner, 1995), which is then measured.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + 3\text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosohate} + O_2 \xrightarrow{\text{Glycerol phosphate}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenzone} + \text{4-chlorophenol} \xrightarrow[\text{Peroxidase}]{\text{Oxidase}} \text{4-(p-benzoquinone–monoimino)- phenazone} + 2H_2O + HCl$$

3.10.7 Determination of Serum High Density Lipoproteins (HDL) and Low Density Lipoprotein (LDL)

In this method, Low Density Lipoprotein fraction is precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ion. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

Procedure:

Pipette into centrifuge tubes:

	Macro	Semi Micro
Sample/Standard	500µl	200µl

Precipitant	1000µl	-
Diluted Precipitant	-	500µl

Mix and allow to stand for 10 minutes at room temperature. Then centrifuge for 10 minutes at 400 rpm. Separate the clear supernatant within two hours to determine the cholesterol content by the CHOD-PAP method.

Cholesterol CHOF-PAP Assay (Friedewald *et al*, 1972):

Wavelength	546nm.Hg
Cuvette	1cm light path
Temperature	37^{o}C
Measurement	against reagent blank

Pipette into test tubes:

	Reagent Blank	Standard	Sample
Distilled water	100ul	-	-
Supernatant	-	-	100ul
Standard Supernatant	-	100ul	-
Reagent	100ul	100ul	100ul

Mix and incubate for 5 minutes at 37^{o}C. Measure the absorbance of the sample (A sample) and standard (A standard) against the reagent blank within 60 minutes.

Calculation:

HDL Cholesterol

When using a factor:

	Macro		Semi Micro	
Wavelength	mmol/l	mg/dl	mmol/l	mg/dl
Hg 546 nm	7.06	274	8.27	320

When using a standard:

Concentration of HDL Cholesterol in supernatant

$$= \frac{\text{AA sample}}{\text{AAsample}} \text{ X Conc. Of standard}$$

LDL Cholesterol:

In mmol/l

$$= \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.2} - \text{HDL Cholesterol}$$

In mg/dl:

$$= \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

3.11 *EXPERIMENT 2B*

Hourly Evaluation of the Hypoglycemic Effect of the Aqueous extracts of *Tithonia diversifolia (Hemsl.) A. Gray, Acalypha wilkesiana* (Mull. Arg), *Lippia multiflora* (Moldenke), *Ocimum gratissimum* Linn and *Morinda morindoides* (Baker) Milne-Redh in rats

3.11.1 *Induction of diabetes mellitus in the rats.*

Diabetes mellitus was induced by single injection of 5% alloxan monohydrate (5g/100ml of normal saline) at a dose rate of 125mg/kg intraperitoneally (Katsumata *et al*, 1999; Trivedi *et al*, 2004; Rajesh *et al*, 2004). Diabetes mellitus was confirmed after 48 hours (on the third day). Only those rats with blood glucose level of 150mg/dl and above were included in the study (day 0). The control group was not administered with alloxan but given normal saline intraperitoneally. Third day after the alloxan injection day zero and the fasting blood glucose of all the groups were measured using electronic glucometer (ACCU chek Advantage, Roche diagnostic GmbH) (Nagappa *et al.*, 2003), with the blood taken from the tail clip of the rats.

3.11.2 *Experimental Design*

Forty-eight rats weighing between 150g -200g and of both sexes used for this study were subdivided in to 8 groups (A-H) of 6 rats per group.

Groups A-E were administered with a single dose of 400mg/kg aqueous plant extract of *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum gratissimum* and *Morinda morindoides* respectively orally, group F was given a single dose of (10 mg/kg) glibenclamide orally while, group G was the untreated diabetic control group and was given a single dose distilled water orally at 10 mg/kg. The group H animals serve as the positive control and were not pretreated with alloxan but administered with distilled water orally.

The fasting blood glucose levels were measured at the following time periods 30 minutes, 1hr, 2hrs, 4hrs, 6hrs, 8hrs and 24 hrs after the administration of the extract

3.11.3 *Determination of fasting blood glucose*

The rats were fasted for 18 hours before the commencement of the experiment and their blood samples were taken and measured by electronic glucometer (ACCU check Active, Roche diagnostic GmbH), (Nagappa *et al.*, 2003) was used to measure the blood glucose. Readings for each rat were repeated thrice and the average reading was used.

3.12 EXPERIMENT 3

Determination of the Analgesic Effects of the Plant Extracts

The two methods commonly employed for the determination of the analgesic effects of plants extracts: (i) The Hot Plate Test and (ii) The Acetic acid induced Writhing Movements Test (Koster *et al.*, 1959; Okolo *et al.*, 1995; Okiemy-Andissa *et al.*, 2004) were used.

3.12.1 *Hot plate test*

The total number of mice used in this study was forty-two and they weighed between 22-25g. They were divided into seven groups of six mice each. The first five groups (A-E) were the test groups, which were administered orally with 400 mg/kg of aqueous extracts of *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora*, *Ocimum gratissimum* and *Morinda morindoides* respectively. The animals were fasted overnight before use for the study. The positive control group (F) was given morphine intraperitoneally at a dose rate of 2 mg/kg and the negative control group (G) was administered distilled water equivalent to the maximum volume of the extract given orally. The hot plate method involves placing the animals on a hot plate maintained at 55^{o}C until the animal reacted to the pain produced by the heat. The reaction time is the time it takes the animal to react to the pain produced by heat and this may involve either rubbing the palms together, licking the palms or jump from

the hot plate. The extracts and drugs were administered one hour before commencing the procedure (Okolo *et al.*, 1995).

3.12.2 Acetic acid induced writhing movement test.

The animals used in this experiment were mice and forty-two in number weighing between 20-26g and were grouped into seven groups of six animals each. Feeds were withdrawn from the animals for 12 hours before starting the study.The first 5 groups (A-E) were administered 400 mg/kg of aqueous extracts of *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum grattissimum* and *Morinda morindoides* orally an hour prior to the induction of the writhing movements. The positive control group (F) i.e the 6th group was given paracetamol (50mg/kg), while group (G) animals, served as the negative control group and were administered distilled water equivalent to the maximum volume of the extract given.

The writhing movements syndrome was induced in the mice by giving 10 ml/kg of 0.6% of acetic acid intraperitoneally (Koster *et al.*, 1959; Okiemy-Andissa *et al.*, 2004).The number of writhing movement, which is the reaction of the mice to the pain induced by acetic acid, of each mouse, was determined for 10 minutes starting 10 minutes after the injection of acetic acid (Okiemy-Andissa *et al.*, 2004).

Percentage inhibition of writhing

The percentage inhibition of writhing as a result of the analgesic effect of the drugs or the plants extract used was calculated according to the formula:

$$\text{Percentage Inhibition} = \frac{\text{Means of writhing}_{(control)} - \text{Mean of writhing}_{(test)}}{\text{Mean number of writhing}_{(control)}} \times 100$$

3.13 EXPERIMENT 4

3.13.1 Determination of anti-inflammatory effects of the extracts

The animals used in this study were 48 rats of the Wistar albino strain weighing between 150 and 220 g. They were divided into eight groups of six rats each. The animals were fasted for 12 hours before the commencement of the study. Groups (A-E) were pretreated with extracts of *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum gratissimum* and *Morinda morindoides* respectively. Group F and G were also pretreated with 150 mg/kg aspirin and indomethacin (10mg/kg) respectively while group H, the negative control group was given distilled water equivalent to the maximum volume of the extract given.

Procedure for the test:

The anti-inflammatory properties of the plants were investigated by using the acute carrageenan induced oedema model (Winter *et al.*, 1962). Inflammation was induced by injection of 0.1ml carrageenan (1% suspension of carrageenan in normal saline) into the planter surface of the right hind paw. Control group was injected with saline (0.9%) into the planter surface of the right hind paws. Immediately after injection the linear circumference of the paw was measured at zero hour on injecting the carrageeenan injection and three hours after carrageenan injection using a loop of thread tied round the paw such that it was neither too

loose nor too tight, the linear circumference after three hours was taken as an index of increase in paw volume, which is a measure of the oedema (Abatan and Adeagbo, 1986). The plant extract, aspirin (150mg/kg) and indomethacin (10mg/kg) were administered one hour before induction of inflammation.

Inhibition of increase in paw volume

The percentage inhibition of increase in paw volume as a result of the anti-inflammatory effect of the drugs or the plants extract used was calculated according to the formula:

$$\text{Percentage Inhibition} = \frac{[C_1\text{-}C_0]_{control} - [C_1\text{-}C_0]_{test}}{[C_1\text{-}C_0]_{control}} \times 100$$

Where Co=Mean paw size at zero hour

C_1= Mean paw size at 3hours after induction of inflammation.

3.14 EXPERIMENT 5

BIOACTIVITY GUIDED FRACTIONATION

Solvent – Solvent Fractionation of Cold methanolic Plant Extract, Purification and Isolation of Active Principles

3.14.1 Introduction

The general chemical composition of an unknown plant product is determined by means of a qualitative chemical analysis by extraction with different solvents. This can be achieved by successive and selective extractions of the plant product with solvent of different polarities. This was therefore carried out to obtain different fractions using Hydromethanol (methanol-H_20), n-Hexane, chloroform and ethyl acetate (Fig B).

3.14.2 *General Experimental Procedures*

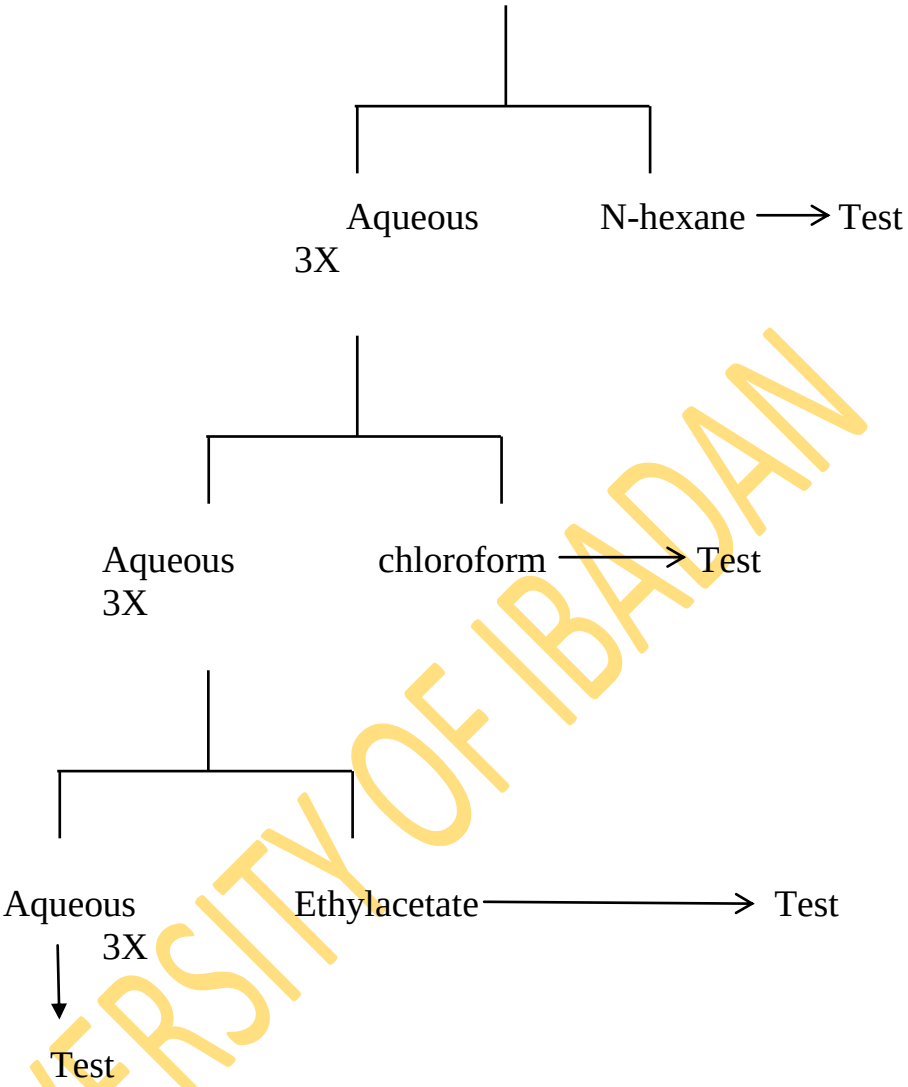
All solvents used for the extraction and chromatographic analysis were of analytical grade.

The analytical and preparative procedure utilized adsorption chromatography. Analytical thin layer Chromatography (TLC) was performed using commercial pre-coated plates (silica gel, Kieselgel, $60PF_{254,}$ 0.25 mm thick on polyester backing, Merck, Damstadt, Germany (Merck Ltd.). Column chromatography was performed on open column silica gel (Merck, Kieselgel 60, 70-230 mesh). The nuclear magnetic

resonance (^{1}H and ^{13}C-NMR) spectra were taken in dimethyl sulphoxide (DMSO) in the Fourier Transform mode on a Bruker WP 120054 Spectrometer (200MHz) for the ^{1}H- NMR and (100 MHz) for ^{13}C-NMR, chemical shift values ,**δ** in ppm with trimethyl silane (TMS) as internal reference. The coupling constant, *J* values were recorded in Hertz.

3.14.3 *Methodology*

The methanolic extract of *A. wilkesiana* leave and *M. morindoides* root bark were subjected to solvent –solvent fractionation in separating funnel. Hundred grams (100 g) of crude methanolic extract of each plant was suspended in 1:3 methanol-water (hydromethanol), n-Hexane, chloroform, and ethylacetate. This was done in a separating funnel at each stage of partitioning, the organic fraction (Phase) were pooled, and the solvent removed by concentrating the fraction on a water bath at 55 ^{o}C. This procedure was carried out for n-Hexane, chloroform, ethyl acetate and hydromethanol respectively.



1. Partition between water and organic solvents

2. Removal of solvent phase to leave solid residue (fraction) for testing.

3X implies procedures repeated thrice

Figure C: Scheme for the solvent – solvent extraction

3.14.4 *Accelerated Gradient Chromatographic Purification Method (Flash chromatography)*

Bioactive fractions obtained from solvent-solvent fractionation, were therefore subjected to further purification using accelerated gradient chromatography.

Methodology

A dry glass column was arranged vertically and supported by clamps. The column was pegged at the base with glass wool to support and hold the slurry of silica gel (Stationary phase) as described by (Stahl, 1980). The column packing was done by pouring the slurry of the silica gel (Merck, Kieselgel 60, 70-230 mesh) into the column and the particles allowed settling. After settling, the top of the column was protected from disturbance when loading the sample or when topping it with solvent by covering it with a small piece of cotton wool. This helped to avoid entrapment of small air bubbles that can cause channeling.

The level of the solvent was never allowed to go below the top of the packing. The flow rate of the solvent was regulated by electrical pump and kept constant.

Forty grams (40g) of chloroform fraction of *M.morindoides*, 24g of n-Hexane fraction of *A. wilkesiana* and 20g of the ethylacetate fraction of

M. morindoides were each separately absorbed on silical gel, allowed to air- dry and packed on the column. The different components were eluted with solvent system combination. The eluent was collected in 50ml conical flasks. Each sample was spotted on the thin layer chromatography (TLC) and the eluent bulked according to their TLC profiles, using their different pooling ratio of solvent system. They were pooled into a total of six (6) sub-fractions (A – F) per extract.

Each of the chromatographic fractions (A-F) was suspended in tween-80 and 100mg/kg dose of each of the chromatographic fractions obtained was tested for their previous bioactive evaluation either for anti-diabetic, anti-inflammatory or analgesic activities.

3.15 Statistical Analysis

The results were expressed as the mean ± standard error of mean (SEM). They were analyzed statistically using One-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. P values <0.05 were considered significant. In all cases SPSS version 12 was used (SPSS instruction manual, 1998).

CHAPTER FOUR

RESULTS

4.1.0 EXPERIMENT - 1A: Acute Toxicity Study

The animals in this study were observed after the administration of 3000 mg/kg of the extracts of the plants for seventy-two hours (3 days). No mortality was recorded in any of the groups, even at seventy-two hours after the administration; there were no serious toxic nervous signs observed.

4.1.1 EXPERIMENT -1B: *Sub-Acute Toxicity Study*

A. Effects of the different doses of Tithonia diversifolia (Hemsl.) A. Gray on haematological parameters of rats

The rats dosed with 400 mg/kg of *T. diversifolia* showed a statistically significant ($P<0.05$) increase in WBC value ($6.44\pm1.1 \times 10^3/mm^3$) and statistically significant ($P<0.05$) reduction in the mean corpuscular haemoglobin (MCH) value (21.18±0.2 pg) compared to control (Table 1).

The rats dosed with 800 mg/kg aqueous extract of *T. diversifolia* produced a statistically significant ($P<0.05$) increase in WBC (6.08 ± 0.4 $10^3/mm^3$) compared with values in the control group (Table 2).

The rats administered 1600 mg/kg dose of *T. diversifolia* exhibited a statistically significant reductions ($p<0.05$) in MCH (21.42±0.10 pg)

while the WBC count (4.94±0.4 x 10^3/mm^3) was statistically significantly (P<0.05) increased when compared with the control (Table 3).

B. Effects of the different doses of Acalypha wilkesiana on haematological parameters of rats

The 400 mg/kg and 800 mg/kg doses of aqueous extracts of *A. wilkesiana* had no effect on haematological parameters when compared with the control (Tables 1& 2).

The rats administered with 1600 mg/kg dose of *A. wilkesiana* exhibited a statistically significant (p<0.05) reduction in neutrophil (29.40±1.9 x 10^3/mm^3) while lymphocytes (71.60±2.1 x 10^3/mm^3) was significantly increased (P<0.05) compared with the control (Table 3).

C. Effects of the different doses of Lippia multiflora on haematological parameters of rats

The 400 mg/kg dose of the aqueous extract of *L. multiflora* produced a statistically significant (p<0.05) reduction in MCH (21.24±0.2 pg) when compared with the control (21.78±0.7 pg) (Table 1).

The rats dosed 800 mg/kg aqueous extract of the *L. multiflora* produced no statistically significant (P<0.05) change in all the haematological parameters when compared with the control group (Table 2).

The rats administered 1600 mg/kg dose of *L. multiflora* exhibited a statistically significant reductions at ($p<0.05$) in MCH (21.38±0.0 pg), WBC (2.04±0.3 x $10^3/mm^3$), and NEUT (25.20±2.9 x $10^3/mm^3$), while the LYMP (74.40±2.8 x $10^3/mm^3$) increased significantly ($P<0.05$) when compared with the control (Table 3).

D. Effects of the different doses of Ocimum gratissimum on haematological parameters of rats

The rats administered with 400 mg/kg dose of aqueous extract of *O. gratissimum* produced statistically significant ($p<0.05$) reduction in WBC (2.08±0.3 x $10^3/mm^3$) when compared with the control (3.68±0.4 x $10^3/mm^3$) (Table 1).

The rats dosed with 800 mg/kg aqueous extract of the *O. gratissimum* produced no statistically significant ($P<0.05$) change in all the haematological parameters when compared with the values in the control group (Table 2).

The rats administered with 1600 mg/kg dose of *O. gratissimum* exhibited a statistically significant reductions ($p<0.05$) in MCH (21.58±0.1pg), WBC (1.96±0.2 x $10^3/mm^3$) and NEUT (24.80±3.1 x $10^3/mm^3$), while the LYMP (75.00±3.1 x $10^3/mm^3$) was significantly at ($P<0.05$) increased when compared with the control (Table 3).

E. Effects of the different doses of Morinda morindoides (Baker) Milne-Redh *on haematological parameters of rats*

The 400 mg/kg and 1600 mg/kg doses of aqueous extract of *M. morindoides* did not produce any statistically significant changes ($p<0.05$) in the haematological parameters (Tables 1 & 3). However, the 800 mg/kg dose of *M. morindoides* produced statistically significant ($p<0.05$) decrease in the neutrophil ($71.60\pm2.1 \times 10^3/mm^3$) when compared with the control ($38.0\pm3.1 \times 10^3/mm^3$) (Table 2).

TABLE 1: EFFECTS OF 400 MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS. (MEAN + STANDARD ERROR OF MEAN)

PLANTS	PCV	RBC	HB	MCV	MCH	MCHC	WBC	NEUT.	LYMP	MONO	BASO	EOSINO
Tithonia diversifolia	29.0± 1.3	4.58± 0.2	9.72± 0.5	63.32± 0.2	21.18± 0.2	33.46± 0.4	6.44± 1.1*	1.67± 2.6	4.73± 2.7	0.01± 0.2	1.43± 22	0.01± 0.2
Acalypha wilkesiana	28.4± 1.6	4.46± 0.3	9.54± 0.6	63.66± 0.6	21.36± 0.1	26.84± 6.6	3.94± 0.4	1.17± 4.5	2.74± 4.8	0.02± 0.4	0.00± 0.0	0.00± 0.0
Lippia multiflora	23.8± 3.6	3.74± 0.6	7.94± 1.2	63.52± 0.3	21.24± 0.2*	20.33± 8.1	3.18+ 0.6	0.88± 1.0	2.28± 0.8	0.00± 0.2	0.00± 0.0	0.00± 0.0
Ocimum gratissimum	26.0± 1.3	4.18± 0.2	8.98± 0.4	63.62± 0.2	21.50± 0.6	33.76± 0.5	2.08± 0.3*	0.59± 3.6	1.48± 3.8	0.00± 0.2	0.00± 0.0	0.00± 0.0
Morinda morindoides	26.6± 0.6	4.16± 1.0	9.06± 0.2	63.94± 0.2	21.78± 0.7	34.08± 0.5	3.08± 0.4	0.82± 3.3	2.25± 3.2	0.00± 0.0	0.00± 0.0	0.00± 0.0
Control	27.0+ 1.5	4.24+ 0.2	9.22+ 0.4	63.66+ 0.2	21.78+ 0.7	20.36+ 8.2	3.68+ 0.4	1.40+ 3.1	2.30+ 2.8	0.01+ 0.4	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume %), Hb = Haemoglobin Concentration (g/dl)

Rbc = Red blood cell ($x10^6/mm^3$), WBC= White blood cell ($x\ 10^3/mm^3$)

Lymp = Lymphocytes, NEUT= Neutrophils, MCV= Mean corpuscular volume (fl)

MCH = Mean Corpuscular Haemoglobin (pg)

MCHC = Mean Corpuscular Haemoglobin Concentration (%)

Note * Superscripted figures are statistically significant at $P < 0.05$.

TABLE 2: EFFECTS OF 800 MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS	PCV (%)	RBC ($x10^6/mm^3$)	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)	WBC ($x\ 10^3/mm^3$)	NEUT.	LYMP	MONO	BASO	EOSINO
Tithonia diversifolia	26.4± 1.5	4.22± 0.2	8.70± 0.6	63.35± 0.2	21.18± 0.2	25.01± 8.2	6.08± 0.4*	2.05± 1.7	4.01± 2.1	0.02± 0.4	0.00± 0.0	0.00± 0.0
Acalypha wilkesiana	28.4± 1.5	4.42± 0.2	9.45± 0.7	64.05± 0.2	21.20± 0.2	33.13± 0.3	3.64± 0.3	1.19± 4.3	2.48± 4.3	0.00± 0.4	0.00± 0.0	0.00± 0.0
Lippia multiflora	28.6± 0.5	4.50± 0.9	9.73± 0.2	63.53± 0.4	21.50± 0.1	25.41± 8.4	3.88± 1.3	1.19± 1.8	2.70± 1.6	0.00± 0.2	0.00± 0.2	0.00± 0.0
Ocimum gratissimum	24.0± 0.7	3.80± 0.1	8.20± 0.3	63.38± 0.3	21.43± 0.1	33.83± 0.1	2.72± 0.2	0.84± 4.3	1.9± 2.9	0.00± 0.0	0.00± 0.0	0.00± 0.0
Morinda morindiodes	29.2± 2.7	4.60± 0.4	9.90± 0.9	63.44± 0.2	21.50± 0.1	27.19± 6.7	3.76± 0.4	1.00± 3.5*	2.70± 3.3	0.00± 0.2	0.00± 0.2	0.00± 0.2
Control	27.0+ 1.5	4.24+ 0.2	9.22+ 0.4	63.66+ 0.2	21.78+ 0.7	20.36+ 8.2	3.68+ 0.4	1.40+ 3.1	2.30+ 2.8	0.01+ 0.4	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume Hb = Haemoglobin Concentration

Rbc = Red blood cell WBC= White blood cell

Lymp = Lymphocytes, NEUT= Neutrophils, MCV= Mean corpuscular volume

MCH = Mean Corpuscular Haemoglobin

MCHC = Mean Corpuscular Haemoglobin Concentration

Note * Superscripted figures are statistically significant at P <0.05.

TABLE 3: EFFECTS OF 1600 MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS	PCV (%)	RBC ($x10^6$/ mm^3),	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)	WBC ($x10^3$/ mm^3)	NEUT.	LYMP	MONO	BASO	EOSINO
Tithonia diversifolia	24.40± 1.2	3.88± 0.2	8.56± 0.5	63.48± 0.1	21.42± 0.1	33.70± 0.1	4.94± 0.4*	1.8± 3.1	3.29± 2.3	0.00± 0.2	0.00± 0.0	0.00± 0.0
Acalypha wilkesiana	25.20± 1.0	3.96± 0.2	8.50± 0.4	63.88± 0.2	21.70± 0.0	33.68± 0.2	3.94± 0.3	1.16± 1.9*	2.82± 2.1*	0.00± 0.00	0.00± 0.0	0.00± 0.0
Lippia multiflora	27.80± 0.7	4.38± 0.1	9.36± 0.3	63.46± 0.2	21.38± 0.0*	33.68± 0.1	2.04± 0.3*	0.51± 2.9*	1.52*± 2.8	0.00± 0.2	0.20± 0.0	0.00± 0.0
Ocimum gratissimum	26.80± 1.7	4.20± 0.3	9.06± 0.5	63.78± 0.3	21.58± 0.1*	33.84± 0.2	1.96± 0.2*	0.49± 3.1*	1.47± 3.1*	0.0± 0.2	0.00± 0.0	0.00± 0.0
Morinda morindiodes	24.20± 1.8	3.78± 0.3	8.28± 0.6	64.08± 0.4	21.94± 0.1	34.20± 0.1	3.98± 0.1	1.24± 2.6	2.70± 2.1	0.02± 0.3	0.00± 0.0	0.00± 0.4
Control	27.00± 1.5	4.24± 0.2	9.22± 0.4	63.66± 0.2	21.78± 0.2	20.36± 8.2	3.68± 0.4	1.40± 3.1	2.30± 2.8	0.01± 0.2	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume Hb = Haemoglobin Concentration

Rbc = Red blood cell WBC= White blood cell

Lymp = Lymphocytes, NEUT= Neutropils, MCV= Mean corpuscular volume

MCH = Mean Corpuscular Haemoglobin

MCHC = Mean Corpuscular Haemoglobin Concentration

Note * Superscripted figures are statistically significant at P <0.05.

4.2 EXPERIMENT 2: SUB- ACUTE EFFECTS OF THE AQUEOUS CRUDE EXTRACTS OF THE PLANTS ON SERUM BIOCHEMISTRY OF RATS

A *Effects of the different doses of Tithonia diversifolia (Hemsl.) A. Gray on serum biochemical parameters of rats*

The rats dosed with 400 mg/kg of *T. diversifolia* showed a statistically significant (P<0.05) increase in the values of the serum aspartate aminotransferase (AST) (103.08±23.6 µ/l), alkaline phosphatase (ALP) (70.05±12.4 µ/l), alanine aminotransferase (ALT) (141.10±2.7 µ/l) and a statistically significant (P<0.05) reduction in the value of blood glucose (GLUC) (80.50±3.4 mg/dl) when compared to control groups (37.27±4.3, 27.92 ± 9.5, 124.00±6.4 µ/l and 116.0±9.8 mg/dl respectively) (Tables 4A&B).

The rats dosed 800 mg/kg aqueous extract of *T. diversifolia* produced statistically significant (p<0.05) increases in the serum urea (UREA) (51.10±4.6mg/dl) and in the serum aspartate aminotransferase (AST) (57.08±7.6 µ/l) while the blood glucose (GLUC) level (86.33±5.6mg/dl) was statistically significantly (P<0.05) decreased when compared with the values in the control group (Tables 5A&B).

The rats administered with 1600 mg/kg dose of *T. diversifolia* exhibited a statistically significant increase (p<0.05) in serum aspartate aminotransferase (AST) (77.38±20.0µ/l), and alanine aminotransferase

(ALT) (129.26±7.2µ/l) while the blood glucose (GLUC) level (70.00±2.5mg/dl) was statistically significantly (P<0.05) decreased when compared with the values in the control group (Tables 6A&B).

B Effects of the different doses of Acalypha wilkesiana on serum biochemical parameters of rats

The rats dosed with 400 mg/kg of *A. wilkesiana* showed a statistically significant (P<0.05) increase in the value of the serum aspartate aminotransferase (AST) (84.96±9.7 µ/l) and statistically significantly (P<0.05) decreased the values of total protein (TP) (74.55±4.3g/dl), globulin (GLOB) 29.14±5.3g/dl and the blood glucose (GLUC) (80.50±3.4mg/dl) when compared to control group (Tables 4A&B).

The rats dosed 800 mg/kg aqueous extract of *A. wilkesiana* produced statistically significant (p<0.05) increase in the serum aspartate aminotransferase (AST) (76.07±5.1 µ/l), while the blood glucose (GLUC) level (73.00±6.6mg/dl) is statistically significantly (P<0.05) decreased when compared with the values in the control group (Tables 5A&B).

The rats administered with 1600 mg/kg dose of *A. wilkesiana* exhibited a statistically significant increase (p<0.05) in the blood urea (UREA) level (48.80±2.7 µ/l), serum aspartate aminotransferase (AST) (58.69±4.6 µ/l), and alanine aminotransferase (ALT) (131.72±4.0 µ/l) while the blood glucose (GLUC) level (81.13±5.7mg/dl) is statistically

significantly (P<0.05) decreased when compared with the values in the control group (Tables 6A&B).

C. Effects of the different doses of Lippia multiflora on serum biochemical parameters of rats

The rats dosed with 400 mg/kg of *L. multiflora* showed a statistically significant (P<0.05) increase in the values of the total protein (TP) (94.69±2.4g/dl), serum aspartate aminotransferase (AST) (86.69±18.5 µ/l), alanine aminotransferase (ALT) (137.18±4.1 µ/l) and a statistically significant (P<0.05) decrease in blood glucose (GLUC) (78.16±2.0mg/dl) when compared to control group (Tables 4A&B).

The rats dosed 800 mg/kg aqueous extract of *L. multiflora* produced statistically significant (p<0.05) decreases in glucose (GLUC) level (77.58±4.2mg/dl) and the plasma potassium ion (2.95±0.1mEq/l) when compared with the values in the control group (Tables 5A&B).

The rats administered with 1600 mg/kg dose of *L. multiflora* exhibited a statistically significant increase (p<0.05) in the serum aspartate aminotransferase (AST) (60.27±5.6 µ/l), and alanine aminotransferase (ALT) (139.42±3.4 µ/l) while the blood glucose (GLUC) level (84.74±5.4mg/dl) was statistically significantly (P<0.05) decreased when compared with the values in the control group (Tables 6A&B).

D. Effects of the different doses of Ocimum gratissimum on serum biochemical parameters of rats

The rats dosed with 400 mg/kg of *O. gratissimum* showed a statistically significant increase ($p<0.05$) in the blood urea (UREA) level (49.63±6.7mg/dl) and alkaline phosphatase (ALP) (52.21±8.5 µ/l). However, the dose produced a statistically significant ($P<0.05$) reduction in the serum globulin (GLOB) (30.70±0.1g/dl) and blood glucose (GLUC) (69.64±3.7mg/dl) when compared to control group (Tables 4A&B).

The rats given 800 mg/kg dose of the aqueous extract of *O. gratissimum* produced statistically significant ($p<0.05$) increases in the serum aspartate aminotransferase (AST) (59.01±4.3 µ/l) while the blood glucose (GLUC) level (76.45±4.0mg/dl) and potassium ions (K^+) (3.73±0.3mEq/l) were statistically significantly ($P<0.05$) decreased when compared with the control group (4.88±0.4 mEq/l) (Tables 5A&B).

The rats administered 1600 mg/kg dose of *O. gratissimum* exhibited no statisticallysignificant changes ($p<0.05$) in all the serum parameters except for the blood glucose (GLUC) level (62.00±1.5 mg/dl) that was statistically significantly ($P<0.05$) reduced when compared with the value in the control group (116.0±9.8mg/dl) (Tables 6A&B).

E. Effects of the different doses of Morinda morindoides on serum biochemical parameters of rats

The rats dosed with 400 mg/kg of *M. morindoides* showed statistically significant ($P<0.05$) decreases in the values of total protein (TP) (74.10±4.8g/dl), globulin (GLOB) (28.08±3.7g/dl) and the blood glucose (GLUC) (70.60±5.5mg/dl) and a statistically significant ($P<0.05$) increase in the value of alkaline phosphatase (ALP) (52.44±1.8 µ/l) when compared to values in the control group (27.92±9.5 µ/l) (Tables 4A&B).

The rats dosed 800 mg/kg aqueous extract of *M. morindoides* produced statistically significant ($p<0.05$) decreases in the serum creatinine (CREAT) (0.58±0.0mg/dl), the blood glucose (GLUC) level (94.62±13.2mg/dl) and potassium ions (K^+) (3.76±0.2 mEq/l) when compared with the values in the control group (Tables 5A&B).

The rats administered 1600 mg/kg dose of *M. morindoides* exhibited a statistically significant increase ($p<0.05$) in the serum cholesterol (CHOL) level (110.45±6.5mmol/l), the low-density lipoprotein (LDL) (60.79±5.5mmol/l) while the blood glucose (GLUC) level (73.64±3.3mg/dl) is statistically significantly ($P<0.05$) decreased when compared with the values in the control group (116.0±9.8mg/dl) (Tables 6A&B).

TABLE 4A: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T.PROTEIN (g/l)	UREA (mg/dl)	CREATININ (mg/dl)	ALBUMIN (g/l)	GLOBULIN (g/l)	AST (u/l)	ALP (u/l)	ALT (u/l)	TOTAL BILIRUBIN (mg/dl)
Tithonia diversifolia	83.22±4.9	49.15±5.6*	0.76±0.07	44.43±0.9	38.80±4.6	103.08±23.6*	70.05±12.4*	141.10±2.7*	0.78±0.2
Acalypha wilkesiana	74.55±4.3*	38.41±3.9	0.78±0.12	44.61±1.3	29.14±5.3*	84.96±9.7*	50.19±4.6	118.98±7.6	0.69±0.3
Lippia multiflora	94.69±2.4*	46.50±3.2	0.88+0.14	47.14±1.3	47.65±3.3	86.69±18.5*	45.10±8.3	137.18±4.1*	0.78±0.1
Ocimum gratissimum	76.62±3.4*	49.63±6.7*	0.74±0.13	45.79±2.0	30.70±4.3*	71.09±9.8	52.21±8.5	125.22±5.5	1.26±0.1
Morinda morindoides	74.10±4.8*	36.64±3.8	1.10±0.25	46.01±2.3	28.08±3.7*	69.22 ±11.74	52.44±1.8	131.28±5.9	0.77±0.2
Control	83.50±4.9	36.23±3.8	0.86±010	40.97±1.7	42.14±5.7	37.27±4.3	27.92±9.5	124.00±6.4	1.08±0.3

Note: * Superscripted items are statistically significant at P<0.05

T. Protein = Total protein, AST = Aspantate Amino transferase; T.BIL= Total Bilirubin

ALP = Alkaline Phosphataze, ALT = Alamine amino transferase

TABLE 4B: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	Ka+ (mmol/l)	Na+ (mmol/l)
Tithonia diversifolia	80.50±3.4*	63.00±10.6	70.99±5.3	30.84±1.5	27.56±3.0	3.99±0.3	68.69±24.3
Acalypha wilkesiana	80.02±5.7*	67.55±3.2	86.08±2.1	50.23±5.0	22.52±3.3	5.32±0.5	70.66±15.7
Lippia multiflora	78.16±2.0*	66.48±8.6	87.29±9.0	49.31±4.2	24.68±4.7	4.56±1.5	117.12±19.9
Ocimum gratissimum	69.64±3.7*	77.04±7.0	106.97±29.3	62.15±28.4	29.43±2.0	4.53±0.4	87.48±8.9
Morinda morindoides	70.60±5.5*	74.44±3.9	108.37±8.1	66.77±10.5	26.69±52	5.67±0.9	104.9±20.3
Control	116.00±9.8	83.41±10.5	87.24±8.2	45.34±7.1	25.22±4.2	4.88±0.4	81.59±19.4

Note: Superscripted figures are statistically significant at P<0.05.

Trig = Trighyceride, Chol = Cholestenol, HDL= High density lipoproteins, LDL =Low density lipoprotein,

K^- = Potassium Na^+ = Sodium ion

TABLE 5A: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T. PROTEIN (g/dl)	UREA (mg/dl)	CREATININE (mg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	AST (u/l)	ALP (u/l)	ALT (u/l)	T.BIL (mg/dl)
Tithonia diversifolia	81.11±4.7	51.10±4.6*	0.70±0.1	46.62±1.1	34.13±4.7	57.08±7.6*	50.38±5.7	132.94±6.0	0.52±0.1
Acalypha wilkesiana	83.63±4.3	32.78±3.5	0.78±0.1	42.41±1.9	41.21±5.1	76.07±5.1*	45.12±8.3	126.40±5.1	0.75±0.2
Lippia multiflora	76.20±3.1	30.10±3.7	0.64+0.1	43.70±0.3	33.07±3.0	47.70±9.7	55.93±3.4	116.66±10.1	0.58±0.2
Ocimum gratissimum	89.79±3.5	38.29±3.8	0.64±0.0	42.68±1.5	45.27±3.1	59.01±4.3*	48.04±12.7	110.92±7.4	0.39±0.1
Morinda morindoides	74.22± 5.6	31.02±3.9	0.58±0.0*	45.09±2.0	29.13±4.9	45.85±6.5	56.76±6.5	127.32±12.5	0.44±0.1
Control	83.51±4.9	36.23±3.8	0.87±0.1	40.97±1.7	42.14±5.7	37.27±4.3	27.92±9.5	124.00±6.4	1.08±0.3

Note: * Superscripted items are statistically significant at P<0.05

T. Protein = Total protein, AST = Aspartate Amino transferase; T.BIL= Total Bilirubin

ALP = Alkaline Phosphatase, ALT = Alanine amino transferase

TABLE 5B: EFFECTS OF 800 MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	K^+ (mmol/l)	Na^+ (mmol/l)
Tithonia diversifolia	86.33±5.6*	76.00±9.0	99.49±13.2	48.90±6.4	35.53±5.4	4.14±0.4	101.88±6.5
Acalypha wilkesiana	73.00± 6.6*	72.59±12.6	89.57±7.8	48.84±5.0	26.22±3.5	4.18±0.2	91.04±15.2
Lippia multiflora	77.58±4.2*	53.94±9.7	95.74±8.7	47.57±7.9	37.38 ±4.0	2.95±0.1*	96.01±8.2
Ocimum gratissimum	76.45± 4.0*	70.83±4.8	87.99±6.2	68.11±29.8	32.94±8.2	3.73±0.3*	67.02±26.0
Morinda morindoides	94.62±13.2*	64.44±5.6	98.91±13.7	50.37±8.1	35.65±3.4	3.76±0.2*	103.10±22.9
Control	116.00±9.8	83.41±10.5	87.24±8.2	45.34±7.1	25.22±4.2	4.88±0.4	81.59±19.4

Note: Superscripted figures are statistically significant at P<0.05.

Trig =Trighyceride, Chol= Cholestenol, HDL= High density lipoproteins, LDL =Low density lipoprotein,

K^- = Potassium Na^+ = Sodium ion

TABLE 6A: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T. PROTEIN (g/dl)	UREA (mg/dl)	CREATININE (mg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	AST (µ/l)	ALP (µ/l)	ALT (µ/l)	T.BIL (mg/dl)
Tithoina diversifolia	77.28±6.3	32.35±1.6	0.70±0.1	44.99±1.0	28.78±6.3	77.38±20.0*	59.41±5.2	129.26±7.2*	0.80±0.1
Acalypha wilkesiana	76.94±2.9	48.80±2.7*	0.78±0.1	44.28±4.1	33.61±5.4	58.69±4.6*	42.96±4.9	131.72±4.0*	1.16±0.2
Lippia multiflora	80.74±8.1	40.19±1.3	0.80±0.1	39.05±5.0	41.69±5.5	60.27±5.6*	40.19±8.6	139.42±3.4*	1.22±0.6
Ocimum gratissimum	71.11±7.2	34.86±3.6	0.62±0.0	44.00±1.2	26.94±7.6	49.69±5.0	44.11±2.8	123.20±7.3	0.45±0.1
Morinda morindoides	73.55±5.3	34.70±5.7	0.64±0.1	42.85±2.7	30.70±4.0	31.23±1.4	41.97±8.2	107.30±8.1	0.56±0.3
Control	83.51±4.9	36.23±3.8	0.87±0.1	40.97±1.7	42.14±5.7	37.27±4.3	27.92±9.5	124.00±6.4	1.08±0.3

T. Protein = Total protein, AST = Aspantate Amino transferase; T.BIL= Total Bilirubin
ALP = Alkaline Phosphatase, ALT = Alamine amino transferase
Note: Superscripted figures are statistically significant at P<0.05.

TABLE 6B: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	K^+ (mmol/l)	Na^+ (mmol/l)
Tithonia diversifolia	70.00±2.5*	65.17±9.5	79.48±6.2	38.56±8.7	27.89±5.3	4.28± 0.5	82.92±16.9
Acalypha wilkesiana	81.13±5.7*	77.55±11.9	103.87±2.7*	49.49±5.2	38.88±4.3	4.33±0.2	97.88±29.6
Lippia multiflora	84.74±5.4*	68.43±6.9	79.99±2.7	39.82±2.8	26.49±4.0	5.49±1.2	68.61±13.4
Ocimum gratissimum	62.00±1.5*	60.00±4.5	91.63±8.9	46.32±7.6	33.31±3.2	3.25±0.2	102.46±50.7
Morinda morindoides	73.64±3.3*	70.75±8.6	110.45±6.5*	60.79±4.5*	35.45±2.7	3.9±0.2	77.42±18.4
Control	116.00±9.8	83.41±10.5	87.24±8.2	45.34±7.1	25.22±4.2	4.88±0.4	81.59±19.4

Note: * Superscripted items are statistically significant at P<0.05
GLYC = Glucose, TRIG = Triglycerides, CHOL = Cholesterol, LDL = Low Density Lipoproteins, HDL = High Density Lipoprotein
Na+ = Sodium ions, K+ = Potassium ions

4.1 Histopathologic changes produced by the crude extract

A. *T. diversifolia*

At the dose of 400 mg/kg and 800 mg/kg body weight, the section of the liver showed mild periportal degeneration of hepatocytes, the section of the kidney showed mild diffuse tubular degeneration and necrosis with evidences of regeneration and section of the spleen showed mild hypoplasia of the lymphoid nodules.

In the group of rats dosed with 1600 mg/kg, section of liver showed diffuse, severe hepatic necrosis with evidences of regeneration i.e cytomegally, binucleation (Plate 7).

The section of kidney showed mild diffuse tubular degeneration of the epithelia cells and mebraneous glomerulonephritis.

B. *A. wilkesiana*

At the dose of 400 mg/kg and 800 mg/kg body weight, most lesions seen in the organs were mild and not significant except for the group of rats dosed with 1600 mg/kg of the crude extract, where the section of the kidney showed diffuse vacuolar degeneration of the tubular epithelia (Plate 8).

C. *L. multiflora*

The crude extract of *Lippia multiflora* at 400 mg/kg and 800 mg/kg body weight did not show any significant organ histopathology except

for the rats dosed with 1600 mg/kg where the section of the liver showed moderate periportal degeneration and necrosis of the hepatocytes (Plate 9).

D. *O.gratissimum*

There were no significant histopathologic changes in the groups of rats dosed with 400 mg/kg and 800 mg/kg body weight of *O. gratissimum*.

At 1600 mg/kg dose, the section of the epididymis showed empty lumina and contains few spermatogonia cells (evidence of severe spermiostasis) (Plate 10).

Other lesions observed include spleen with mild hypoplasia of the lymphoid nodules and hyperplasia of the megakaryocytes and there no significant lesion observed in the heart.

E. *M. morindoides*

At the doses of 400 mg/kg and 800 mg/kg body weight, there were no significant lesion in the liver, spleen, testis and epididymis; there was mild tubular nephrosis of the kidney and at 1600 mg/kg dose, significant pathological lesions were observed in the heart with the section showing thickened epicardium and mononuclear cells infilteration (Plate 11).

Other lesions observed at this dose are liver section showing diffuse degeneration and necrosis of the hepatocytes and the kidney showing mild tubular nephrosis.

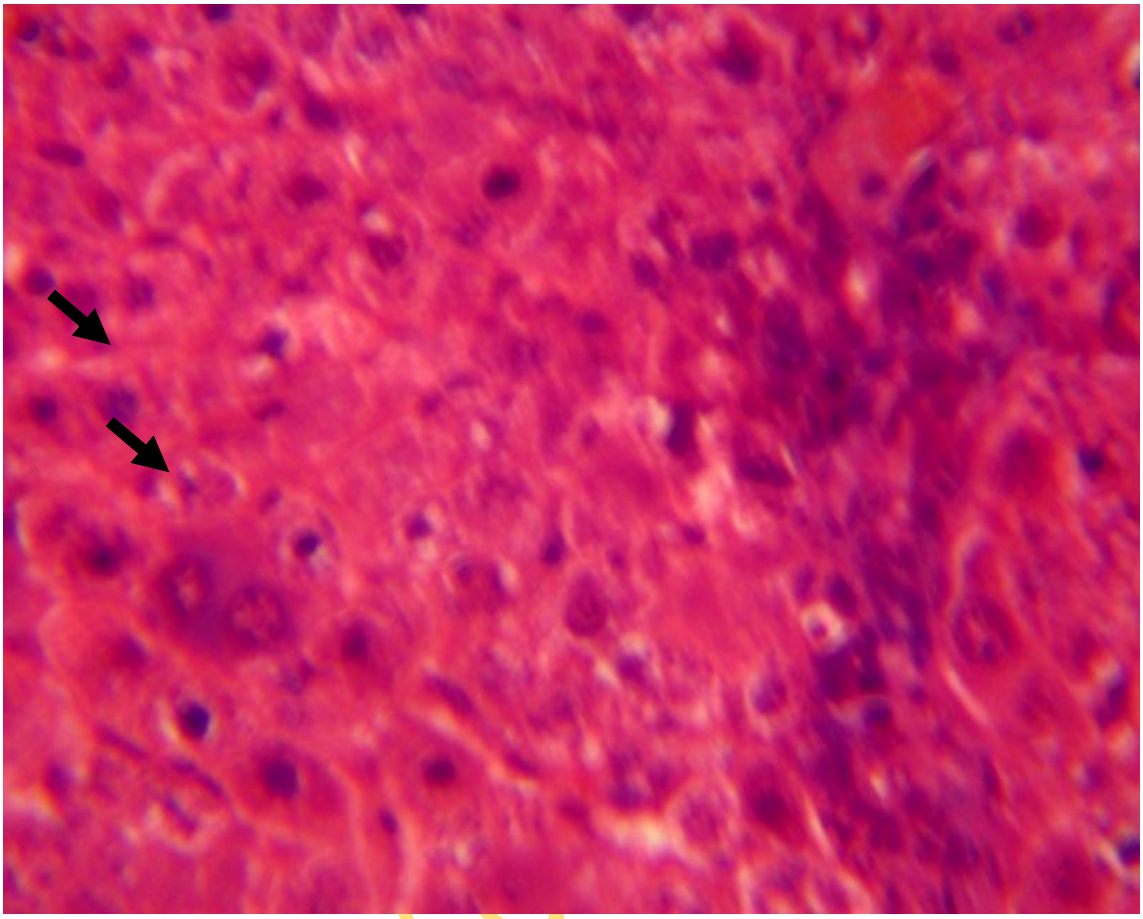


Plate 7: showing section of liver with diffuse severe hepatic necrosis and evidences of regeneration i.e cytomegally, binucleation (arrow) (H & E X250).

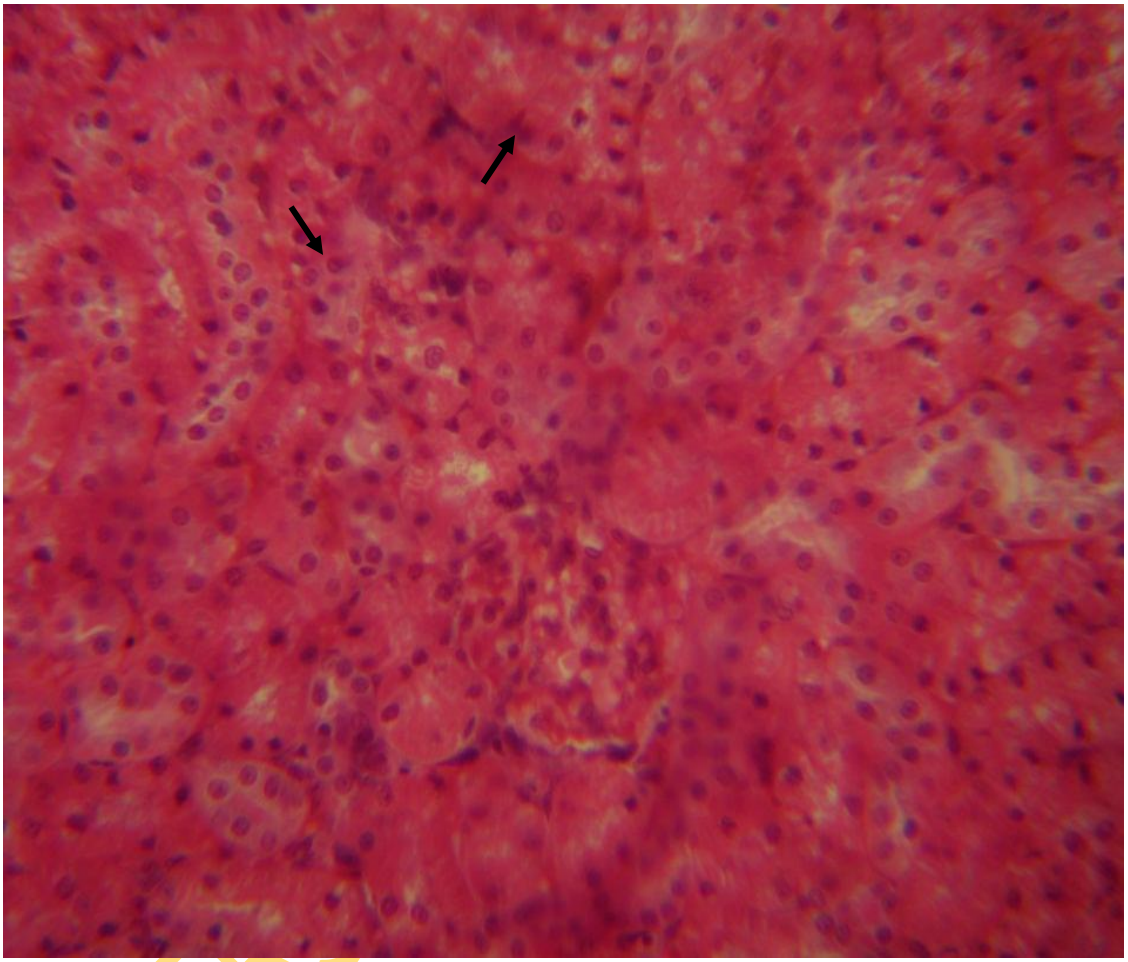


Plate8: showing section of kidney with diffuse vacuolar degeneration of the tubular epithelial cells (H & E X 250).

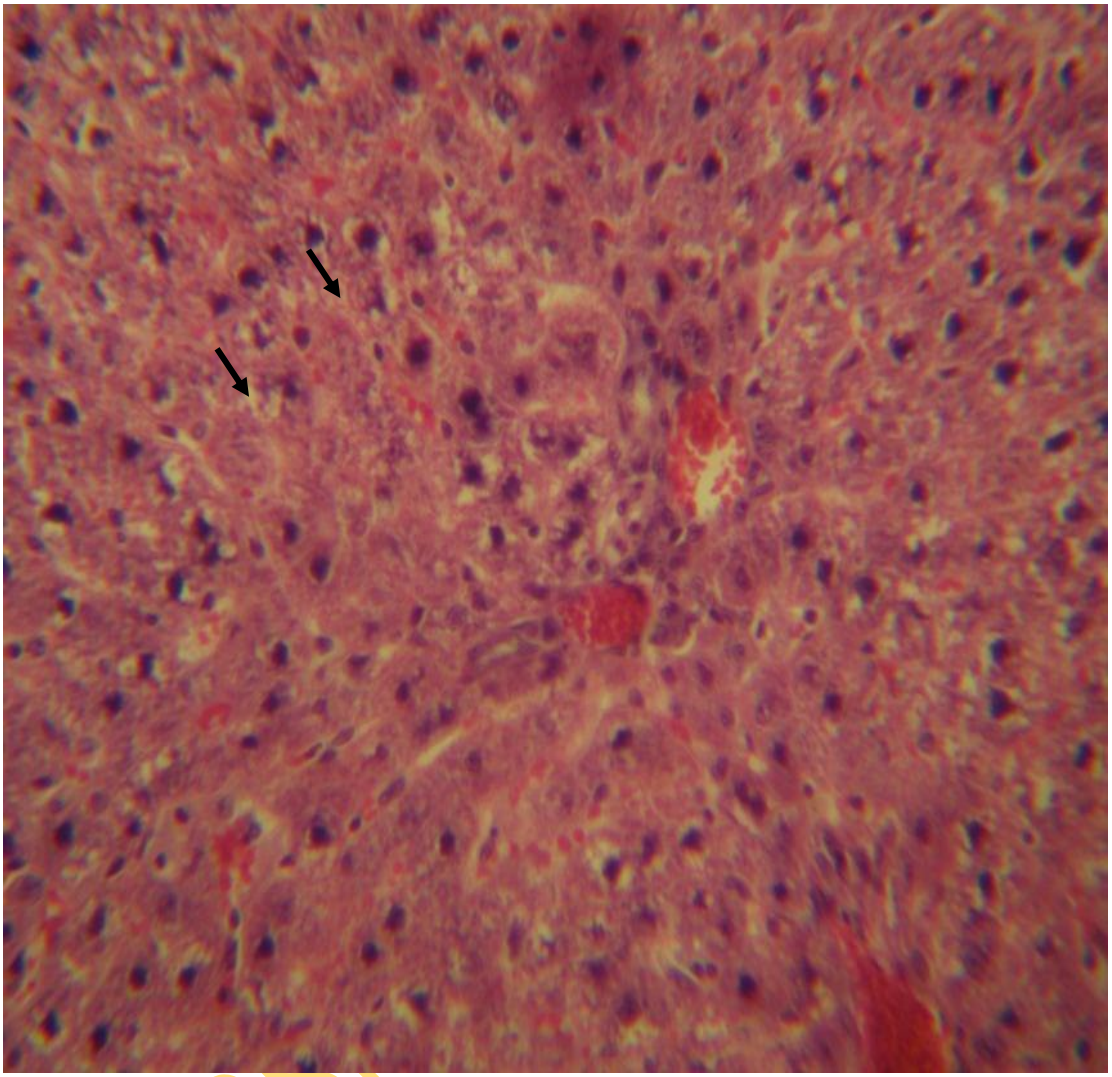


Plate 9: showing section of the liver with moderate periportal degeneration and necrosis of the hepatocytes (H & E X250)

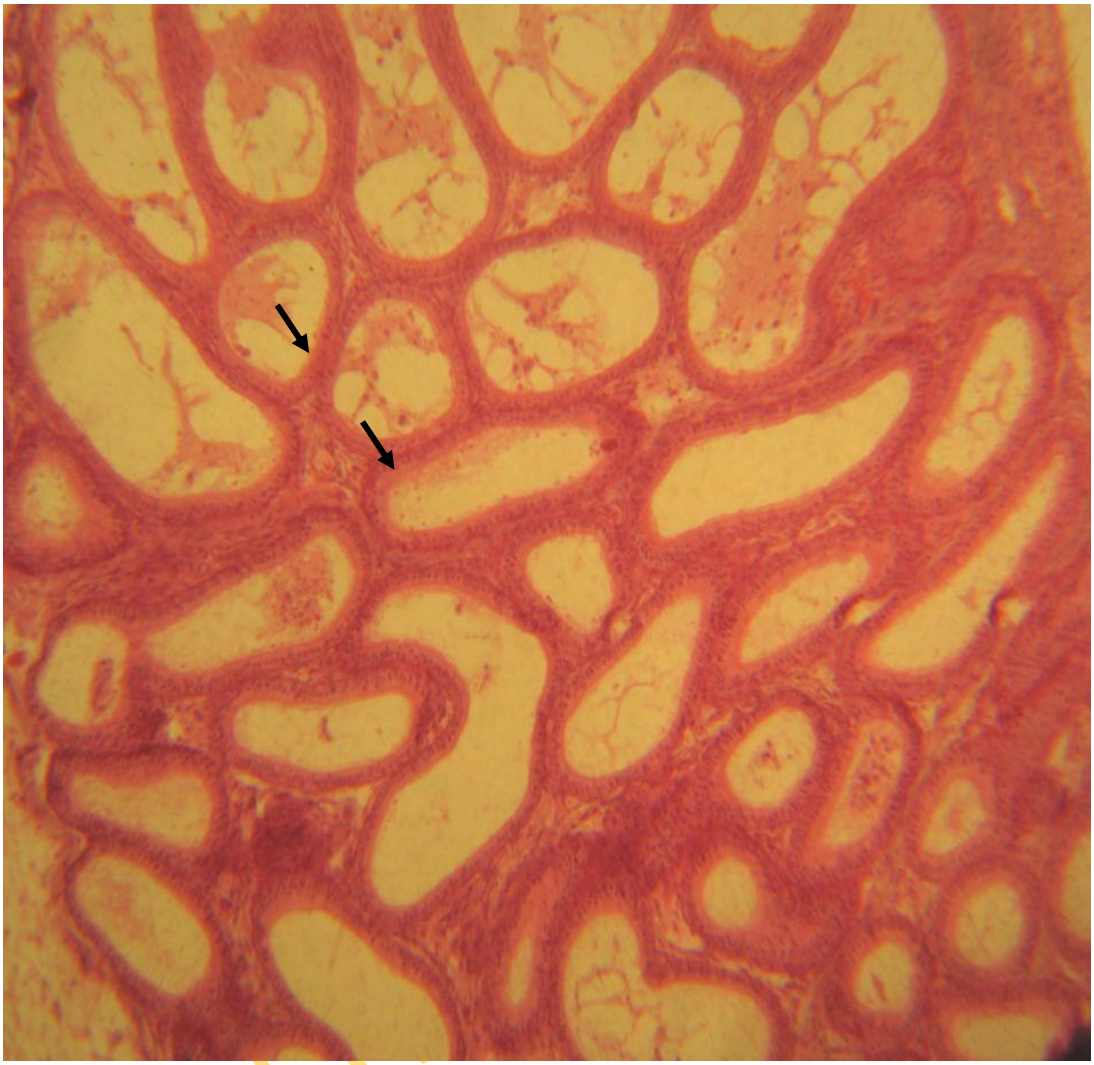


Plate 10: showing section of the epididymis with empty lumina and containing few spermatogonia cells (evidence of severe spermiostasis) (H & E. X350).

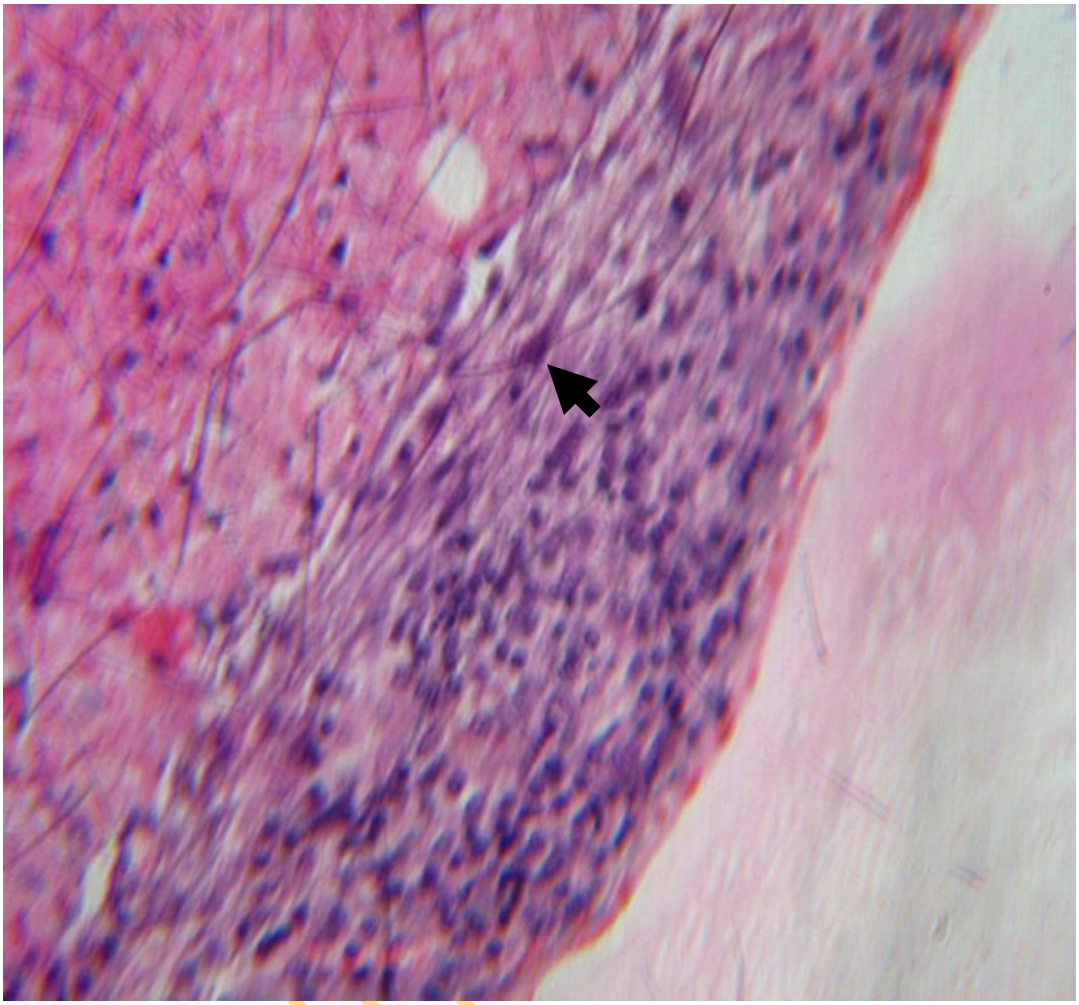


Plate 11: showing section of the heart with thickened epicardium and mononuclear cells infilteration (H & E X200).

4.1 Effects of aqueous leaf extracts on semen morphology and charateristics in male Wistar albino rats

A. *Tithonia diversifolia*

The effects of three different doses of aqueous extract of *T. diversifolia* leaves on semen morphology and characteristics in male Wistar rats is shown in Tables 7A&B, 8A&B and 9A&B.

There was a dose - dependent decrease in the progressive sperm motility of the Wistar rats. There was a significant ($P<0.05$) decrease in sperm motility (62.50±7.5%) in the group dosed with 1600 mg/kg when compared with the control (93.75±1.3%) (Table 9B).

There was no significant ($P<0.05$) change in the percentage life sperm cells/ death ratio in the rats treated with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses when compared with the control value (Tables 7B,8B&9B).

Similarly, there was no significant ($P<0.05$) change in the values of the sperm volume in the rats treated with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses when compared with the control value (5.18±0.0µl) (Tables 7B,8B &9B).

However, there was a dose - dependent decrease in the sperm count of the Wistar rats following oral administration of aqueous extract of *T. diversifolia* leaves. The decrease in sperm counts (69.75±0.5sperm cells/ml) in the group dosed with 1600 mg/kg was significant at ($P<0.05$) when compared with the control (129.0±5.0sperm cells/ml) (Table 9B).

There was no significant ($P<0.05$) changes in the total number of abnormal spermatozoa in the group treated with 400 mg/kg when

compared with the control. The abnormalities observed were mainly secondary, characterized by tailless head, headless tail, rudimentary tail, bent tail, curved tail, curved midpiece, bent midpiece and looped tail.

Also, there was no significant ($P<0.05$) change in the total number of abnormal spermatozoa in the group treated with 800 mg/kg except in the secondary abnormalty of the curved midpeice value (3981.7±330.3 abnormal cells) when compared with the control group (2696.25±300.0 abnormal cells). The other secondary abnormalities observed were characterized by tailless head, headless tail, rudimentary tail, bent tail, curved tail, bent midpiece and looped tail.

There was also no statistically significant ($P<0.05$) change in the total number of abnormal spermatozoa in the group treated with 1600 mg/kg when compared with the control. However, there were significant ($P<0.05$) increases observed in the following abnormalities: headless tail (321.5±0.3 abnormal cells), tailless head (171.00±50.0 abnormal cells) and bent tail (3766.0±320.5 abnormal cells) whereas, others like rudimentary tail, curved tail, curved midpiece, bent midpiece and looped tail were not statistically significant ($P<0.05$) when compared to the control values.

B. *Acalypha wilkesiana*

The effects of three different doses of aqueous extract of *A. wilkesiana* leaves on semen morphology and characteristics in male Wistar rats is shown in Tables 7A &B, 8A&B and 9A&B.

There is a dose - dependent decrease in the progressive sperm motility of the Wistar rats given 400 mg/kg dose of *A. wilkesiana*, though this decrease was not significant ($P<0.05$) in all the treated groups when compared with the control (Tables 7B,8B& 9B).

There was no significant ($P<0.05$) change in the percentage life sperm cells/ death ratio and sperm volume in the groups of rats treated with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of *A. wilkesiana* when compared with the control value (Tables 7B,8B & 9B).

Similarly, there was no significant ($P<0.05$) change in the sperm count of the rats treated with 400 mg/kg and 800 mg/kg doses when compared with the control value (129.00±5.0 sperm cells/ml) (Tables 7B, 8B & 9B).

However, there was a difference in the sperm count of the Wistar rats following oral administration of 1600 mg/kg aqueous extract of *A.wilkesiana* leaves. The decrease in sperm count value of (71.50±3.5 sperm cells/ml) the group dosed with 1600 mg/kg was significant at ($P<0.05$) when compared with the control (129.00±5.0 sperm cells/ml) (Table 9B).

At 400 mg/kg there were statistically significant (P<0.05) increases in the values of bent tail (3796.3±274.9 abnormal cells) and curved tails (3519.0±268.2 abnormal cells) when compared to the control (Table 7A).

There was no significant (P<0.05) change in the total number of abnormal spermatozoa in the group treated with 800 mg/kg when compared with the control except in the curved tail value (3517.25±269.2 abnormal cells) which was significantly (P<0.05) higher than the control. The other abnormalities observed were mainly secondary and include tailless head, headless tail, rudimentary tail, bent tail, curved midpiece, bent midpiece and looped tail.

However, at 1600 mg/kg dose, there were significant (P<0.05) increases in the values observed in the following abnormalities: headless tail (239.3±27.3 abnormal cells), tail less head (239.25±27.6 abnormal cells), bent tail (4157.0±475.45 abnormal cells), curved tail (4099.3±260.0 abnormal cells) and curved midpiece (4557.5±259.3 abnormal cells), whereas, others like rudimentary tail, bent midpiece and looped tail did not exhibit any significant (P<0.05) changes when compared to the control values.

C. *Lippia multiflora*

The effects of three different doses of aqueous extract of *L.multiflora* leaves on semen morphology and characteristics in male Wistar rats is shown in Tables 7A &B, 8A&B and 9A&B.

There was a non significant ($P<0.05$) decrease in the progressive sperm motility of the Wistar rats given 400 mg/kg and 1600 mg/kg doses of *L.multiflora*, however at 800 mg/kg dose, there was a significant ($P<0.5$) decrease in the sperm motility (60.00±0.0%) when compared with the control (Tables 7B, 8B & 9B).

There was no statistically significant ($P<0.05$) change in the percentage life sperm cells/ death ratio and sperm volume in the groups of rats treated with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of *L. multiflora* when compared with the control value (Tables 7B, 8B& 9B).

However, there was statistically significant non dose - dependent decreases in the sperm count of the Wistar rats following oral administration of 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of the aqueous extract of *L. multiflora* leaves. These decreases in sperm count values of 78.50±.2.5, 60.50±1.4 and 68.00±2.9 sperm cells/ml respectively were significant ($P<0.05$) when compared with the control (129.00±5.0 sperm cells/ml) (Tables 7B, 8B &9B).

At 400 mg/kg there was a significant ($P<0.05$) increase in the values of bent tail (3471.3±249.9 abnormal cells) when compared to the control (Table 7A).

There was no significant (P<0.05) change in the total number of abnormal spermatozoa in the group treated with 800 mg/kg when compared with the control. The other abnormalities observed were mainly secondary and include tailless head, headless tail, rudimentary tail, curved tail, curved midpiece, bent midpiece and looped tail.

However, at 1600 mg/kg dose, there were significant (P<0.05) increases in the values observed in the following abnormalities: tailless head (211.50±0.3 abnormal cells), bent tail (3777.5±320.1 abnormal cells), curved tail (4269.5±33.3 abnormal cells) and curved midpiece (4826.5±285.5 abnormal cells), whereas, others like headless tail, rudimentary tail, bent midpiece and looped tail did not exhibit any statistically significant (P<0.05) changes when compared to the control values.

E.Ocimum gratissimum

The effects of three different doses of aqueous extract of *O. gratissimum* leaves on semen morphology and characteristics in male Wistar rats is shown in Tables 7A &B, 8A&B and 9A&B.

There was a non dose - dependent statistically insignificant (P<0.05) decrease in the progressive sperm motility of the Wistar rats at 400 mg/kg and at 800 mg/kg doses. However, there was a significant (P<0.05) decrease in sperm motility (50.00±7.1%) in the group dosed

with 1600 mg/kg when compared with the control (93.75±1.3%) (Table 9B).

There was no significant (P<0.05) change in the percentage life sperm cells/ death ratio and sperm volume in the groups of rats treated with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of *O. gratissimum* when compared with the control value (Tables 7B, 8B & 9B).

Similarly, there was no significant (P<0.05) change in the sperm count of the rats treated with 400 mg/kg and 800 mg/kg doses when compared with the control value (Tables 7B, 8B & 9B).

However, there was a decrease in the sperm count of the Wistar rats following oral administration of 1600 mg/kg aqueous extract of *O. gratissimum* leaves. The decrease in sperm count value of (68.25±3.80 sperm cells/ml) is statistically significant (P<0.05) when compared with the control (Table 9B).

At 400 mg/kg, there was significant (P<0.05) increase in the values of curved tail (4576.5±248.2 abnormal cells) when compared to the control (Table 7A).

There were no significant (P<0.05) change in the total number of abnormal spermatozoa in the group treated with 800 mg/kg when compared with the control except in the curved tail value of (4448.8±45.5 abnormal cells) which was significantly (P<0.05) higher than the control. The other abnormalities observed were mainly secondary and include

tailless head, headless tail, rudimentary tail, bent tail, curved midpiece, bent midpiece and looped tail.

However, at 1600 mg/kg dose, there were significant (P<0.05) increases in the values observed in the following abnormalities: headless tail (339.0±27.3 abnormal cells), bent tail (3649.0±262.0 abnormal cells), curved tail (4452.8±45.1 abnormal cells) and curved midpiece (4654.8±228.9 abnormal cells), whereas, others like tailless head, rudimentary tail, bent midpiece and looped tail did not exhibit any statistically significant (P<0.05) changes when compared to the control values.

F.Morinda morindoides

The effects of three different doses of aqueous extract of *M. morindoides* root on semen morphology and characteristics in male Wistar rats is shown in Tables 7A &B, 8A&B and 9A&B.

There were decreases in the progressive sperm motility of the Wistar rats given 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of *M. morindoides*, the values of 22.50±13.2, 47.50±6.4 and 12.50±7.5 percent respectively. These decreases observed were significant at (P<0.05) when compared with the control (Tables 7B, 8B & 9B).

There were no significant (P<0.05) change in the percentage life sperm cells/ death ratio and sperm volume in the groups of rats treated

with 400 mg/kg, 800 mg/kg and 1600 mg/kg doses of *M. morindoides* when compared with the control (Tables 7B, 8B & 9B).

However, there was statistically significant dose - dependent decreases in the sperm count of the Wistar rats following oral administration of 400 mg/kg, 800mg/kg and 1600mg/kg doses of the aqueous extract of *M. morindoides* root. These decreases in sperm count (45.75±5.1 sperm cells/ml), (40.75±1.1 sperm cells/ml) and (35.50±2.4 sperm cells/ml) respectively are statistically significant at ($P<0.05$) when compared with the control (Tables 7B, 8B &9B).

At 400mg/kg, there were significant ($P<0.05$) increases in the values of bent tail (4017.3±236.5 abnormal cells) and curved tail (3903.8±273.6 abnormal cells) when compared to the control (Table 7A).

There were no significant ($P<0.05$) changes in the total number of abnormal spermatozoa in the group treated with 800 mg/kg except for the following: curved tail (3822.3±288.5 abnormal cells) and curved midpiece (4567.00±220.3 abnormal cells) when compared with the control (Table 8A). The other abnormalities observed were mainly secondary and included tailless head, headless tail, rudimentary tail, bent tail midpiece and looped tail.

At 1600 mg/kg dose, there were significant ($P<0.05$) increases in the values observed in the following abnormalities: headless tail (340.0±27.0 abnormal cells), tailless head (239.25±27.3 abnormal cells),

bent tail (5822.5±225.5 abnormal cells), curved tail (4435.00±57.7 abnormal cells) and curved midpiece (5347.3±25.3), whereas, others like rudimentary tail, bent midpiece and looped tail did not exhibit any statistically significant ($P<0.05$) changes when compared to the control values.

TABLE 7A: EFFECTS OF 400 MG/KG DOSE OF 5 PLANTS EXTRACTS ON MEAN SPERM MORPHOLOGY OF TREATED RATS

	HEADLESS TAIL	TAILESS HEAD	RVDIMENTALLY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithonia diversifolia	191.25± 62.3	143.75± 74.4	13.50± 4.8	2461.8±24 9.7	1464.3± 748.4	2727.3± 288.3	2991.75± 261.1	11.00± 4.1	6.00± 5.0	401.3± 1.3
Acalypha wilkesiana	191.25± 62.3	118.75± 56.5	11.0± 4.1	3796.3± 274.9*	3519.00± 268.2*	2769.3± 323.8	3102.00± 260.9	18.50± 2.5	11.00± 4.1	406.3± 2.4
Lippia multiflora	163.75± 47.6	191.3± 62.3	11.0± 4.1	3471.3± 249.9*	3047.00± 239.7	3596.00±2 42.8	3822.00± 258.8	8.50± 4.8	8.50± 4.8	401.3± 1.3
Ocimum gratissimum	239.00± 27.3	164.00± 47.7	11.0± 5.8	2766.5± 248.2*	4576.5± 248.2*	3746.3± 274.8	4101.8± 219.9	11.0± 4.1	6.00± 5.0	401.3± 1.3
Morinda morindoides	166.00± 48.4	239.50± 27.1	13.50± 4.8	41017.3± 236.5*	3903.8± 273.6*	4422.5± 57.0	3871.5± 263.6	11.0± 5.8	13.50± 4.8	402.5± 2.5
Control	116.25± 54.9	68.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75± 2.4

** Mean ± SEM based 5 observations

TABLE 7B: EFFECTS OF 400 MG/KG DOSE OF 5 PLANTS EXTRACTS ON MEAN SPERM CHARACTERISTICS OF TREATED RATS.

EXTRACT	SPERM MOTILITY (%)	LIVE/DEAD (%)	SPERM COUNTS (sperm cells/ml)
Tithonia diversifolia	90.00±0.0	95.8±0.8	97.25±2.1
Acalypha wilkesiana	80.00±7.0	96.00±2.0	82.75±2.7
Lippia multiflora	77.50±6.3	93.25±2.8	78.50±2.5
Ocimum gratissimum	85.00±2.9	96.50±0.8	82.50±2.7
Morinda morindoides	22.50±13.2*	86.3±2.4	45.75±5.1*
Control	93.75±1.3	98.00±0.0	129.00±5.0

* Superscripted items are significant at P <0.05.

** Mean ± SEM based on 5 observations.

TABLE 8A: EFFECTS OF 800 MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON MEAN SPERM MORPHOLOGY OF TREATED RATS.

	HEADLESS TAIL	TAILESS HEAD	RUDIMENTARY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithonia diversifolia	148.33± 63.7	147.67± 63.3	17.67± 3.3	3217.7± 3.3	2882.33± 329.7	3965.00± 367.00	3981.7± 330.3*	14.33± 3.3	1.00± 0.00	405.00± 2.9
Acalypha wilkesiana	164.00± 47.7	188.50± 60.6	16.00± 2.9	3546.00± 259.4	3517.25± 269.2*	3216.8± 408.1	3256.5± 28.6	16.00± 2.9	11.00± 5.8	403.75± 24
Lippia multiflora	266.00± 31.8	166.50± 48.6	11.00± 5.8	3721.5± 283.2	3227.00± 573.9	3822.0± 288.1	3763.00± 259.2	11.0± 5.8	6.00± 2.9	402.5± 1.4
Ocimum gratissimum	164.00± 47.7	238.75± 27.4	11.0± 4.1	4074.00± 251.0	4448.8± 45.5*	4099.00± 226.0	4096.5± 258.9*	8.50± 4.8	11.00± 4.1	401.3± 1.3
Morinda morindoides	239.00± 27.3	163.75± 47.6	16.00± 2.9	4629.00± 235.5	3822.3± 288.5*	4544.8± 226.9	4567.00± 220.3*	13.50± 4.8	11.00± 4.1	400.00± 0.0
Control	116.25± 54.9	68.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75± 2.4

TABLE 8B: EFFECTS OF 800 MG/KG DOSE OF 5 PLANTS EXTRACTS ON MEAN SPERM CHARACTERISTICS OF TREATED RATS.

EXTRACT	SPERM MOTILITY (%)	LIVE/DEAD (%)	SPERM COUNTS (sperm cells/ml)
Tithonia diversifolia	70.00±11.6	98.00±0.0	77.0±1.7
Acalypha wilkesiana	75.00±2.9	94.50±1.7	76.50±3.8
Lippia multiflora	60.00±0.0*	96.50±0.9	60.50±1.4*
Ocimum gratissimum	87.50±2.5	96.50±0.9	74.00±2.8
Morinda morindoides	47.50±6.34*	90.00±2.0*	40.75±1.1*
Control	93.75±1.3	98.00±0.0	129.00±5.0

Superscripted items are significant at P<0.05

** Mean ± SEM based on 5 observations.

TABLE 9A: EFFECTS OF 1600 MG/KG DOSE OF 5 PLANTS EXTRACTS ON MEAN SPERM MORPHOLOGY OF TREATED RATS.

	HEADLESS TAIL	TAILESS HEAD	RUDIMENTARY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithonia diversifolia	321.5± 0.3*	171.00± 50.0*	13.50± 2.5	3766.0± 320.5*	4072.00± 811.3	4052.00± 280.00	3792.00± 526.7	18.50± 2.50	11.00± 5.8	400.00± 0.00
Acalypha wilkesiana	239.3± 27.3*	239.25± 27.6*	18.50± 2.5	4157.0± 475.4*	4099.3± 260.0*	4379.00± 388.0	4557.5± 259.3*	16.00± 2.9	11.00± 4.0	403.75± 2.4
Lippia multiflora	117.00± 55.4	211.50± 0.3*	16.25± 4.7	3777.5± 320.1*	4269.5± 33.3*	3836.5± 221.9	4826.5± 285.5*	16.00± 2.9	16.00± 2.9	400.00± 0.00
Ocimum gratissimum	339.00± 27.3*	116.0± 54.9	16.00± 2.9	3649.0± 262.0*	4452.8± 45.1*	4204.0± 258.1	4654.8± 228.9*	11.00± 5.8	16.00± 2.9	403.75± 2.4
Morinda morindoides	340.0± 27.0*	239.25± 27.3*	16.25± 4.7	5822.5± 225.5*	4433.00± 57.7*	4827.50± 286.7	5347.3± 25.3*	18.50± 2.5	8.50± 2.5	402.50± 1.4
Control	116.25± 54.9	68.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75 ±2.4

TABLE 9B: EFFECTS OF 1600 MG/KG DOSE OF 5 PLANTS EXTRACTS ON MEAN SPERM CHARACTERISTICS OF TREATED RATS

EXTRACT	SPERM MOTILITY (%)	LIVE/DEAD (%)	SPERM COUNTS (sperm cells/ml)
Tithoina diversifolia	62.50±7.5*	97.25±0.1	69.75±0.5*
Acalypha wilkesiana	85.00±5.0	96.50±0.9	71.50±3.5*
Lippia multiflora	92.50±1.4	98.00±0.0	68.00±2.9*
Ocimum gratissimum	50.00±7.1*	92.50±1.4	68.25±3.8*
Morinda morindoides	12.50±7.5*	70.00±5.8	35.50±2.4*
Control	93.75±1.3	98.00±0.0	129.00±5.0

Superscripted items are significant at P<0.05

** Mean ± SEM based on 5 Observations.

4.4 EXPERIMENT 3:

Hypoglycaemic Effects of the Plant Extracts (4A)

A. Tithonia diversifolia

The rats administered extract of *T. diversifolia* showed a reduction in blood glucose level from 584.40±10.6 mg/dl at day-0 to 102.95±2.6 mg/dl at day-21, the decrease is statistically significant ($p<0.05$) when this value was compared to the value of the untreated diabetic control group; 269.5±10.6 mg/dl (Table 11). Also at day 21, there were significant ($p<0.05$) decrease in the serum cholesterol value (127.43±0.7mmol/l) compared to control group (159.62±2.6 mmol/l) and serum low density lipoprotein (LDL) value (57.95±4.2 mmol/l) compared with of the control group (116.98±3.0 mmol/l). A significant ($p<0.05$) increase was observed in serum high-density lipoprotein (HDL) value (52.42±0.6 mmol/l) in comparison with control group (25.70±0.9 mmol/l) and an insignificant increase in serum triglycerides value (101.98±5.8 mmol/l) when compared to the control group (83.90±4.3 mmol/l) (Table11).

B.Acalypha wilkesiana (Mull. Arg)

The group of rat treated with extract of *A. wilkesiana* showed a statistically significant decrease ($p<0.05$) in the blood glucose level from (168.00±2.8mg/dl) at day-0 to (82.72±3.9mg/dl) at day-21, the decrease

was also statistically significantat ($p<0.05$) when the value was compared to that of the control group (269.5±10.6mg/dl) (Table 11). Also at day 21, the serum cholesterol value (121.80±0.5 mmol/l) and serum low-density lipoprotein value (61.70±1.4 mmol/l) showed a statistically significant ($p<0.05$) reduction compared to control values (159.62±2.6 mmol/l) and (116.98±3.0 mmol/l) respectively (Table 11), while the serum high-density lipoprotein value (42.95±1.0 mmol/l) increased significantly ($p<0.05$) when they were all compared to the values in the untreated diabetic control group (Table11).

C. Lippia multiflora

The blood glucose level of the rats administered with extract of *L. multiflora* decreased at day-21 to 351.00±19.1 mg/dl from 539.70.±50.4 mg/dl at day-0, but the reduction was not statistically significant ($p>0.05$) when compared to the glucose value (324.53±22.3mg/dl) of the untreated diabetic control group. The serum cholesterol, triglycerides and low-density lipoprotein levels also decreased insignificantly at ($p>0.05$) when compared to the values in the control group, while the serum high density lipoproteins increased insignificantly at ($p>0.05$) when also compared with the control (Table 11).

D. *Ocimum gratissimum*

The rats administered the extracts of *Ocimum gratissimum* showed a statistically significant ($p<0.05$) reduction in the blood glucose level from 567.68±49.6mg/dl at day-0 to 195.15±42.5mg/dl at day-21 when compared with the control group value of 324.53±22.3mg/dl. A statistically insignificant ($p<0.05$) reduction in the values of serum cholesterol, serum triglycerides and serum-low density lipoprotein were observed while the serum-high density lipoprotein value obtained (41.27±2.0 mmol/l) was statistically significantly ($p<0.05$) increased when compared with the value of the untreated diabetic control group (Table 11).

E. Morinda morindoides

There was a reduction in blood glucose level of the rats treated with the extract of *M. morindoides* from (277.87±17.5mg/dl) at day-0 to (103.00±2.8mg/dl) at day-21, this reduction was statistically significant ($p<0.05$) when compared with the control group (324.53±22.3mg/dl) (Table 11). Also at day 21, there were statistically significant ($p<0.05$) decreases in the values of serum cholesterol (133.48±1.1 mmol/l) and serum low density lipoproteins (66.38±2.5 mmol/l) when compared to 159.62±2.6 mmol/l and 116.98±3.0 mmol/l, the diabatic control values for cholesterol and low density lipoprotein respectively. The serum high-

density lipoprotein value of 51.03±3.0 mmol/l was statistically significantly ($p<0.05$) higher than 25.70±0.9 mmol/l in the control group (Table 11).

F. Glibenclamide

The group of rats treated with Glibenclamide showed a statistically significant ($p<0.05$) reduction in the blood glucose values from 268.63±14.8 mg/dl at day-0 to 78.85±2.9 mg/dl at day-21 when compared with the values of blood glucose in the untreated diabetic control group at day-21 (324.53±22.3 mg/dl). There was statistically significant ($p<0.05$) reduction in the serum cholesterol value (134.13±1.5 mmol/l) and serum low-density lipoprotein value (68.70±3.5 mmol/l) when compared with the values in the control group, while the high-density lipoprotein value (47.32±2.2 mmol/l) was statistically significantly ($p<0.05$) increased as compared with the untreated diabetic control group and a slight statistically insignificant increase recorded in the serum triglyceride value (Table11).

TABLE 10: EFFECTS OF 400MG/KG DOSE OF THE EXTRACT OF EACH PLANT ON ALLOXAN INDUCED DIABETIC RATS.

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	BLOOD GLUCOSE (mg/dl) "DAY O"	BLOOD GLUCOSE (mg/dl) "Day 21"	SERUM CHOLESTEROL (mmol/l) "Day 21"	SERUM TRIG (mmol/l) "Day 21"	SERUM HDL (mmol/l) "Day 21"	SERUM LDL (mmol/l) "Day 21"
Tithonia diversifolia	584.40±10.6	102.95±2.6*	127.43±0.7*	101.98±5.8	52.42±0.6*	57.95±4.2*
Acalypha wilkesiana	168.00±2.8	82.72±3.9*	121.80±0.5*	84.23±2.6	42.95±1.0*	61.70±1.4*
Lippia multiflora	539.70±50.4	351.00±19.1	130.43±3.3*	83.45±2.0	37.25±0.7	78.15±3.2*
Ocimum gratissimum	567.68±49.6	195.15±42.5*	137.57±4.3*	82.75±2.8	41.27±2.0*	79.88±3.5*
Morinda morindoides	277.87±17.5	103.00±2.8*	133.48±1.1*	80.00±4.6	51.03±3.0*	66.38±2.5*
Glibenclamde	268.63±14.8	78.85±2.9*	134.13±1.5*	86.45±4.5	47.32±2.2*	68.70±3.5*
Untreated Diabetic (-VE control)	269.50±10.6	324.53±22.3	159.62±2.6	83.900±4.3	25.70±0.9	116.98±3.0
Non- Diabetic Control	113.18±2.3	108.00±3.1	128.82±4.1*	91.87±4.8	40.80±2.5	69.08±4.9*

* Superscripted figures are statistically significant at P <0.05

4.5 EXPERIMENT 4: HOURLY ANTI-DIABETOGENIC STUDY

Hourly evaluation of hypoglycemic effect of 400 mg/kg dose aqueous extract of Tithonia diversifolia

In the groups of rats dosed with 400 mg/kg of *T. diversifolia*, there were reductions in blood sugar level at 30min, 1 hour, which were not statistically significant at P>0.05.

At 2 hours, 4hours and 24 hours after administration, statistically significant (P<0.05) reductions in the sugar level were observed (Table 11A).

The hourly percentage reductions obtained increased steadily from 3.2% reduction at 30min to 21.4% reduction in 4 hours. Although there was a drop in the percentage reduction of blood sugar at 6 hours to 2.8%, it later increased to 12.6% at 8 hours and 36.0% reductions in blood sugar level at 24 hours after administration (Table 11B).

Hourly evaluation of hypoglycemic effect of 400 mg/kg dose aqueous extract of Acalypha wilkesiana

The group of rats treated with 400 mg/kg extract of *Acalypha wilkesiana* showed a statistically significant (P<0.05) reductions in blood sugar level at 30 min, 1 hours, 2 hours 4 hours, 6 hours, 8 hours and 24 hours (Table 11A).

The hourly percentage reductions obtained revealed a steady increase from 10.7% at 30mins post administration to 43.8% reduction at 24 hours (Table 11B).

Hourly evaluation of hypoglycemic effect of 400 mg/kg dose aqueous extract of Lippia multiflora

The group of rats dosed with 400 mg/kg extract of *Lippia multiflora,* showed no significant reduction in the blood sugar level at the time periods the blood samples were evaluated except at 2 and 24 hours after the extract administration (Table 11A).

The percentage hourly reduction of blood sugar level revealed 0% for 30min and 1 hour, 14%, 0%, 1.5%, 1.1% 2hours, 4hours, 6 hours and 8 hours respectively and 12.3% at 24 hours post administration (Table 11B).

Hourly evaluation of hypoglycemic effect of 400 mg/kg dose aqueous extract of Ocimum gratissimum

In the group with rats dosed with 400 mg/kg extracts of *Ocimum gratissimum*, the only statistically significant reductions observed was at 24 hours after administration (Table 11A).

The percentage hourly reductions in the sugar level were 2.8% at 30 min, 9.6% at 2 hours, 7.9% at 4 hours, thereafter 0%, 1.5%, 1.1% at 4, 8 hours and 13.0% at 24 hours.

Hourly evaluation of hypoglycemic effect of 400 mg/kg dose aqueous extract of Morinda morindoides

Aqueous extracts of *Morinda morindiodes* at the single dose of 400 mg/kg body weight produced a statistically significant ($P<0.05$) reductions in the blood sugar level of wistar albino rat at 1, 2, 4, 6, 8 and 24 hours (Table 11A).

The percentage hourly reductions in blood sugar level were 2.5% at 30mins and there after, 17.7%, 33.1%, 45.9%, 40.4% and 41.8% in 1, 2, 4, 6, and 8 hours respectively.

A significant percentage reduction of 56% was observed at 24 hours (Table 11B).

Hourly evaluation of hypoglycemic effect of Glibenclamide

The group with rats dosed with single oral dose of 10 mg/kg *glibenclamide*, showed a steady reduction in blood sugar level from 30mins after administration.

A statistically significant ($P<0.05$) reductions in blood sugar level were observed at 1 hours, 2hours, 3 hours, 4 hours, 6 hours, 8 hours and 24 hours respectively (Table 11A).

The percentage hourly reduction of the level of blood sugar, revealed 6.2%, 22.5%, 27.1%, 33.4%, 20.7%, 18.2% and 52.4% at 30min, 1 hour, 2hours, 4hours, 6hours, 8 hours and 24 hours respectively (Table 11B).

TABLE 11A: HOURLY ASSESSMENT OF THE EFFECT OF 5 PLANTS EXTRACTS ON BLOOD SUGAR LEVEL IN ALLOXAN INDUCED DIABETIC WISTAR ALBINO RATS [BLOOD SUGAR LEVEL (mg/dl) MEAN + STANDARD ERROR OF MEAN].

PLANTS EXTRACTS	0 HOURS	30 MIN, AFTER	1 HOUR AFTER	2 HOURS AFTER	4 HOURS AFTER	6 HOURS AFTER	8 HOURS AFTER	24 HOURS AFTER
Tithonia diversifolia	528.30± 29.3	511.50± 32.0	472.80± 40.4	448.50*± 40.3	415.50*± 40.6	513.30± 33.4	461.70± 33.4	338.100*± 34.4
Acalypha willkesiana	500.10± 50.2	446.70*± 56.3	393.00*± 65.5	358.50*± 73.2	343.50*± 73.7	344.40*± 86.6	315.90*± 76.4	285.10*± 89.5
Lippia multiflora	612.00± 0.0	612.00± 0.0	612.00± 0.0	522.00*± 54.2	612.00± 0.0	602.70± 5.5	605.10± 3.3	537.00*± 68.2
Ocimum gratissimum	612.00± 0.0	595.20± 16.8	567.00± 28.5	553.50± 35.9	563.40± 28.0	612.00± 28.0	591.60± 20.4	532.50*± 35.6
Morinda morindades	238.80± 15.2	232.80± 16.0	196.50*± 17.4	159.90*± 13.5	129.23*± 6.6	142.43*± 12.4	138.90*± 15.3	105.00*± 35.6
Glibenclamide	399.60± 61.3	374.70± 54.3	309.90*± 57.5	291.30*± 62.5	266.27*± 57.3	316.80*± 75.3	327.0± 75.8	190.20*± 32.8
Non diabetic untreated	199.13± 5.0	121.80± 4.9	121.80± 4.6	117.37± 3.0	114.60± 3.3	111.00± 3.9	108.30± 4.1	102.00± 4.5
Diabetic untreated	558.51± 25.0	580.89± 13.2	590.66± 8.3	578.71± 9.3	588.09± 5.8	603.26± 5.2	610.20± 1.3	610.50± 1.0

Superscripted items are statistically significant at P<0.05

TABLE 11B: HOURLY PERCENTAGE REDUCTIONS OF MEAN BLOOD GLUCOSE LEVEL BY THE AQUEOUS EXTRACTS OF 5 PLANTS IN ALLOXAN INDUCED DIABETIC RATS VALUES IN %

PLANTS EXTRACTS	0 HOUR	30 MIN, AFTER	1 HOUR AFTER	2 HOURS AFTER	4 HOURS AFTER	6 HOURS AFTER	8 HOURS AFTER	24 HOURS AFTER
Tithonia diversifolia	0%	3.2%	10.5%	15.1%	21.4%	2.8%	12.6%	36.0%
Acalypha willkesiana	0%	10.7%	21.4%	28.3%	31.3%	31.1%	36.8%	43.8%
Lippia multiflora	0%	0%	0%	14.0%	0%	1.5%	1.1%	12.3%
Ocimum gratissimum	0%	2.8%	7.4%	9.6%	7.9%	0%	3.3%	13.0%
Morinda morindoides	0%	2.5%	17.7%	33.1%	45.9%	40.4%	41.8%	56.0%
Glibenclamide	0%	6.2%	22.5%	27.1%	33.4%	20.7%	18.2%	52.4%

4.6 EXPERIMENT 5: Analgesic Effects of the Plant Extracts

4.6.1 Hot Plate Test

The reaction time statistically significantly ($p<0.05$) increased for the groups treated with the aqueous extract of *Tithonia diversifolia* (25.73±0.7 sec), *Acalypha wilkesiana* (30.00±0.6 sec), *Lippia multiflora (*26.26±2.8 sec) and *Morinda morindoides* (25.73±10.0 sec) when compared to the untreated control group reaction time (18.22±0.3 sec). However, these reaction times were lower than that of mice in the positive control group treated with morphine (31.81±1.5 sec) (Table12). The increase in reaction time of mice treated with aqueous extract of *Ocimum gratissimum (*21.55±0.9 sec) was statistically insignificant compared with the untreated control group (Table 12).

4.6.2 Acetic Acid induced writhing movements

The mean number of writhing movements was significantly reduced in groups of mice treated with *Tithonia diversifolia* with percent inhibition of 43.92%; *Acalypha wilkesiana* with percent inhibition of 44.65%; *Lippia multiflora* with percent inhibition of 42.15%; *Ocimum gratissimum* with percent inhibition of 38.57% and *Morinda morindoides* with percent inhibition of 44.29% when compared with the mean number of writhing of the control (46.7±1.4%). The mean number of writhings obtained from all the plants extracts value was higher than that of mice given the standard analgesic, paracetamol (23.5±1.3%) (Table 12).

TABLE12A: THE MEAN ANALGESIC EFFECTS OF 400 MG/KG DOSE OF FIVE PLANTS EXTRACTS ON MICE TREATED WITH THE EXTRACT

PLANTS EXTRACT	HOT PLATE REACTION TIME (sec)
Tithonia diversifolia	25.18 ± 07*
Acalypha wilkesiana	30.00 ± 0.6*
Lippia multiflora	26.26 ± 2.8*
Ocimum gratissimum	21.55 ± 0.9*
Morinda morindoides	25.73 ± 10.0*
Morphine	31.8 ± 1.5*
Untreated Control	18.22 ± 0.3

TABLE12B: THE MEAN ANALGESIC EFFECTS OF 400 MG/KG DOSE OF FIVE PLANTS EXTRACTS

PLANTS EXTRACT	NO OF WRITHINGS	% INHIBITION OF WRITHING
Tithonia diversifolia	26.17 ± 0.7*	43.92
Acalypha wilkesiana	25.83 ± 1.3*	44.65
Lippia multiflora	27.00 ± 2.2*	42.15
Ocimum gratissimum	28.67 ± 1.0*	38.57
Morinda morindoides	26.00 ± 0.7*	44.29
Paracetamol	23.50 ± 1.3	49.65
Untreated Control	46.67 ± 1.4	

4.7 EXPERIMENT 6

Anti-inflammatory Effects of the Plant Extracts

The percentage inhibition of increase in paw volume of the rats with different plants extract treatments is shown in Table 13.

Acalypha wilkesiana showed the highest percentage inhibition of increased rat paw volume of 74.1±10.0% which was higher than that of aspirin 65.5±12.0% but slightly lower than that obtained for indomethacin 85.6±3.0% (Table 13).

The percentage inhibition of increase in paw volume of rats given 400 mg/kg dose of *Morinda morindoides* was 65.5±12.0% and this is equivalent to value obtained for aspirin (65.5±12.0%) but much lower than that of indomethacin (85.63±3.0%) (Table13).

Tithonia diversifolia extract at 400 mg/kg dose exhibited a percentage inhibition of increase in rat paw volume of 62.3±13.0% which is lower compared with that of aspirin (65.5±12.0%) and much lower than that of indomethacin 85.6±3.0% (Table 13).

The least percentage inhibitions of increase in rat paw volume were obtained from the extracts of *Lippia multiflora* with a value of 54.2±16.0% and *Ocimum gratissimum with a* value of 45.4±21.0%.

TABLE 13: THE MEAN ANTI-INFLAMMATORY EFFECTS OF 400 MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON CARRAGENAAN INDUCED PAW VOLUME IN MICE.

TREATMENTS	CBF	CAF	% INHIBITION
Tithonia diversifolia	2.82±0.1	3.03±0.1*	62.3±13.0
Acalypha wilkesiana	2.83±0.2	2.98±0.0	74.1±10.0
Lippia multiflora	2.90±0.5	3.13±0.3	54.2±16
Ocimum gratissimum	2.78±0.2	3.10±0.6	45.4±21.0
Morinda morindoides	2.82±0.7	3.02±0.7	65.5% ± 12.0
Aspirin	3.03±0.0	3.23±0.4	65.5%±12.0
Indomethacin	2.90±0.0	2.97±0.0	85.3±3.0
Untreated control	2.82±0.0	3.38±0.0	0.00

CBF: Circumference of the rat paw at "O Hr" of injection of Carragenaan

CAF: Circumference of the rat paw at "3Hrs" after carragenaan injection

% inhibition of increase in rat paws volume.

TABLE 14: SUMMARY OF PHARMACOLOGIC ACTIVITIES AND TOXIC POTENTIAL OF THE PLANT EXTRACTS

Extracts of	Antidiabetic effect	Analgesic Effect	Antiinflammatory effect	Toxic potential	Antifertility effect
Tithonia diversifolia	+	+++	+++	+	–
Acalypha wilkesiana	++	+++	+++	–	–
Lippia multiflora	–	+++	++	–	–
Ocimum gratissimum	–	+	+	–	–
Morinda morindoides	+++	+++	+++	–	+

N.B: +++ = strong; ++ = good;

\+ = mild; - = weak;

4.8 EXPERIMENT 7

PURIFICATION AND IDENTIFICATION OF ACTIVE COMPONENTS OF PLANTS

From Table 14, it became obvious that *Acalypha wilkesiana* and *Morinda morindoides* extracts showed more pharmacological potentials and least toxicity potentials. Also, this is the first report of the antidiabetic potential of *A. wilkesiana* and *M. morindoides* and also the first report of analgesic and anti-inflammatory potentials of *M. morindoides*.

These 2 plant extracts were thus subjected to further studies to determine the active principles and possibly elucidate the structures and the nomenclatures of the active compounds in the plants.

4.8.1 *Phamacological testing of the fractions for anti-diabetic effect M. morindoides:*

Each solvent fraction was tested for anti-diabetic potential at 200 mg/kg and 400 mg/kg. The 400mg/kg dose group of chloroform fraction of *M. morindoides* showed the highest activity, reducing blood sugar level from 350.6±75mg/dl at Ohr to 235.1±41mg/dl at 4hrs. This implies a 33% reduction of blood sugar level within 4hrs, a value higher than that of glibenclamide (31.5%) (Fig 1A& B)

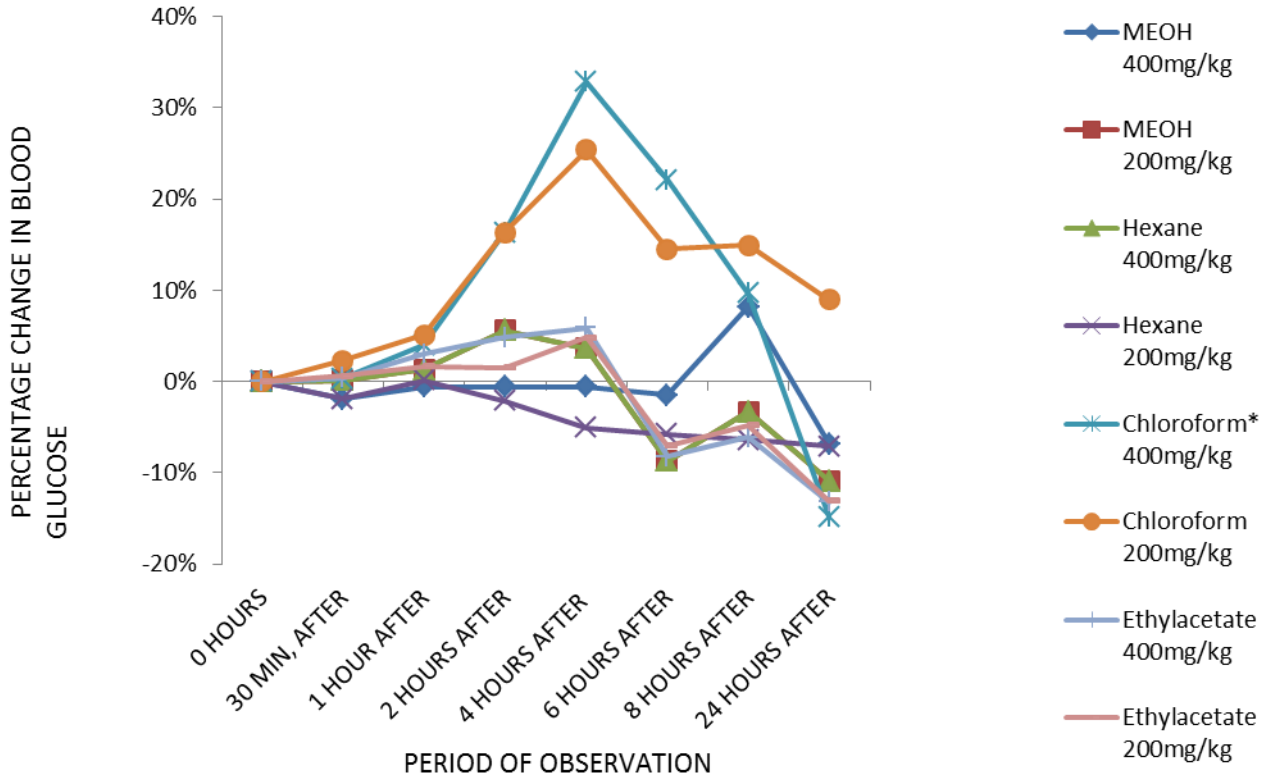


FIG. 1A : PERCENTAGE HOURLY REDUCTION OF BLOOD SUGAR LEVEL IN ALLOXAN INDUCED DIABETIC WISTAR ALBINO RATS BY VARIOUS ORGANIC FRACTIONS *OF EXTRACTS* OF *M. MORINDOIDES*

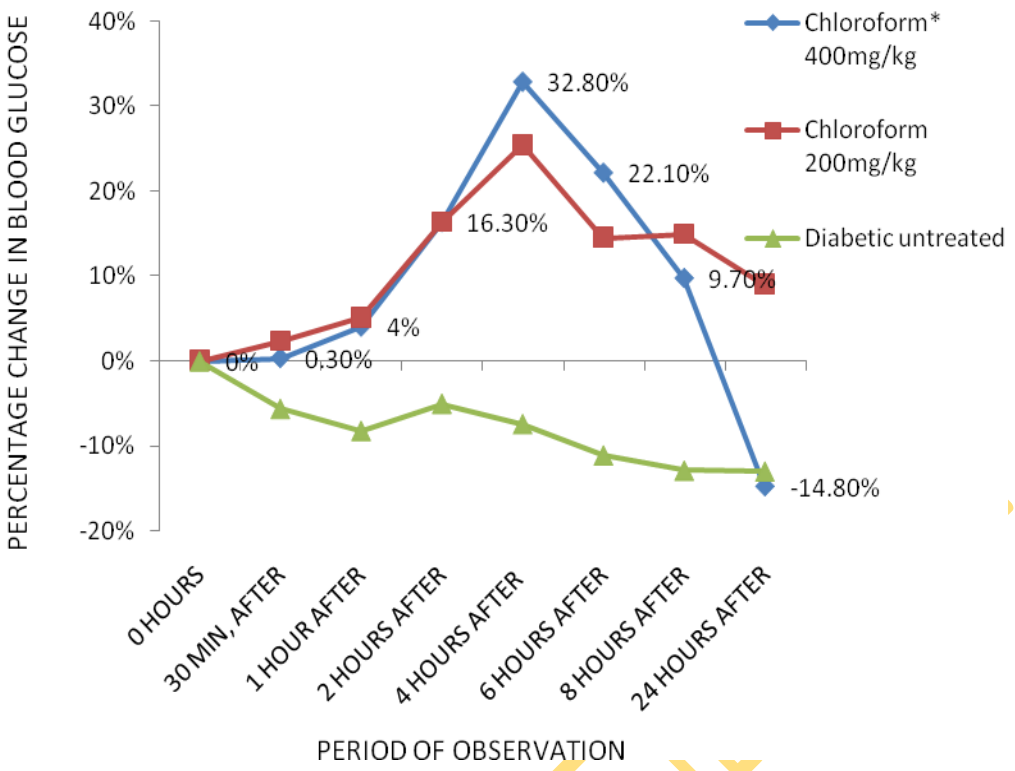


FIG.1B: EFFECTS OF 200 MG/KG AND 400 MG/KG DOSES OF CHLOROFORM EXTRACT OF M. MORINDOIDES ON DIABETIC RATS

4.8.2 *Phamacological testing of the fractions for anti-diabetic effect Acalypha wilkesiana:*

The N-hexane fraction of *A. wilkesiana* at 400 mg/kg showed the highest activity reducing blood sugar level from 525.10±37.0mg/dl at Ohr to 333.7±7.4mg/dl at 4hrs indicating a 37% reduction of blood sugar level within 4hrs a value higher than that of glibenclamide (31.5%) (Figs 2A & 2B).

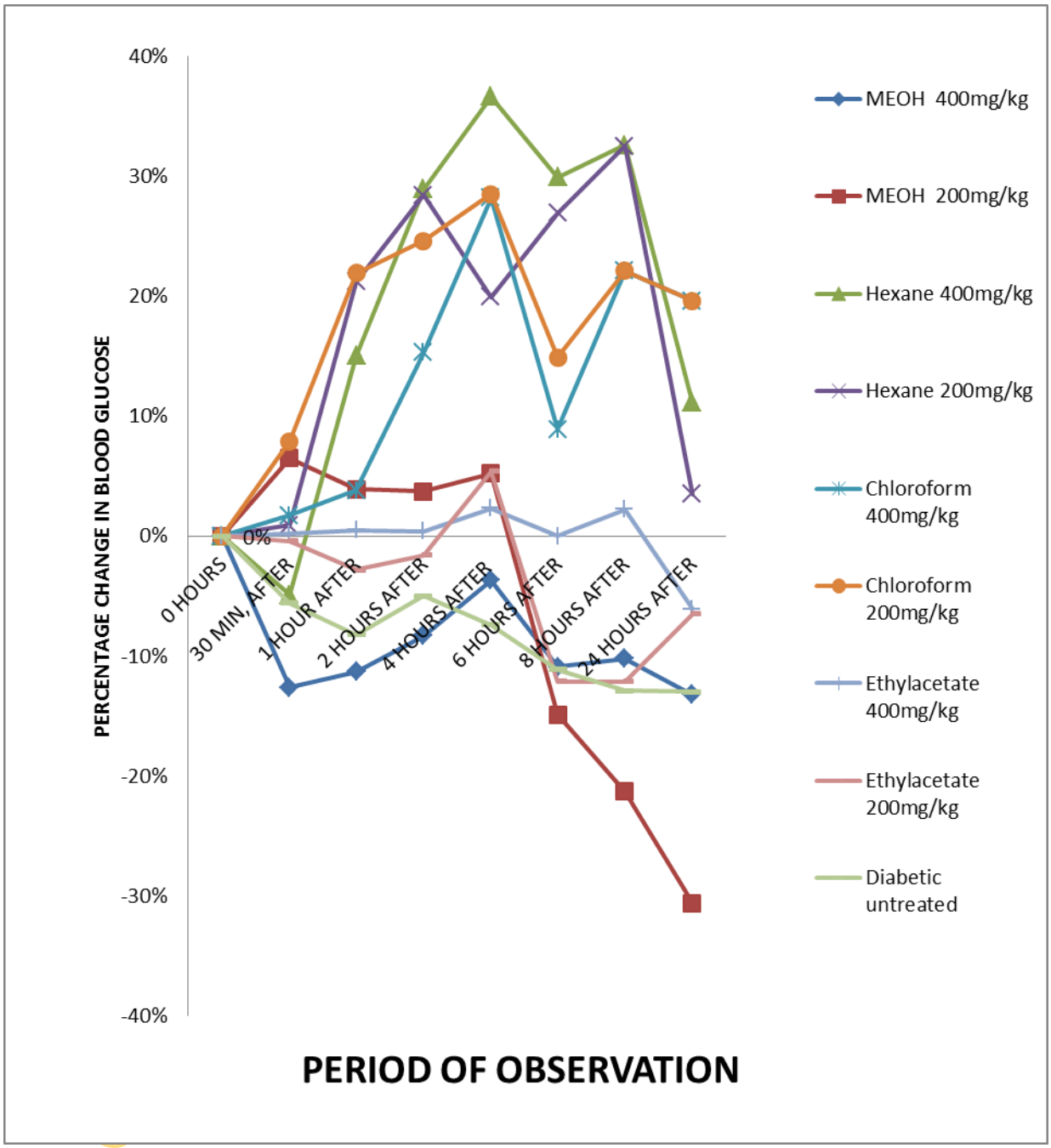


FIG 2A: PERCENTAGE HOURLY REDUCTION OF THE EFFECT OF *MEOH, HEXANE, CHLOROFORM, & ETHYLACETATE* EXTRACTS OF *A.WILKESIANA* ON BLOOD SUGAR LEVEL IN ALLOXAN INDUCED DIABETIC WISTAR ALBINO RATS

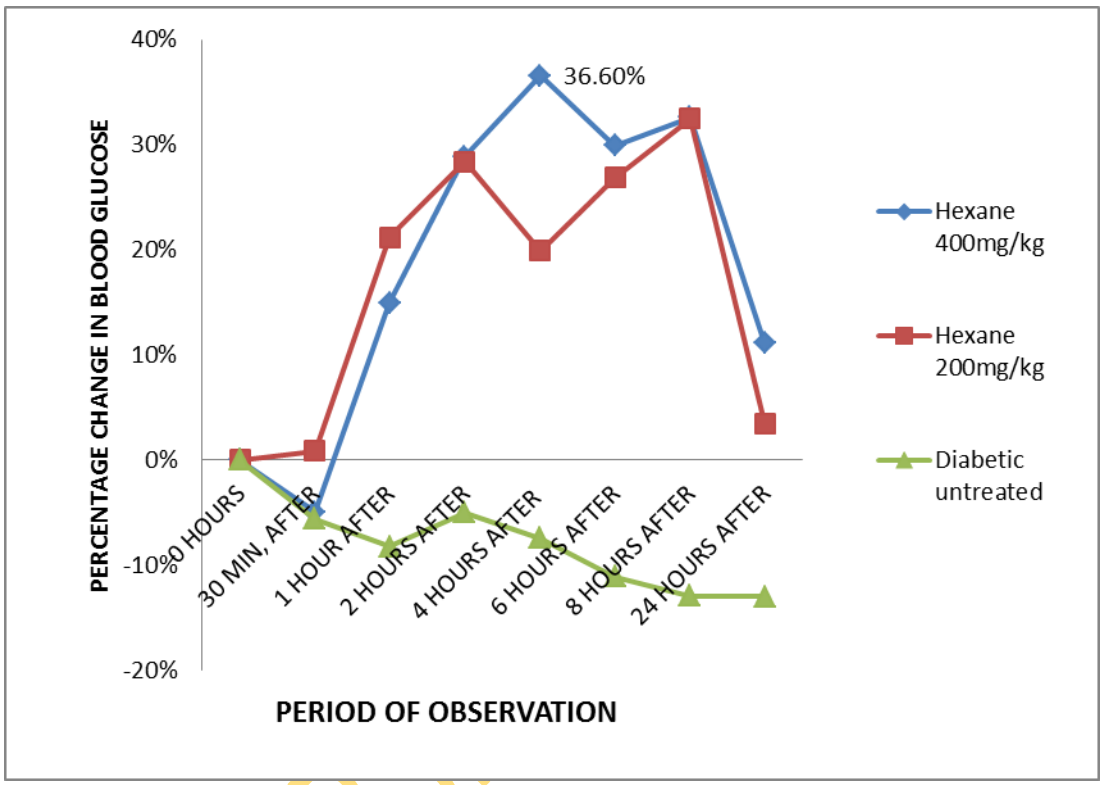


FIG 2B: EFFECTS OF 200 MG/KG AND 400 MG/KG DOSES OF HEXANE EXTRACT OF A. WILKESIANA ON DIABETIC RATS

4.8.3 *Phamacological testing of the fractions of M. morindoides for anti-inflammatory effect*

Each solvent fraction was tested for anti-inflammatory potential and the 200 mg/kg dose group of chloroform fraction of *M. morindoides* showed activity with the highest percentage inhibition of increase in paw volume (92.80%) a value higher than the performance of standard anti-inflammatory agents; indomethacin (85%) and aspirin (66%) (Fig 3).

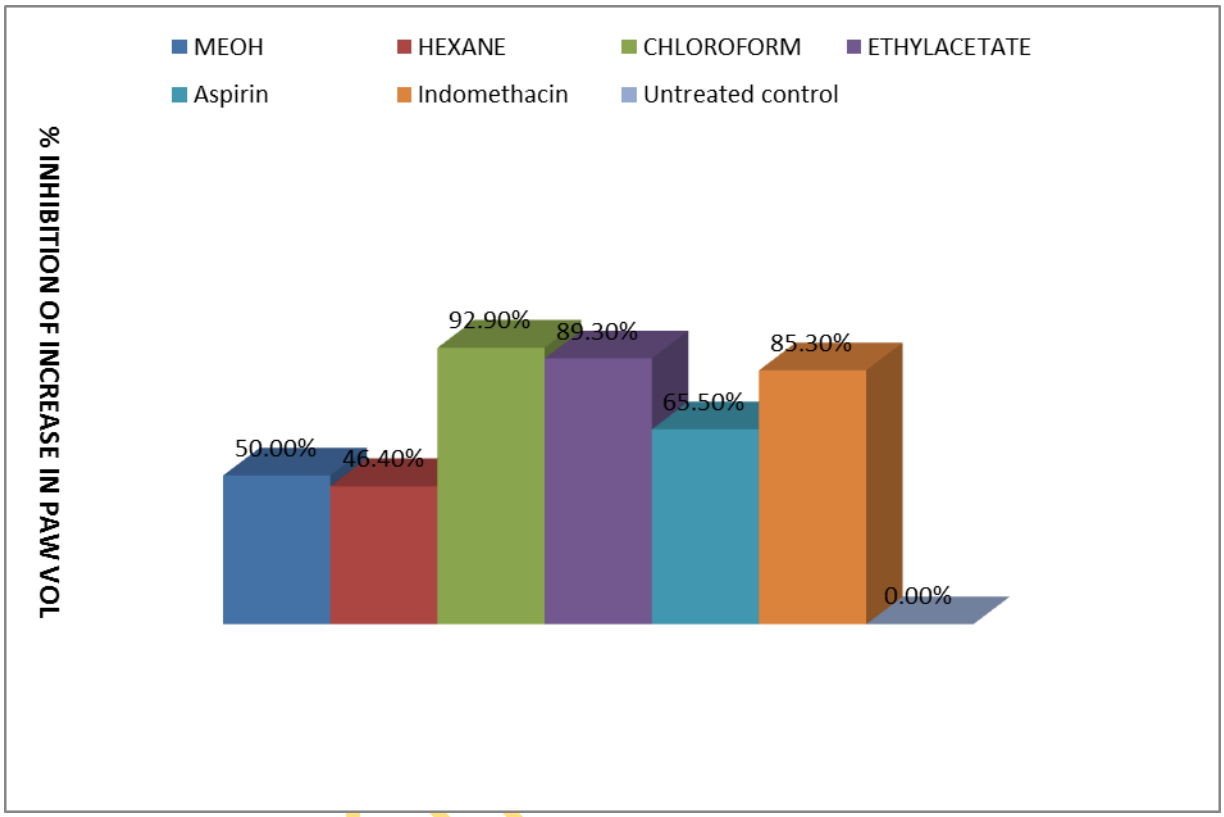


FIG 3: EFFECTS OF 200 MG/KG DOSE OF *MEOH, HEXANE, CHLOROFORM &ETHYL ACETATE* EXTRACTS OF *M. MORINDOIDES* ON CARRAGENAAN INDUCED RAT PAW OEDEMA

4.8.4 *Phamacological testing of the fractions of A. wilkesiana for anti-inflammatory effect*

The 200 mg/kg dose group of chloroform fraction of *A.wilkesiana* showed activity with highest percentage inhibition of increase in paw volume (93%) a value higher than the performance of standard anti-inflammatory agents; indomethacin (85%) and aspirin (66%) (Fig 4)

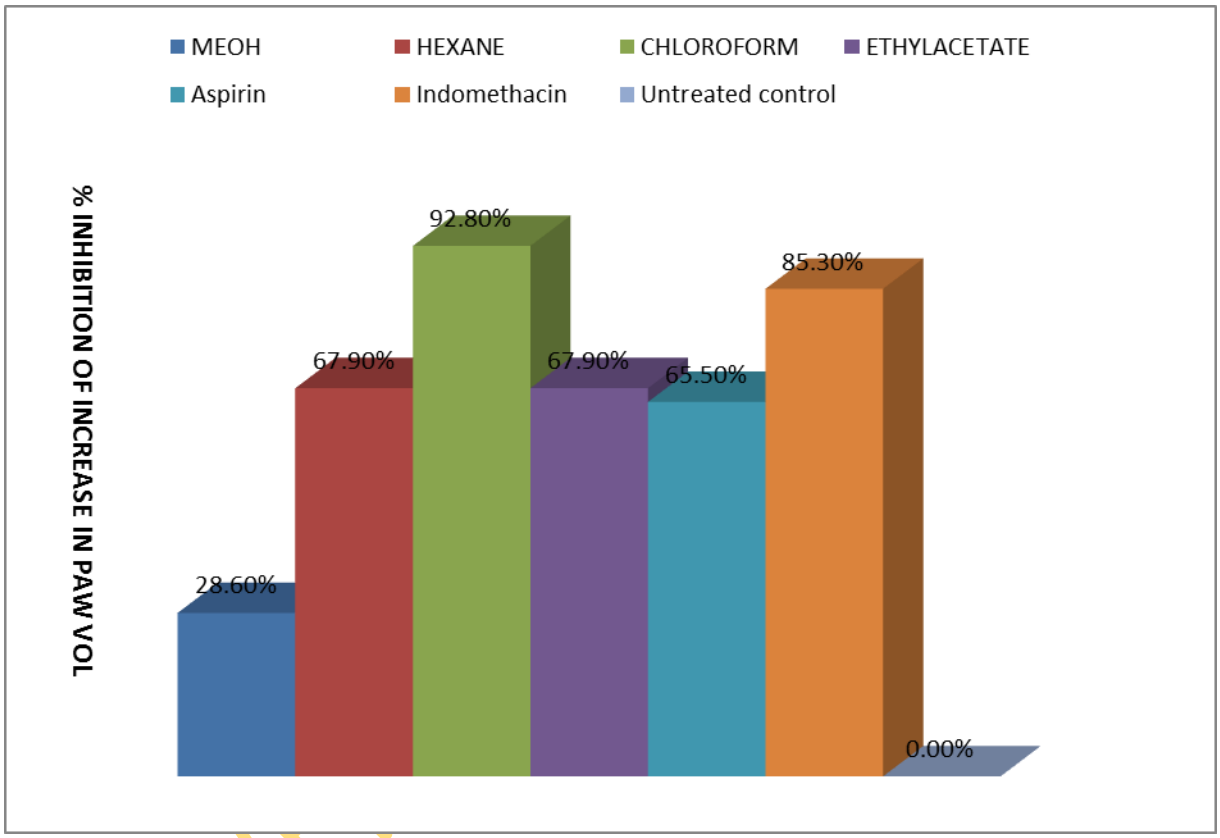


FIG 4: EFFECTS OF 200 MG/KG DOSE OF MEOH, HEXANE, CHLOROFORM &ETHYL ACETATE EXTRACTS OF *A. WILKESIANA* ON CARRAGEENAN INDUCED RAT PAW OEDEMA

4.8.5 *Phamacological testing of the fractions of M. morindoides for analgesic effect*

Each solvent fraction was tested for analgesic potential and the 200 mg/kg dose of ethylacetate fraction of *M. morindoides* showed activity with the highest percentage inhibition of writhing (93.20%) a value higher than the performance of reference analgesic agent paracetamol (50%) (Fig 5).

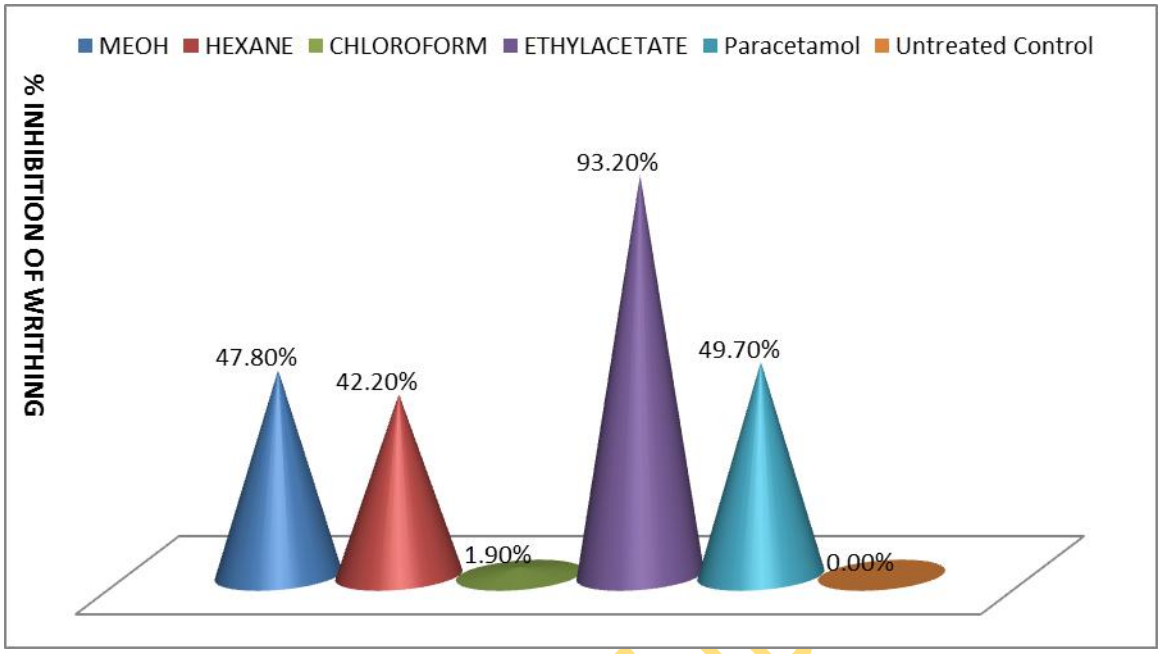


FIG 5: EFFECTS OF 200MG/KG DOSE OF MEOH, HEXANE, CHLOROFORM AND ETHYL ACETATE EXTRACTS OF *M. MORINDOIDES* PLANT ON ACETIC ACID INDUCED WRITHING TEST IN MICE.

4.8.6 Accelerated Gradient Chromatography of the Chloroform fractions of *M. morindoides* and *A. wilkesiana* and Ethyl acetate Fraction of *M. morindoides*

Introduction

The chloroform and n-hexane fractions of both *M. morindoides* and *A. wilkesiana* respectively showed the most potent activities in the hourly assessment of anti-diabetic effects using alloxan induced diabetic rats. Also the chloroform fraction of both *M. morindoides* and *A. wilkesiana* showed most potent anti-inflammatory activity in the carrageenan induced rat paw oedema model of inflammation test.

The ethyl acetate fraction of *M. morindoides* showed the most potent activities in the acetic acid induced writing test.

4.8.7 *Phamacological testing of the chromatographic fractions for anti-diabetic effect*

Each of the 6 subfractions of chloroform fraction of *M. morindoides* and n-Hexane fraction of *A. wilkesiana* was tested for anti-diabetic potential at 100 mg/kg dose.

M. morindoides:

The chloroform chromatographic fraction B of *M. morindoides* showed the highest activity reducing blood sugar level from 472.8±57.0mg/dl at O hr to 218.9±65.0mg/dl at 4hrs and to 121.2±13.4 mg/dl (Table 6A& B). This implies a 53.7% reduction of blood sugar level at 4hrs and a 74.4% reduction after 24 hrs. These values were higher than that obtained for glibenclamide (31.5% and 52.1% respectively) (Fig 6).

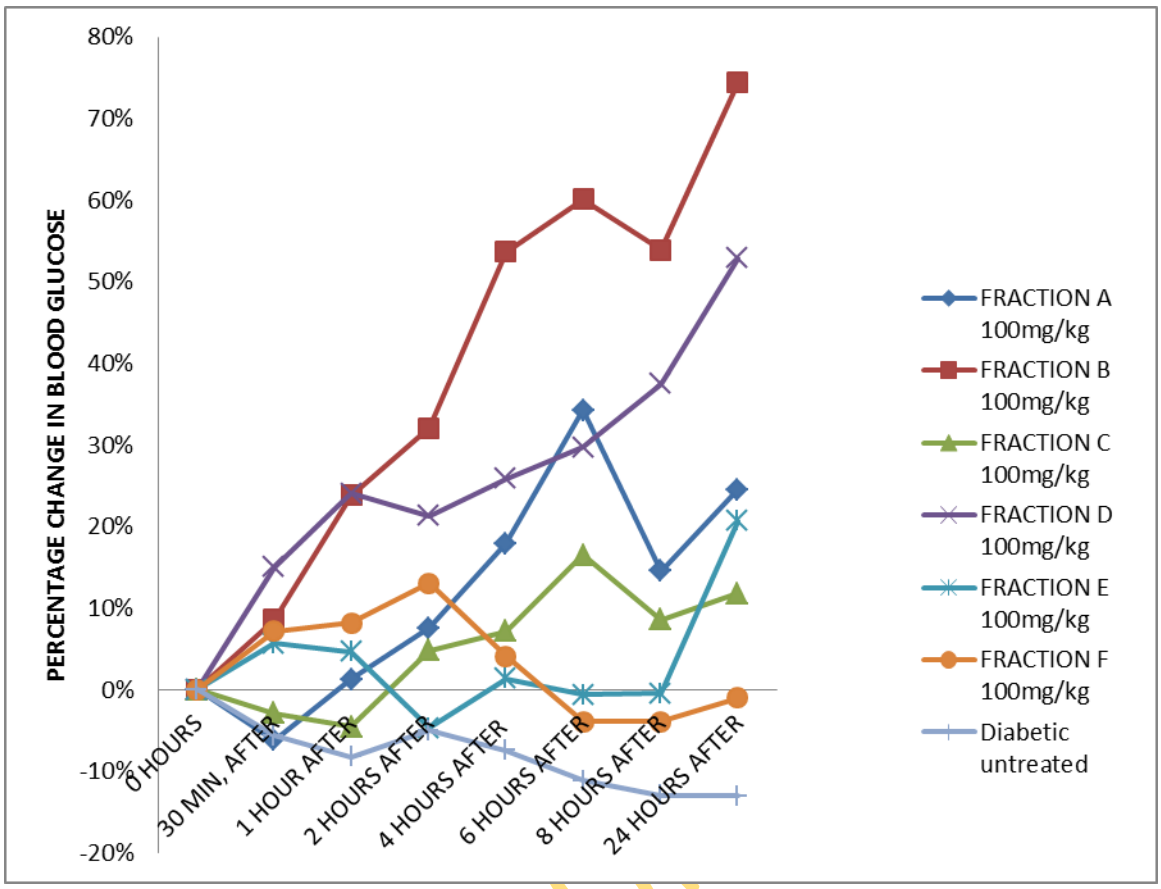


FIG. 6: EFFECT OF CHROMATOGRAPHIC FRACTIONS OF M. MORINDOIDES ON BLOOD GLUCOSE

Acalypha wilkesiana:

The n-Hexane chromatographic fraction D of *A. wilkesiana* showed the highest activity reducing blood sugar level from 430.9±31.0mg/dl at O hr to 267.1±3.0mg/dl at 4 hrs and to 181.4±11.1mg/dl (Fig. 7). This implies a 38% reduction of blood sugar level at 4hrs and a 58% reduction after 24 hrs. These values were higher than that obtained for glibenclamide (31.5% and 52.1% respectively) (Fig 7).

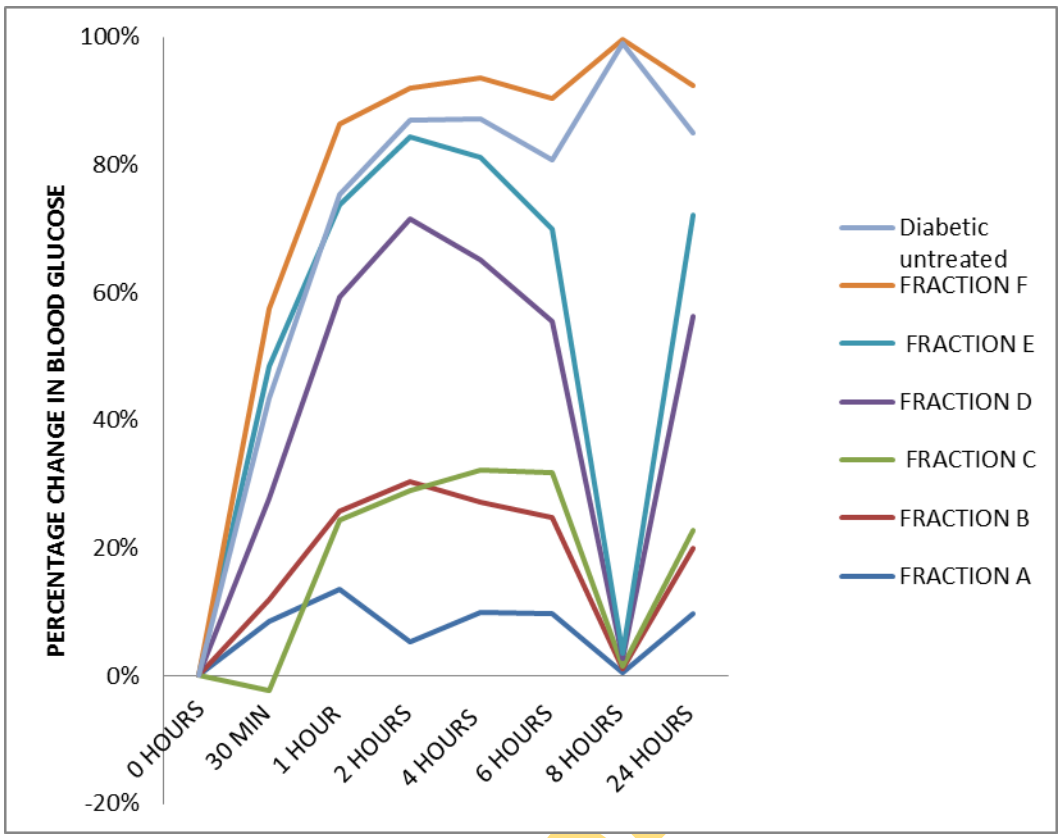


FIG. 7A: EFFECTS OF CHROMATOGRAPHIC FRACTIONS OF *A. WILKESIANA* ON BLOOD GLUCOSE

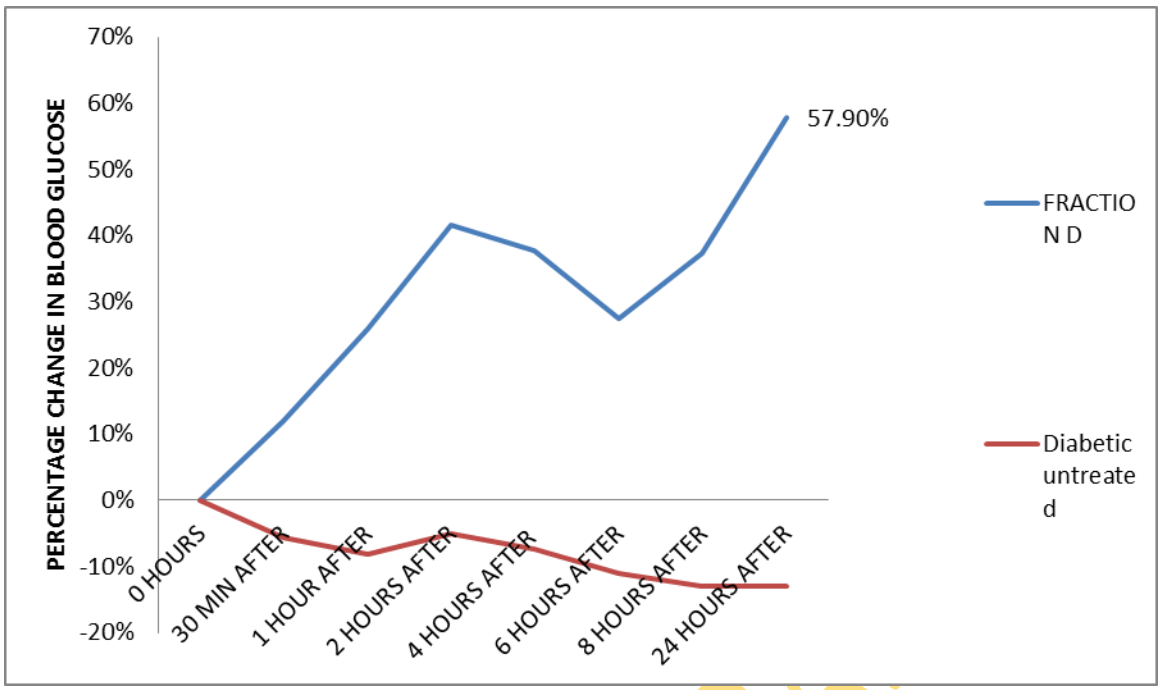


FIG 7B: COMPARING CHROMATOGRAPHIC FRACTION D OF *A. WILKESIANA* AND UNTREATED DIABETIC GROUP

4.8.8 *Phamacological testing of the Chromatographic fractions for anti-inflammatory effect*

Each of the 6 subfractions of chloroform fraction of *M. morindoides* was tested for anti-inflammatory potential at 100mg/kg dose. The chloroform chromatographic fraction A of *M. morindoides* showed the highest activity with percentage inhibition of increase in paw volume (93%), a value higher than the performance of standard anti-inflammatory agent indomethacin (85%) and aspirin (66%) (FIG 8)

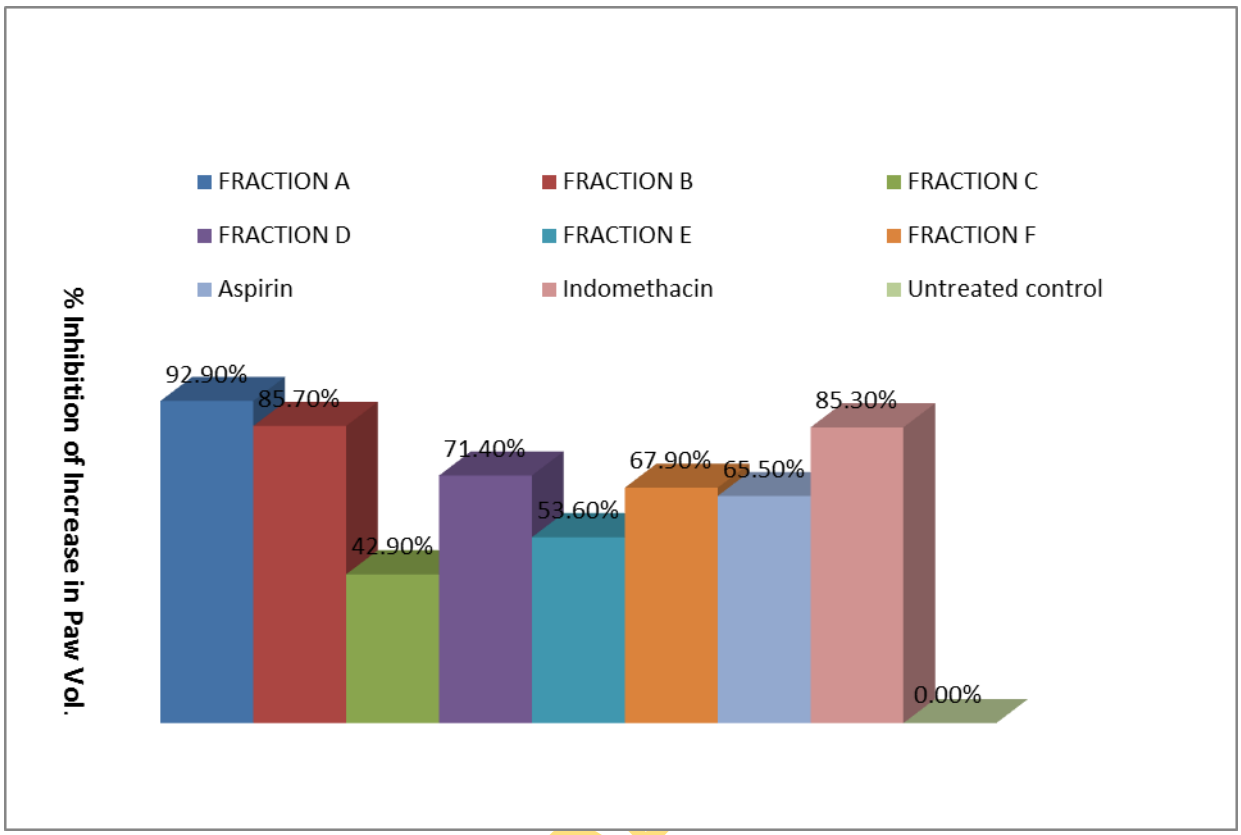


FIG 8: EFFECTS OF 100MG/KG DOSE OF CHROMATOGRAPHIC FRACTIONS OF CHLOROFORM EXTRACT OF *M. MORINDOIDES* ON CARRAGENAAN INDUCED RAT PAW OEDEMA.

4.8.9 *Phamacological testing of the chromatographic fractions for analgesic effect*

Each of the 6 subfractions of ethylacetate fraction of *M. morindoides* was tested for analgesic potential and the 100mg/kg dose of ethylacetate chromatographic fraction D of *M. morindoides* showed activity with the highest percentage inhibition of writhing (71%), a value higher than the performance of reference analgesic agent paracetamol (50%) (Fig 9).

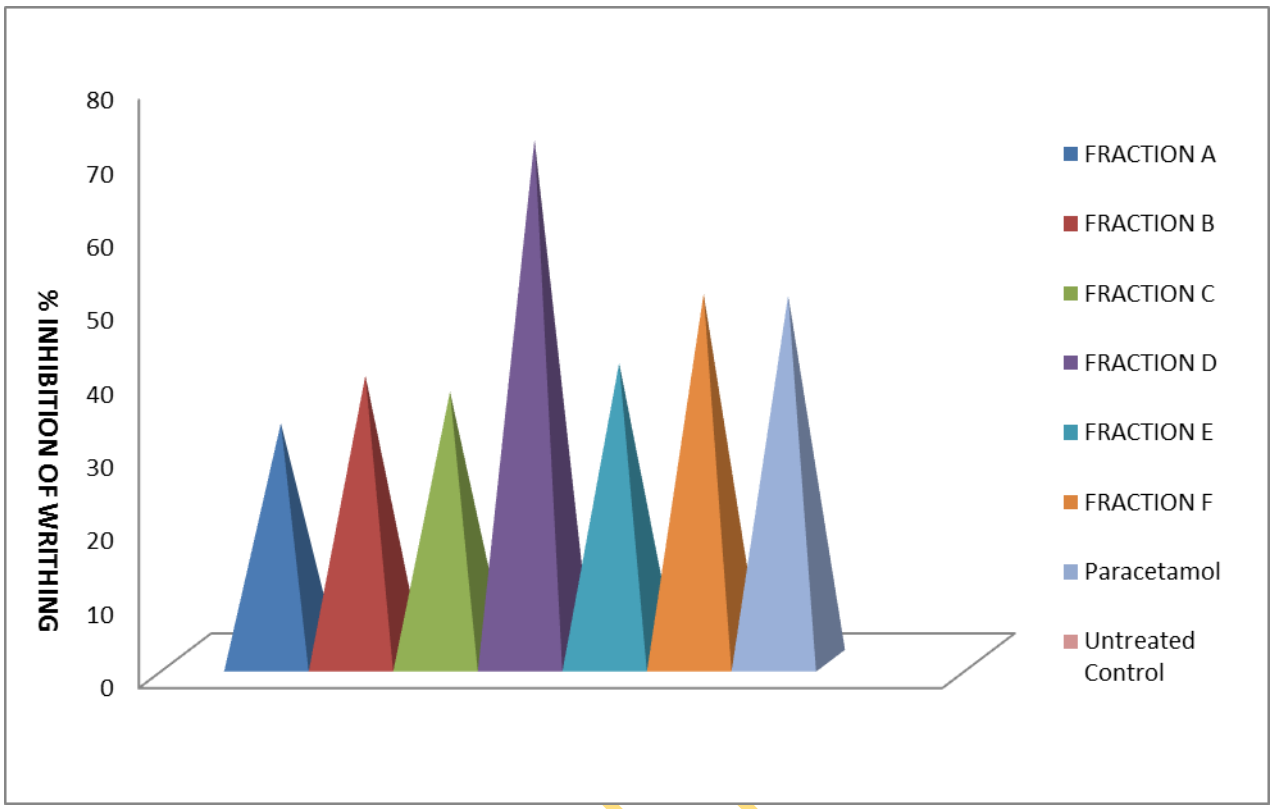


FIG 9: EFFECTS OF 100MG/KG DOSE OF CHROMATOGRAPHIC FRACTIONS OF *ETHYL ACETATE* EXTRACTS OF *M. MORINDOIDES* ON ACETIC ACID INDUCED WRITHING TEST IN MICE

4.9 Determination of Structures and Nomencalture of the Isolated Compounds

Structural determination of the active principles of medicinal plants is a way of knowing which part of the structures is or are responsible for the activities. An attempt was made to determine the structures of the four isolated compounds so as to name them and know the parts of the structure responsible for the above reported activities.

The structures and names of the 1st and 2nd compounds have been established with the aid of spectroscopic techniques using the high resolution electron ionization mass spectroscopy (NMRS). While the 3rd and the 4th compounds NMR spectrum given is impure and cannot be presented now, there are still on going efforts to elucidate the structures and the names of compounds 3 and 4.

In line with TLC analysis, Fractions [124 from chloroform extracts of *M. morindoides* using hexane/EtOAc solvents combinations; 68 fractions from ethyl acetate extract of *M. morindoides* using solvent combination of EtOAc/ MeOH and 66 fractions from hexane extract of *Acalypha wilkesiana* using solvents combination of chloroform/MeOH] obtained were grouped into 6 sub fractions coded A-F.

All fractions were evaluated for antidiabetic, anti-inflammatory and analgesic activities.

2 compounds were isolated from the chloroform extract of *M. morindoides*,

Compound 1 (fraction A) obtained from eluents 2-47, eluted with (98% hexane+2%EtOAc) afforded a yellowish-white powdery solid, Compound 1 (850 mg) after recrystallising in EtOAc/hexane (3:7, vol/vol, 30 ml). Compound 1 displayed remarkable anti-inflammatory activity in carragenaan-induced rat paw inflammation.

Compound 2 (fractions B) isolated from chloroform extract *of Morinda morindoides* consists of eluents 73-80 (eluted with 10% EtOAc in hexane) afforded yellow powder which on further purification by recrystallisation in EtOAc/hexane solvent mixture (3:7. vol/vol) yielded yellow powder (760 mg). Compound 2 exhibited hypoglycemic activity in alloxan-induced diabetic rats.

Compound 3 (fraction D) obtained from ethyl acetate extract of *M. morindoides* consists of Fractions 21-28 (eluted with 80% EtOAc in MeOH) yielded yellow solid. Further purification and recrystallisation in EtOAc/MeOH solvent system (3:7, vol/vol, 200ml) afforded amorphous yellow powder of compound 3 (775mg) but the NMR spectrum obtained requires futher work be done to obtain a more reliable result.

Compound 4 (fraction D) obtained from hexane extract of *A. wilkesiana* consists of Fractions 22-35 (eluted with 60% EtOAc in hexane) yielded a

yellowish-brown solid. Further purification and recrystallisation in EtOAc/hexane solvent system (3:7, vol/vol, 200ml) afforded brownish-yellow powder of compound 3 (775mg) the NMR could not be obtained yet as futher work needs to be done to obtain a more reliable result.

The physicochemical and spectral data of the fully isolated compounds 1 and 2 are reported below.

Compound **1**: Quercetin 7- dimethylether (Fig. 10A)

and Compound **2**: Glucoside of 4, 7-dimethoxy quercetin (Fig. 10B)

The compounds tested positive to Ferric chloride test, giving a yellow precipitate, indicating its phenolic nature (i.e OH on aromatic ring).

It equally gives positive test with Shibata reaction, which indicates its flavonoidal nature.

Shibata's test

Five mg (5 mg) each of the extract was dissolved in MeOH, warmed with three pieces of magnesium turning and mixed with few drops of concentrated H_2SO_4. Presence of an orange pink coloration confirmed the presence of flavonoids in the extract (Harborne, 1999).

Elution of the column with n-hexane diethyl ether (Et_2O) (3:1) furnished white powder of 1, Quercetin; R_f 0.35 (analytical TLC, silica gel, Merck 60 $PF_{254+360}$ developed in Hexane/EtOAC 3:1 vol/vol); ^{1}H-NMR [400MHz , $CDCl_3$, δ (ppm)]: 7.8 (1H, d, *J* = 2.0 Hz, H-5'),7.4 (1H,

d, *J* = 2.1 Hz, H-8), 7.1 (1H, d, *J* = 2.0 Hz, H-1'), 6.7 (1H,dd, *J* = 8.1, 2.0, H-2'), 6.60 (1H, dd, 7.05, 2.0, H-6), 10.3-10.6 (1H each, -OH), 3.92 [-OCH_3); ^{13}C-NMR [400MHz , $CDCl_3$, δ (ppm)]: 1193.0(C-4), 182.5 (C-8),165.1 (C-3),140.8(C-2), 135.7.1(C-5), 134.4 (C-9), 132.1 (C-4'), 127.1 (C-5'),111.0 (C-3'),111.9 (C-1'), 92.6.1 (C-6'), 1 (C-10), 105.2 (C-2'), 98.9 (C-6), 93.0 (C-8), 39.4(CH_2), 38.9(CH_3).

The NMR spectrums look similar except the glucose moiety on the C-5.

Compound 1

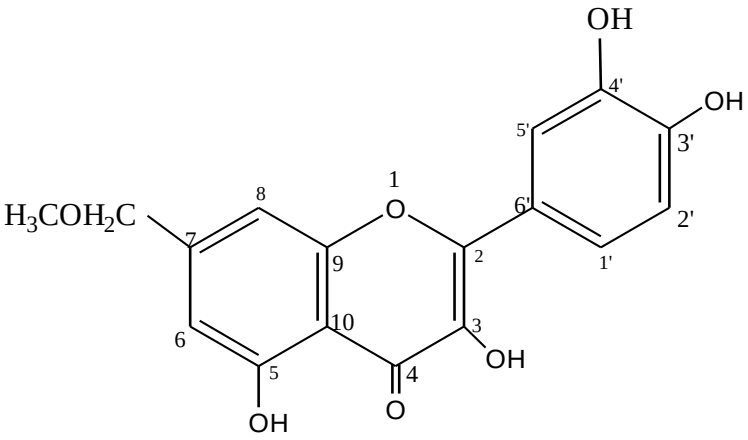
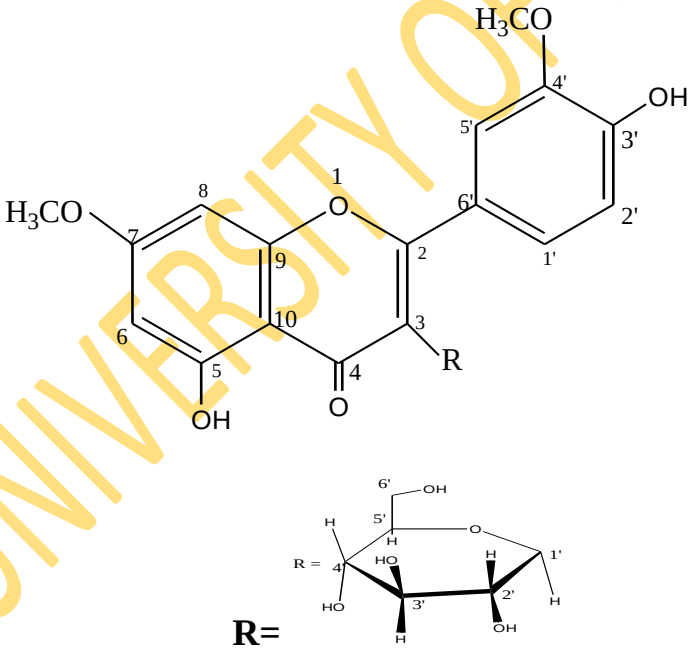


Fig.10A. Compound **1**: Quercetin 7- dimethylether

Compound 2



R=

Fig.10B. Compound **2**: Glucoside of 4',7-dimethoxyl quercetin

CHAPTER FIVE

DISCUSSION

In the most general terms, pain is a symptom of some form of dysfunction and resultant inflammatory processes in the body (Edmond, 1987). In more specific terms, pain is the result of an injury to tissue either from physical or chemical trauma. *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora* and *Morinda morindoides* plant extracts produced significant analgesic effects in both chemical and thermal-induced pain in mice (Tables 12) respectively. In chemical induced pain, which is more of an inflammatory pain that involve production of pro-inflammatory mediators such as bradykinin, kinin, histamine and serotonin (Keel, 1969), these mediators stimulate nociceptors therefore having a pain producing effect (Colher *et al.*, 1968). The analgesic activity of non-steroidal anti-inflammatory drugs (NSAIDS) might be ascribed to their ability to inhibit the cylooxygenase and lipoxygenase activities at the periphery (Mc Queen *et al.*, 1991; Kiruchi *et al.*, 1992).

This work has shown that *Tithonia diversifolia, Acalypha wilkesiana Lippia multiflora* and *Morinda morindoides* plant extracts reduced pain chemically induced by acetic-acid in a comparable manner to that of aspirin and indomethacin (NSAID) (Table 12). It can therefore be inferred that *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora*

and *Morinda morindoides* plant extracts may exert their analgesic effects by:

1. Reduction of production of pro-inflammatory mediators (Fernandez and Boyle, 2001).
2. Inhibition of cyclooxygase and lipoxygenase activities.

Thermal induced pain by the hot plate method is usually considered suitable for centrally acting analgesics (Melcod, 1970). *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora* and *Morinda morindoides* plant extracts at 400 mg/kg produced a significant prolongation of reaction time comparable to those reported for morphine (Table 12).

Like in the hot plate test, *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora* and *Morinda morindoides* plant extracts also exhibited analgesic effect by inhibition of numbers of spasmodic abdominal contraction exhibited by mice administered with acetic acid (Table 12). These models are useful tools to access the potency of drugs and their probable sites and mechanism of action. It is therefore possible that the extracts may be producing its effects both peripherally through acetic-acid induced writhing and centrally through hot plate test (Martins Do Monte *et al.*, 2004).

Inflammation is typically characterized by increased permeability of endothelial tissue and influxes of blood leukocytes into the interstitium resulting in oedema. Many different biological mediators influence each step of inflammation and typically, anti-inflammatory agents exhibit therapeutic properties by blocking the actions or synthesis of some of these mediators. Some of the phytochemical found in certain herbs and plants are reported to demonstrate pain and inflammation-reducing properties (Havsteen, 1983). Like aspirin and indomethacin, many are presumed to work by blocking the cyclooxygenase and lipoxygenase pathways and possibly by other mechanisms as well (Arora *et al.,* 1971; Srivastave and Mustafa, 1992; Kiruchi *et al.,* 1992; Ammon *et al.,* 1993). Carrageenan has been shown to induce the release of kinin-like substances, especially bradykinins (Elizabetsky *et al.,* 1995). It has also been shown to induce a protein-rich exudates containing large number of neutrophils (Lo *et al.,* 1982). Carrageenan-induced neutrophils migration is a process dependent on the release of chemotactic mediators by resident cells (Souza *et al.,* 1988). The 400 mg/kg dose extracts of *Tithonia diversifolia, Acalypha wilkesiana,* and *Morinda morindoides* exhibited an effective suppressant activity on acute inflammatory model of carrageenan-induced paw eodema in rats (Table 13).

It is possible that the anti-inflammatory effects of *Tithonia diversifolia*, *Acalypha wilkesiana* and *Morinda morindoides* could be as a

result of an inhibitory action of release of kinin-like substances or prevention of the release of chemotactic agents or by inhibition of neutrophils migration to the site of inflammation.

The persistent and consistent reduction in the values of the blood glucose, which was seen also to be mostly dose dependent and exhibited by all the plant extracts, was then exploited for an antidiabetic study (Tables 1B, 2B and 3B).

It was observed that the 400 mg/kg dose of *Tithonia diversifolia, Acalypha wilkesiana, Ocimum gratissimum* and *Morinda morindoides* plant extracts significantly reduced blood glucose level in alloxan induced diabetic rat with *Acalypha wilkesiana, Tithonia diversifolia* and *Morinda morindoides* extracts exhibiting outstanding antidiabetic and hypolipidaemic performance (Table 11). They also significantly lowered the concentration serum of LDL and increase the serum concentration of HDL and this is comparable with the performance of glibenclamide (a standard oral antidiabetic drug).

It should be noted that Momoh *et al*., (2006) reported the antidiabetic effect of *Laportea ovaliofolia* based on the reduction of the blood glucose level, serum cholesterol, serum LDL and increased serum HDL. To further reaffirm the earlier assertion on the hypoglycemic and anti-diabetic effect of the 400 mg/kg aqueous extracts of *T. diversifolia, A. wilkesiana*, *L. multiflora. O. gratissimum* and *M. morindoides*, a

further study of the time dependent variation in blood glucose level was carried out.

In this study, a statistically significant reduction in the level of blood sugar were observed, at the 2, 4 and 24 hrs in the group of rats dosed with a single oral administration of 400 mg/kg. This result indicates that the extract of *T. diversifolia* at the dose of 400 mg/kg will produce up to 15%, 21% and 36.0% reductions in blood sugar within 2 to 24 hours of administration.

The performances observed implies that the extract of *T. diversifolia* possess anti diabetic potential. This is consistent with the study of Miura *et al.*, (2005), who reported that the extract of *T. diversifolia* produced very significant hypoglycemic activity 4 hours after administration.

Also, it was observed that 400 mg/kg of *A. wilkesiana* extract produces significant reductions in blood sugar levels with percentage reductions up to 43.8% after 24 hrs. The performance of *A. wilkesiana* at 400 mg/kg is fairly comparable with that of a standard anti-diabetic drug, glibenclamide.

An interesting observation was made with the study done on 400 mg/kg dose *Morinda morindiodes*. From the result, the performances of *Morinda morindiodes* was outstanding in the time dependent rate of variation observed in the blood sugar level, at 1 hours after

administration, the performance of *M. morindoides* extract strongly competed with that of glibenclamide, and with further studies at 2 hours, the hypoglycemic potential of the extract of *Morinda morindiodes* had outclassed that of glibenclamide and this outstanding performance of *M. morindoides* continued steadily till 24 hrs after administration when 56% of the blood glucose level had been reduced in a manner that far outweigh the performance of even the known standard oral hypoglycemic agent glibenclamide.

Based on this outcome, it is concluded that the extracts of *Morinda morindiodes* and *Acalypha wilkesiana* possess reliable hypoglycemic potential at 400 mg/kg that is comparable to that of standard oral anti-diabetic agent glibenclamide, with the extracts of *Ocimum gratissimum* and *Lippia multiflora* showing slight reductions of blood sugar level.

The observed anti-diabetic (i.e hypoglycemic) effect of the extract of the plants may be due to presence of phytochemical substances in the plants that potentiates increase pancreatic secretion of insulin from B-cells.

The possible mechanism by which these extracts bring about the observed hypoglycemic effects may be due to stimulation of pancreatic beta cell function and extra pancreatic action, by increasing the peripheral utilization of glucose.

The subacute toxicological study carried out on the plants is desirable and necessary to determine if these claimed medicinal

antimalaria herbs i.e. *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum gratissimum* and *Morinda morindoides* have any serious toxic potential that may do more harm than the medicinal benefit envisaged. It was discovered that at doses of 400 mg/kg, 800 mg/kg and 1600 mg/kg of these plants, the toxicity indices observed were negligible. This is obvious from the haematology and serum biochemistry study outcome of the aqueous extracts of the plants. *Tithonia diversifolia, Acalypha wilkesiana, Lippia multiflora, Ocimum gratissimum* and *Morinda morindoides* aqueous extracts are relatively safe because there were no significant toxic changes manifestations in the PCV, Hb and WBC at the 3doses of the extracts (Tables 1, 2 &3).

Also the results of the serum biochemistry study at 400 mg/kg, 800 mg/kg and 1600 mg/kg doses showed that the extracts of all these plants except for *Tithonia diversifolia* are relatively safe at 400 and 800 mg/kg doses. From the histopathology results, it could be observed that most lesions seen are mild and occurred at high doses with the exception of the lesions caused by the extract of *Tithonia diversifolia.* Expectedly of any true pharmacologically potent agent, some signs of toxicity will manifest at very high doses or at extra - pharmacological dose. So this implies that the extracts of these plants are safe when given at reasonable doses (Tables 4B, 5B &6B).

Also from the out come of the reproductive toxicity study, the observed dose - dependent reductions in sperm count, progressive sperm motility and few secondary morphological sperm abnormalties do not appear to be seriously out of place, firstly they are dose -dependent and are usually milder at the lower doses of the 400 and 800 mg/kg of the extracts (Tables 7, 8, 9(A&B)

Secondly since all these plants are reported to have antimalaria capability (Valentin *et al.*, 1995; Zirihi *et al.*, 2005; Tona *et al.*, 2001; Goffin 2002; Udobang *et al.*, 2010), various studies have reported that effective antimalaria drugs produce reversible anti-fertility effects in male animals.

It has been reported that chloroquine, quinine and quinacrine inhibit leydig cell steroidogenesis and fertility in male (Sairam, 1978). Furthermore, chloroquine was reported to reduce sperm motility and hence fertility by a reduction in the average number of fetuses of cohabited female rats (Adeeko and Dada, 1998). Raji *et al.*, (2005b) also reported significant reduction in sperm motility, viability, count and testosterone levels in the rat treated with artemeter (an artesunate brand).

Moreover, antimalaria medicinal plants extracts like *Quassia amara, Astonia boonei* and *Azadiratcha indica* have also been reported in experimental male infertility (Ojewole, 1984:, Raji and Bolarinwa, 1997, Olajide *et al.*, 2000; Raji *et al.*, 2005b).

The isolation and purification of the active compounds in *Morinda morindoides* and *Acalypha wilkesiana* yielded flavonoidal compounds.

Morinda morindoides chloroform extract yielded 2 flavonoids; quercetin 7- dimethylether (compound 1) and glucoside of 4,7-dimethoxyl quercetin (compound 2). The two compounds were obtained by direct comparison with similar compound previously synthesized and elucidated spectroscopically as constituents from *Morinda morindoides* by Cimanga *et al.*, 1999.

Counpound 1, isolated from the chloroform fraction of *M. morindoides* has been established to be a flavonoid named quercetin 7, 4 dimethylether which was found to be the active principle in the plant extract responsible for the anti-inflammatory effects of the root bark extract in carrageenan – induced rat paw inflammation.

It should be noted that Teresita *et al.*, 2002 reported that quercetin significantly inhibit both acute and chronic inflammation models in rats.

An important effect of flavonoids is the scavenging of oxygen-derived free radicals. (Van Acker, 1995; Shettya *et al*., 2004) and thus decrease oxidative stress caused by NF-κB activation, a factor that has been found responsible for the coding of oxidative cytokines in most inflammatory conditions.

Several flavonoids, including quercetin, result in a reduction in ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity (Shoskes, 1998). Nitric oxide is produced by several different types of cells, including endothelial cells and macrophages.

Although the early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels (Huk *et al*., 1998), the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. In these circumstances, activated macrophages greatly increase their simultaneous production of both nitric oxide and superoxide anions.

Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane. When flavonoids are used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage (Shutenko *et al*., 1999).

Compound 2, a Glucoside of 4,7-dimethoxyl quercetin was found to be active principle responsible for the antidiabetic effects of *M. morindoides* root bark extract.

It should be noted that Xian-Kang *et al*., (2007) investigated the possible mechanism of antidiabetic activity of *Euonymus alatus* and reported that quercetin which significantly improve insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes potentially act at multiple targets to ameliorate hyperglycemia in diabetics mellitus.

Also, it has been reported by Vessal *et al*., (2003), that quercetin, a flavonoid with antioxidant properties brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozocin-induced diabetic rats; thus exerting its beneficial antidiabetic effects.

This study thus proposes that the mechanism of action of antidiabetic activity of *Morinda morindoides* root bark extract is by regeneration of the of pancreatic islets and the improvement of insulin-stimulated glucose uptake due to the activity of the constituent flavonoid in the root bark extact i.e Glucoside of 4,7-dimethoxyl quercetin.

CONCLUSION

In this study, the analgesic, the anti-inflammatory and antidiabetic properties of *T. diversifolia*, *A. wilkesiana* and *M. morindoides* were demonstrated in different models of analgesia, inflammation and diabetes. This study therefore establishes the ethnomedicinal uses of *T. diversifolia*, *A. wilkesiana* and *M. morindoides* as analgesic, antiinflammatory and antidiabetic agents.

RECOMMENDATION FOR FURTHER STUDIES

It is important to know how the active principles of these ethnomedicinal herbs carry out the activity in the body.

So I recommend that the mechanism of action of the isolated bioactive compounds be looked into to be able to make a more globally acceptable scientific assertion of their therapeutic usefulness.

REFERENCES

Abatan M.O. (1992). The toxic effect of six commonly occurring plants in Nigeria pastures. Ph.D Thesis, *University of Ibadan Unpublished.*

Abatan M.O. and Adeagbo S.O., (1986) Studies on the acute inflammatory response induced in the rat hind paw by carrageenan and calcium antagonists. *Animals Technology* 37(3): 231- 238.

Adesina, S.K. (1998): Research and Development into Herbal Medicines, *Nig. J. Nat. Prod. And Med. 2:9-15.*

Adesina S. K., O. Idowu, A. O., Ogundaini, H. Oladimeji, T. A. Olugbade, G. O.Onawunmi M. Pais. (2000). Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytotherapy Res.* 14(5): 371–374.

Addison, R.G. (1984): Chronic pain Syndrome *AM. J. Med*. 77: 54-64.

Akerele, O. (1993). Summary of World Health Organization guidelines for the assessment of herbal medicines. *Herbalgram.* 28:13-16. (Reprinted with permission from Fitoterapia. Volume LXIII. November 2, 1992. 99-110).

Akinde, B.E (1986). Phytochemical and Microbiological evaluation of the oils from the leaves of *Acalypha wilkesiana* In: Sofowora A,

(editor). The state of medicinal plant Research in Nigeria, University of Ibadan Press, Nigeria.:362 363.

Akinyemi K. O, O. K. Oluwa, E. O. Omomigbehin (2006). African Journal of Traditional Complementary and Alternative Medicines Vol. 3, No. 4

Agardh, C.D Bjorgell, P., Nlson, E.P. (1999). The effect of tolbutamide on lipoproteins and lipoprotein lipase and hormone sensitive lipase-Diabetes. *Res Clin Pract Antibiol Bull* 46: 99-108.

Arthur, L.C. (1981). Cooperative Extension University of California *Enviromental Toxicologic Newsletter* vol.1, No.4.

Aikawa, J.A. (1971): The Relationship of Magnesium to Disease in Domestic animal and Humans DILC. Thomas Publication. Springfield.

Ammon HPT, Maek T., and Singah G.B, (1991): Inhibition of leukotriene B4 formation in rat peritoned neutrophils by an enthanol extracts of the gum resin exudes of Bostivelhia Serrata *Planta Medica*. 57:203-7

Ammon HPT, Safayhi H, Mack T (1993): Mechanism of anti-inflammatory actions of Curemine and boswellie acids *J. Ethnopharmacol* 38:143-9.

Ammon HPT, (1996): Salani Guggal Boswellia Sertula from an herbal medicine to a specific inhibitor of leukotriene hiosynthesis. Phytomediine 3: 67-70.

Arora, R.B., S.B. Vohora and M.S.Y, Khan (ed), (1984): Proceedings of the first International Conference on Elements in Health and Disease, World Health Organisation and Institute of History, Medicine and Medical Research, New Delhi.

Augusti K.T., and Joseph P, Babu T.D. (1995): Biologically active principles isolated from salacia oblonga wall. *Indian J. Physiol Pharmacol* 39: 415-417.

Awe, S.O. Olajide O.A., Oladiran, O.O., Makinde, J.M. (1998): Antiplasmodia and antipyretic screening of *Mangifera indica* extract, *Phytotherapia Research* 12 (6) 437-438.

Awe, E.O., Makinde J.M., Olajide O.A., Wahkeel, O.K. Adeoye, A.O. (2005): Evaluation of Analgesic , anticonvulsant and hypnotic activities of *Pyrenacanthia staundtii Af. J. Trad*. CAM 2 (9) 122-128.

Awe, S.O. (1998a) Evaluation of anti-malarial activities of some Nigeria Medicinal Plants using in-vivo and in-vivo techniques Ph. D. Thesis, University of Ibadan.

Awe, S.O. and Makinde, J.M. (1991b): Anti-malaria effect of aqueous stem bark extracts of three *Khaya soecies. Fitoterapia* 62: 467-473.

Avlessi F, Alitonou GA, Sohounhloue DK, Bessiere, JM, Menut C (2005). Aromatic plants of tropical West Africa. Part XV. Chemical and biological evaluation of leaf essential oil of *Commiphora Africana* from Benin. *J. Essen. Oil Res*., 17(5): 569-571.

Babu, T. D., Kultant, G. Padikkala J. (1995). Cytotoxic and anti-tumor properties of certain Laxa of Umbelliferae with special reference to (*Camellia sinensis*) extract on the rat diapharm. *J. Ethopharmacol* 57:197-201.

Badam, L. (1994) La Vitro studies on the effect of glycyrrhizin from Indian *Glycyrrhiza glabra* Linn on some RNA and DNA Viruses *Indian J. Pharmacol* 28: 194-99.

Banerjee S., Prashar R., Kumar A, and Rao A.R. (1998): Modulatory influence of alcoholic extract of Ocimum leaves on carcinogen metabolizing enzyme activities and reduced glulathione levels in mouse. *Nutr Cancer* 25:205-17.

Bardin I. (avarenine) Eschalier (2000): Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. *Pain* 85, 11, 18

Bardin, L, Bardin, H; Lavarenne, J. Eschaher A. (1997a): Effect of intratheal Serotonin on nociception in rats: influence of the pain used Exp. *Brain Res*. 103: 31-87.

Bardin, L., Jourdan, D. Alloui, A. Leavened, J. Eschalier A. (1997b): Differentials influence of two serotonin, 5th receptors antagonists on special Serotonin induced analgesia in rats. *Brains Res*. 765: 267-272.

Barnes, P.J and Adeock I. (1993): Anti-inflammatory actions of steroids: Molecular mechanism. *Trends Pharmacol Sci*. 14:436.

Barrata, M.T. Doraman, H.I.D. Drans, S.G. Pigeuredo A.C. Barroso J.G. Ruberto G. (1998): Autimicrobial and Antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal* 13(4) :235-244.

Belcher, G. Ryall R.W. Schaffner, R. (1978): The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurons-in the cat. *Brain Res*. 151-307-321.

Bertram, G. Katzung (2004a). Botanicals and Nutritional Supplements. Basic and Clinical pharmacology, 9th edition Mc Graw Hill p. 1076

Bertram, G. Katzung (2004b). Pancreatic hormones and antidiabetic drugs. Basic and clinical pharmacology 9th edition Mc Graw Hill pp 706-710

Beesley W. N. (2002). Oxford Concise Veterinary Dictionary Ed: Oxford University Press, London (UK).

Bopanna, K.N., Kanna, J., Sushma, G., Balaraman Rathod, S.P., (1997). Antidiabetic and antihyperlipidemic effect of neem, Kernal powder in alloxan diabetic rabbits. *Ind. J. pharmacol.* 162: 162-167.

Burkill, H.M. (1985). The useful plants of west tropical Africa, Vol 2:197.

Burkill, H.M., (1997). The useful plants of West Tropical Africa. Families M-R. Royal Botanic Gardens, Kew, Richmond, United Kingdom 2, 104-22

Büssing A., G. M. Stein, I. Herterich-Akinpelu and U. Pfüller.(1999). Apoptosis-associated generation of reactive oxygen intermediates and release of pro-inflammatory cytokines in human lymphocytes and granulocytes by extracts from the seeds of *Acalypha wilkesiana (*Mull Arg*) J. Ethnopharmacol*. 66(3): 301–309.

Bucolo, G., David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem*. 19:478.

Calixto, J. B. (2000) Twenty-five years of research on medicinal plants in Latin America. A personal view. *Journal of Ethnopharmacology* 100:131–134.

Carlsson, K.H., Helmreich, J. and Jurnal (1986): Activation of Inhibition from the Periaquadaetal gray matter medicates central analgesic effect of metamizol (dipyrone). Pain 27.373-390.

Carlson, G.P. (1996). Clinical Chemistry tests. In: Smith BP ed. Large Animal Internal Medicine, 2nd Ed Baltimore, Bosby, 463.

Clark, A.M., Jurgens, T.M. and Hufford C.D., (1990) Antimicrobial activity of Jugione *Phytother Res*. 4, 11-14.

Chauhan R. S. and D.K. Agarwal (2006). Text of Veterinary Clinical and Laboratory Diagnosis. Second edition. 201-202.

Chavali, S.R and Campbell, J.B. (1987): Adjuvant effects of orally – administered saponins on humoral and cellular immune responses in mice. *Immunobiology* 174(3) 347-359.

China Cooper Research Group on Qinghaosu and its Derivatives as Antimalarials (1982): Antimalarial efficacy and mode of action of Qinghaosu and its derivatives in experimental models. *J. Tradit. Chin. Med*. 2:17-24

China Cooperative Research Group on Qingbaosu and its Derivatives as Antimalaries (1982): Chemical Studies on quigbaosu (artemisinine) *J. Tradit. Chin. Med*. 2:3-8.

Chopra R. N, Nayguar S. L, Chopra IC (1956). Glossary of Indian Medicinal Plants Council of Science Industrial Research New Delhi, 160.

Chakraborty, A., Chowdhury B.K., Bhattacharya P., (1995): Clausenol and clausenine two carbazole alkaloids from clausena anisata. *Phytochemistry* 40: 295-298.

Chalterjee S. and Das S.N., (1996): Anti-arthritic and anti-inflammatory effect of a poly-herbal drug (Easy (B) is mechanism of action. *Indian J. Pharmacol* 28: 116-119.

Chapman, C.R. Casey, K.L. Dubner, R. Folley, K.M., Gracely, R.H. and Reading, A.E., (1985). Pain measurement, an overview. *Pain* 22:31.

Checke, P.R. and Shull, L.R. (1985). Natural toxicants in feeds and poisonous plants, Westport Conn, AVI Publishing.

Cimanga, R.K., De Bruyne, T., Lasure, A., Quinn, L.L., Pieters,L. (1995). Flavonoid O-glycoside from the leaves of *Morinda morindoides. Phytochemistry* 38, 1301-1303.

Cox, P.A. and Balick, M.J. (1994): The ethnobotanical approach to drug discovery, Scientific American 60-65.

Coppock, R.W. Reynbolds, J.d and Buck, W.B. (1989). Acute aflaxoxicosis in feeder pigs resulting from improper storage of corn. *J Am vet Med 1Assoc.* 195: 1380-1381.

Cornelius, C.E. (1989). Serum enzyme activities and other markers for detecting hepatics necrosis, cholestasis or carcinoma In: *Biochemistry of Domestic Animals*. 4th ed. Academy press, 381-386.

Crocker, C.L. (1967). Rapid determination of urea nitrogen in serum or plasma without deproteinization. *Am J Med. Technol*. 334: 361-363.

Dahanukar S.A. and Kulkarni, N.N. (2000): Pharamacology of Medicinal Plants and Natural Products. *Indian J. of Pharmacology* 32: 81-118.

Dahanukar S.A., Rege N.N., Thatle, U.M., (1997): Adaptogens, In: Ed, Medical plants, the bio-activity, screening and evaluation. Proceedings of the international Workshop CDRI (India) 25: 143-163.

Dahanukar, S.A. and Thatle U.M., (1997): Current status of Ayurieda in *Phytomedicia Phytomedicine* 4: 559-568.

Drake J.J., Butterworth K.R., Gaunt I.F., Hooson J.,Evans J.G Gangolli S.D. (1982).Long-term toxicity study of quillaia extract in rats. *Food Chem Toxicol*. 20 (1):15-23

Dhuley, J.N., (1998): Therapeutic efficacy of Ashwagandha against experimental aspergilosis in mice. *Immunopharmacol Immunotoxicol* 20: 191-198.

Dubner R and Bennellt, G.J., (1983): Spinal and Trigeminal mechanisms of nociception. *Ann. Rev. Neurose*. 6:384-418.

Dubner, R. Sumino R and Wood, W.I. (1975): A peripheral 'cold' fibber population responsive to innocuous and noxious thermal stimuli applied to monkey's face. *J. Neurophysiol*. 38: 1373-1379.

D"Arcy P.F. (1991). Adverse reactions and interactions with herbal medicines, Part 1, *Adverse Drug Reactions and Toxicology Reviews*.10 (4) 189-208.

Dobrowoski, J.W. and S.P. Vohora (1987): New Horizons of Health Aspects of Elements IHMMR, New Delhi.

Douidar S.M., Richardson, J., Snodgrass W.R., (1988): Role of indomethacin in ducts closure: An update evalution, *Dev. Pharmacol Ther* 11:196.

Elangovan, V., Shammamoorthy, N., Nalasu-bramanian S., *et al* (1994) Studies on the anti-proliferative effect of some naturally oocuring broflavanoidal compound against human carcinoma of larynx and sarcoma-180 cell lines. *Indian J. Pharmacol*: 26: 6-9.

Elford, B.C., Roberts, M.F. Philipson, J.D. and Wilson R.J.M (1987) Potentiation of the anti-malaria activity of qinghnosu by methoxylated flavones. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81: 434-640.

Edmond, J. (1987): The physiology and pain (review). *Progress in Neuro psychopharmacology and Biological Psychiatry* 7: 413-483.

Eisenberg D. N, Kessler R. C, Foster C, Norlock E. E, Calkins D. R, Delbano T. L. (1993). Unconventional medicine in the United States: Prevalence, costs and patterns or use. *New Engl. Med. Journal* 328: 246-252.

Eggstein, M. kreutz, F. (1996) Klin wsv=chhr. 44:262-267.

Expert Committee on the Diagnosis and classification of Diabetes mellitus (2002): report Theexpert committee on the diagnosis and classification of diabetes mellitus. Diabetes care;25 (Suppl 1) : S5.

Edward F. Gilman (1985). Useful plants of West Tropical Africa. 1003-1005.

Elujoba, T. (2005) Book Review "Traditional Medicinal Plant and Malarial". *African Journal of Traditional, Complementary and Alternative Medicines* 2 (2): 206-207.

Elizabetsky, E. Amador, T.A. All-querque, R.R. Nunes, O.S. Carvalho, ACT (1995): Analgesic activity of Psyenotria Colorate (Wildes R & S) Muell Arg. Alkaloids. *Journal of Ethnopharmacology* 48:77-83.

Farnsworth, N.R. (1990): The role of ethnopharmacology in drug development. In Bioactive compounds from Plants (Edited by Chadwick D.J. and Marh J.) Wiley Ghichester.

FAO (1994): A Systemic approach to tsetse and trypanosonomiasis control. Proceedings of the FAO Panels of Experts, Rome, 1-3 December 1993. *FAO Animal Production and Health paper* No 121.

Farnsworth, N.R. and Bingel, A.S. (1977): New Natural Products and Plants Drugs with Pharmacological, Biological or Therapeutic

Activity (Edited by Wagner H and Wolff, P.) Spronger, New York 61-73.

Fernandez, E., Boyle, G.J. (2001) Affective and evaluative descriptors of pain in the McGill Pain Questionnaire: reduction and reorganization. *J Pain. 2(6):318-25. Erratum in: J Pain. 2002 Feb;3(1):69. Corrected and republished in: J Pain. 2002 3(1):70-7.*

Flymn D.I., Rafferty M.F (1986): Inhibition of human neutrophil 5-lipoxygenase activity by geingerehone, shogaol, capsaicin and related pungent compounds. *Prostagladins Leukotr Med* 24: 195-198.

Fields, I.L, Bryed Bental, L. and Zeraum (1983): The activity of neurous in the roasted redulla of the during it rewal from noxious heat J. Neurosci 545-452.

Friendwald, W.L., levy R.I., Fredrickson, D.S. (1972). Estimation of the concentration of low denswity lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem*. 18:499.

Florianni A,E, O; Treira J., Sandos A.R. S. Delk- Monachie E, Yunes, R.A. Ceehir Fiho; V. (1998). Analgesic Compounds from Etidendmum cosmic Stems Pharmazic 53(6): 126-127.

Fujioka, T. Kashiwada Y, Kilkuskie R.E. Cosentino L.M. Ballas L.M Jiang J.B. Jansen W.F., Chem I.S. Lee K.S. (1994): Anti-aids agent,

11 Betulinic acid and platonic acid as anti-HIV principles from Syzigium claviflorum, and the anti-HIV activity of structurally related tritepenoids. *J. Nat. Prod* 57: 243-247.

Galeffi, C. (1980). New trends in the separation of active principles from plants. *Journal of Ethnopharmacology*. 2(2): 129-134.

Gaunch, H.G. (1971): Inorganic Plant Nutrition, Dowden, Hutchinson and Ross, I.O. Stroudsburg Pa. U.S.A.

Gathumbi, P.K., Mwangi, J.M. Megera C.M., and Njire S.M. (2000): Biochemical and Haematological Changes Medicated by a Chloroform Extract of Erunis African stem bark in rats. *Pharmacol Bidej* 28(5): 374

Ganguli S. Hota d., Coel R.K. et al (1996): Neuro-pharmacological studies on Fusarium toxin 111. Neurochemical investigation on total toxin extract from F-Monilifrome and F. Oxysporum. *Indian J. Exp. Biol*. 34:531-534.

Geerts S, Holmes PH (1998). Drug management and parasite resistance in Bovine Trypanosomiasis in Africa. *Food and Agricultural Organisation Document Repository*, 31.

Goffin, E., Zierns, E., De Molps de Madureira, M.C., Martins, A.P., da Cunha A.P., Philippe, G., Tits, M., Angenot Angenit L., Frederich, M., (2002): In vitro anti-plasmodial activity of *Tithonia diversifolia*

and identification of its main active constituent: Targitinin C Planta Med. 68 (6): 543-545.

Golden, M.H. (1988): Trace Elements in Human Nutrition, Hum, Clin. Nutri. 6:488-455.

Godwin LG, Green DG, Guy MW and Voller A (1972). Immunosuppression during trypanosomiasis. *Br. J. Exp. Path.* 53:40-43.

Goodman and Gilman (1996). Opioid analgesic and antagonists: in the pharmacological basis of therapeutic 9th Edition 529-537.

Grumbach, L. (1966). The production of analgesic activity in man by animal testing. Inkinghton R.S., Dunke, P.R. (Eds). Fain Little brown Boston massachysells 163-182.

Gutteridge W.E. (1985). Existing Chemotherapy and its Limitation. *Br. Med. Bull*. 41:162-168.

Hamburger, M. and Hostellmanu, K. (1991): Bioactivity in plants. The link between. *j*30:3864-3874.

Harborne, J. B. 1998. Phytochemical Methods, Guide to Modern Techniques in Plant Analysis, 3rd. Edition, Chapmann and Hall, London. 42-58.

Harks G.W. and Hoskin, P.J. (1987): Opioid analgesics in the management of pain patients with cancer; A review Palliative Med 1:1-25.

Hardison, W.G.M., Weiner, R.G., Hattoff, D.E., Miyaki, K. (1983). Bile acid and Bilirubin.Hepatology. 3;383.

Harnack, L.J, Rydall, S.A, and Stang J. (2001) Prevalence of use of herbal products by adults in the Minneapolis/St. Paul, Minn, metropolitan area. *Mayo Clin Proc* **76:**688–694.

Havesteen, B. (1983). Flavanoids,a class of natural products of high pharmacological potency. Biochem. Pharmacol. 30: 1141-1148.

Herbert, W.J., and Lumsden, W.A., (1976): *Trypanosome brucei*A trapid matching method for estimating the host's parasitaemia ExpH. Parasitol, 40:427-431.

Hess, S.M., Miloni R.C. (1972): Assay for anti-inflammatory drugs in: Lepow, L.H. Ward, P.A. (Eds) Inflammation, Mechanism and Control Academic Press New York pp. 1-12.

Huk I, Brovkovych V, Nanobash VJ, et al. Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. British Journal of Surgery 1998; 85:1080–5.

Huang Z.S. and Wang Z.W.(1998). Study on emodin in plant distribution and Pharmacological research, International Journal of Oriental Medicine. 23(4) 177-182

Hirano, T., Kokka and M. Akiba (1989). Effects of synthetic and natural flavonoids on Na, K-ATPases: Aspects of the structure activity relationship and action mechanism. Life Sci.,45:1111-1117.

Holaday, J.W. (1985); Endogenous opioids and heir receptor.Upjohn Kalamazoo.

Ho, P.C. and Orange R.P. (1978): Indireact evidence of phospholipase A activitation in purified rat mast cells during reverse anaphylactic challenges EASEB Proceedings 37:1667.

Homewood, C.A., J.M., Jewsbury and M.L. Chance (1972): The pigment formed during haemoglobin digestin of malarial and schistosomal parasites. Comp. Biochem. Physiol. B. 43:517-523.

Ibrahim M. A., Nwude N., Ogunsusi R. A., Aliu Y. O. (1984). Screening of West African plants for antihelminthic activity. ILCA Bull. 17:19-23.

Jacobs, N. and P. VanDen Mark, (1960):Arch. Biochem. Biophys. 88:250-255.

Iga, T. and klassen, C.D (1982).bile Acids in rats Biochem pharmacol. 31:205.

Iggo A., (1982): The physiological approach to pain and pain relief. Proceedings of the association of veterinary Anaesthetists 10 (supplement) 32.

Ismail T. S., Gopalakrishman S., Hazena B.V. (1997) Biochemical modes of action of Cimelina in inflammation. Indian J. Pharmacol 29 306-9

Iwu, M.M. (1996): Development and Production of Herbal Medicines. Book of Abstracts. XXIIIRD. Internation Conference of the West African Society for Pharmacology PP 1-2.

Iwu, M.W. 1993Handbook of African Medical plants.CRC press, Boca Raton, FL.

Iwu, M.W., Duncan, A.R. and Okunji, C.O. 1999New antimicrobials of plant origin. Pp 457-462. In. J. Janick (Ed). *Perspectives in New Crops and New Uses,* ASHS Press, Alexandria V.A.

Jendrassik, L. and Graf, P. (1938).Vireinfacilite photometrische. Methoden zur Bestimmung des Blutbilirubin Biochem Z: 297- 81.

Jekayinfa A. O., A. O. George and K. T. Jaiyeoba. 1997. *Acalypha wilkesiana*: preliminary in vitro microbiological and clinical trial on dermatitis. Afr J Health Sci. 4(1): 39–42.

Johri R.K., Singh S., Kaul B. (1995): *Vernonia lasiopus* and *Vernonia galamensis.* A medicinal perspective Res. Ind. 45:327-8.

Kanko, C. Sawaliho, B.E.H . Kone, C G. Koukoua and Y.T. N'Guessan (2004). Étude des proprieties physico-chimiques des huiles

essentielles de *Lippia multiflora*, *Cymbopogon citratus*, *Cybopogon nardus*, *Cymbopogon giganteus*. *C. R. Chemie* **7**, 1039-1042.

Kaoru Kinoshita, Michico Akiba, Massaki Saitoh, Yang. Ye, Kiyotaka Koyama, Kunio Takahas hi, Norio Kondo and Hirosi Yuasa (1998): Antinociptive effect of triterpenes from cacti. Pharmaceutical Biology 36(1) 50-57.

Karunakar N. Pillai K.K. Husan S.Z. Rao M. (1997): Investigations of anti-inflammatory activity of Jigrine. Indian j.Physiol Pharmacol. 41:134-8.

Kashiwada, Y; Wang huikang; Nagao, T; Kitanaka, S., Yasuda, I., Fujioka, T., Yamagisli, T., Cosentino, L.M. Kozuka, M., Okabe, H., Ikeshiro, Y., Hu chanqgi; Veh E., Lee Kuohsiung: (1998): Anti-AIDs agents, 30, anti-HIV activity of Oleanolic acid, promolic acid, structurally related triterpenoids.

Katiyar C.K., Brindavanam N.B. 11 Tiwar P. Narayana DBA. (1997) Immunomodulatory products from ayuveda current status and future protectives.In SN Upadbyay ED immunomodulation, Narosa publishing House, New Delhi 141-8.

Kambu, K (1990): Elements de phytotherapie compance plants medicinales Africanes CRP-Kinshasa 20-22, 38-39, 40-41, 51-62.

Kamuanga, M. (2003) Socio-economic and cultural factors in the research and control of Trypanosomiasis pp. 1-10.Information division FAO, Rome.

Kazi T.G., T.O. Ansari and G.H. Kazi (1999): ACGC Chemical Research Communication. 9:51-56.

Kaylaap, S.O. (1998): Rasyonel Tedaci Tibbi Farmakoloji Sekizinci Baski. Cilt 2.Pp 991.

Keel, C.A. (1969): Clinical and pathological aspects of Kinins in man Proceedings of the Royal Society of Biology 173:361-369.

Kiruchi F., Iwakami S., Shibuya M., Hanaoka F., Sankawa U (1992): Inhibition of prostaglandin and leucotriene biosynthesis by gingeerols and diaryheptanoids. Chem Pharm Bull 40:387-391.

Klayman, D.L. (1993): Artemisia annua from weed to respectable anti-malaria plant. In: Human medicinal Agents from plants (Edited by Kinghorn, A.D. and Balandrin, M.F) American Chemical Society Pp. 242-255.

Kujubu D.A., Herschman H.R. (1992): Dexamethasone inhibits the mitogen induction of TES10 prostragladin synthase cyclooxygenase gene. *J. Biol. Chem*. 267-7991.

Kulkarni S.K., Ninan (1997): Inhibition of morphine tolerance and dependence by *Withania sominafera* in mice. J. Ethnopharmacol 57:213-7.

Kumar A., Singh R.K. (1996): Effect of *Pongamia pinnata* on perturbed central cholinergic makers of cognition in experimentally demented rats. D. Basic APPC Biomed 4:43-8.

Kumar V.I, Basu N. (1994): Anti-inflammatory activity of the latex of Calotropis procera. J. Ethnopharmacol 44:123-5.

Kuzoe FAS (1993): Current situation of African Trypanosomosis. Acta Tropica 54:153-162.

Kokko, J. P. (1984). Site and mechanism of action of diuretics. American Journal of Medicine, 77: 11-17.

Kurumbal R.C., Steve A.M. and Giesse J.K. (1996): Structural basis for selective inhibition of cox-2 by anti-inflammatory agents. Nature 364:644-648.

Le strange, R. (1977). 'A History of Herbal Plant". Angus and Roberson London.

Lecomte M., Lancuvilic O. Jic (1994): Acetylation of human endoperoxide synthase-2 by aspirin J. Boil Chem 269:13207-13215.

Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J, Buscher P (2002). Treatment of human African trypanosomiasis present situation and needs for research and development. *Lancet Infect. Dis*. 2: 437-440.

Linder M.C. (1991): ed Nutritional Biochemisty and Metabolism 2nd ed Elsciver.

Lima, T.C. DE: Klueger, P.A. Pereira P.A. Macedoneto, W.P. Morato, A.S., Farias, M.R. (1998): Behavioural effects of crude and semi-perfied extracts of *Syzygium cumini* Linn Skeels. Phytotherapy Research 12 (7) 488-493.

Liu, K.C.S.C., Yang Roberts, M.F. Efford, B.C and Philipson, J.D. (1992) antimalaria activity of *Artemisia annua* flavonoids from whole plants cell culture Plant cell Reports 11:637-640.

LIU Ju-Chi ; HSU Feng-Lin ; TSAI Jen-Chen ; CHAN Paul ; LIU Jenny Ya-Hsin ; THOMAS G. Neil; TOMLINSON Brian ; LO Ming-Yu ; LIN Jung-Yaw.(2003) Life sciences, vol. 73, n°12, pp. 1543-1555.

Loci, A.S., Shabha, M., Khazraji, A. L., Husain, A., Twaija, A, (1994). Hypoglycemic effect of valuable extract of artemisia herb. Alba II. Effect of valuable extract on some blood parammeters in diabetic animals. *J Ethnopharmacol*. 43: 167-171.

Loeb, W.F. (1989). In Human Risk Assessment-The Roles of Animals selection and Extrapolation (M.V. Roloff. Ed): p81-86. Taylor and Francis, N.Y.

Lyer M.B. Pal Sc. Kasture, V.S. Kasture S.B. (1998): Effect of *Lawsonia inermis* on Memory and behaviour mediated via menoamine neurotransmitters. *Indian J.Pharmacol* 30:18-5.

Madureira Mdo, C., Martins, A.P, Gomes, M, Paiva, J. Cunha. A.P, and Rosario, V (2002): Antimalaria Activity of Medicinal plants used

in traditional Medicine in Sao Tome and Principle Islands. *Journal of Ethnopharm*. 81:1, 23-29.

Makinde, J.M and Obih P.O. (1985): Sreening of *Morinda lucidal* leaf extracts for antimalarial action on plasmodium berghei in mice. African Journal of medicine and medical science Vol. 14 No Y2 pp 54-63.

Makinde J.M. Adesogan E.K. and Amusan C.O (1987): The Schizontocidal activity of *Spathodea campanulata* leaf extract on plasmodium berghei in mice. Phytochemical Research Vol. 1 No 2 pp 65-68.

Makinde J. M., Obih 'O and Jimol A.A. (1987): Effect of *Solanum erianthum* aqueos leaf contracts on plasmodium berghei in mice Vol. 16 pp 193-196.

Makinde *et al*, J.M., Awe S.O. and Agbedehunsa J.M. (1988): Effect of *khaya grandifoliola* extract on plasmodium berghei berghei in mice Phytotherapy Research Vol. 2 No 1 pp 30-32.

Makinde, J.M., Adesogun E.K., And Amasa O.O. (1988): Anti-malarial activity of *Spathodea campanulata* stem bark extract on *Plasmodium berghei berghei* in mice. Phytotherapy Research Vol. 2 No 1 pp 30-32.

Makinde J.M., Amusan O.O and Adesogan E,K. (1990): The antimalarial activity of chromatographic fractions of stem bark extract against

Plasmodium berghei berghei in mice. Phytotherapy Research Vol. 4 No 2 pp 53-56.

Makinde J.M, S.O. Awe and Alako L.A. (1994): Seasonal variation in the antimalarial activity of *Morinda lucida* on *Plsmoduim berghei bergheiinh* mice. Fitotherapia Vol.1 No 2 pp. 124-130

Machaomei; Nakamura, N. Miyashiroh; Hattori M. shimotohno, K. Inhibitory effects of ursolic acid derivatives from Cyanomorium songaricum, and related tritepenes on human immunodeficiency viral protease.In Proceedings of the second International symposium on natural drugs, Maretea, Italy; 28 Sept-1 October, 1997.

Malhora, V.K. (1987). Metabolism of lipis. Biochemistry for students: 10th ed. *Jaypee Press*. India. P. 159

Makwana H.G. Ravishankar b., Shekia V.J. *et al*.,(1994): General Pharmacology of *Vitex leucoxion* linn leaves Indian. J. Physiol. Pharmacol. 38:95-100.

Manobjyoti, Bordoloi. Barua-N.C., Ghosh, A.c. and Bordoloi, M. (1996): An arteminisinic acid analogue from Tithonia diversifolia. Phytochemistry 41:2. 557-559.

Manocha A., Pillal K.K., Hussein Z. (1996): Influence of *Gingo biloba* on the effect of ant convulsants Indian. J. Pharmacol 28:84-7

Manocha A., Pillial K.K., Hussein Z. (1997): Effect of *Ginko biloba* in Chemishock in mice. Indian K. Pharmacol 29:200.

Martins DO Monte F.H, Guilherme dos Santos J, Russi M, Lanziotti VMNB, Leal LKAM, Cunha GM, (2004). Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L in mice. Pharmacol. Res. 49: 239-243.

Mayer, D.J. Wife T.L., Akil, H., carder, Blieberskind, J.C., (1971): Analgesia from electrical stimulation in the brainstem of the rat. Science 174, 1351-1354.

Mayfield, J., (1998). Diagnosis and classification of diabetes melltus: new criteria. An Fam Physician. 58: 1355.

Mazzaanti, G., Battinelli, L., Salavatone, G., (1998): Anti-microbial properties of the Linalool-rich essential oil of Hyssopus officinalis, *Lvar decumben*, (Lamiaceae). Flavour and Fragrance Journal 13(5) 289-294.

Mazumber. U.K., Malaya Gupla, Nandeta Ruth (1998): CNS Activities of *Casia Fistula*in Mice Phytotherapy Research 12(7) 520-522.

McCormark, K., and Burne, K. (1991): Dissociation between antinociceptive and anti-inflammatory effects of the non-steroidal anti-inflammatory drugs. A survey of their analgesic efficacy.Drugs 41, 533-517.

McQueen, D.S., Iggo A., Birmel G.I. et al (1991): Effects of paracetamol and aspirin on neural activity of joint mechanociceptors in adjuvant arthritis, British *J. Pharmacol*, 104, 178-182.

Mereto, E., Ghia, M.and Brambilla, G.(1996): Evaluation of the potential carcinogeni activity of Senna and Cascara glycosides for the rat colon. Cancer Letters. 101 (1) 79-83.

Menut,C. . Lamaty,G. Bessière J.M, Koudou J.and. Maidou, J (1995). Aromatic plants of tropical Central Africa. Part XVII. 6,7-Epoxymyrcene, the major unusual constituent of *Lippia multiflora* S.l. Moldenke essential oil from the Central African Republic. *Flavour Fragr. J.* **10**, 75-77.

Metaallfe D.D., Kaliner M. and Donton M.A. (1981): The most cell CRC critical Review of immurology 3:23-74.

Melzack, Rad Wall, P.O. (1965): Pain Mechanisms A new theory Scient 150:972-972.

Millan and Colpact (1991): 5-Hydroxytrptamine (ITT) A Receptors and the Tail-Flie's Response 11. High Efficacy 5-HTA Agonists Attenuate Morphine-Industry Aptinociception in Mice in a Competitive like Manner, Pharmacol Exp. Ther. 256(3), 983-992.

Mitchell, C.I. (1964): A comparison of drug effects upon the jaw jerk response to electrical stimulation of the tooth pulp in clogs and cats. *J. Pharmacol* Exp. 146: 1-6.

Mitchell, J.A., Akarqsereenont P. Thiemermanne (1994): Selectivity of non-steriodal anti-inflammatory drugs as inhibitors of constitinitive

and inducible cyclooxygenase. Pro. Natl Acad. Sci U.S.A. 90: 11693- 11697.

Mitra S.K. Chrakrabort A, Bhatterya S.K. (1996): Neuropharmacological studies on *Panax ginseng*. Indian J. Exp. Biol. 36:187-91.

Montagnae, A.J., Provost, M., Litaudon, M., and M., Pais (1997): Planta medical 63: 305-306.

Morat H. (1985): The inflammatory reaction Elsvier. Amsterdum pp. 1-8

Moncade. S. Gryglewski R., Bunting S., and Vane J.R. (1976): an enzyme isolated from arteries transforms prostaglandin endoperoxide to an unstable substance that inhibits platelets aggregation nature 263:663.

Miura, T. Furuta, K, Yasuda,A., Iwamoto. N; Kato,M., Ishihara, E., Ishida, T.and .Tanigawa,K. (2002): Antidiabetic Effects of Nitobeniku in KK-Ay Diabetic mice. Am.J. of Chinese Medicine. 30:1, 81-86.

Mukherjee P.K. Suhak, Pal, M Saha B.P., (1997): Studies on the anti-inflammatory activity of rhizomes of Nelumbo nucifera. Planta Med. 63:367-9.

Mykherjee R.K., Saha K., Balasubramaniar R., *et al*., (1996): Studies on psycho-pharmacological effects of Nelumbo meifera Gaertn. Rhizome extract. D. Ethnopharmacol 54:63-7.

Muyibi S.A., Olorode B.R., Onyeyili P. A., Osunkwo U.A., Mohammed B.Y., Ajagbonna (2000): Haemotological and histopathological changes of *Cassia occidentalis* leaf extract in rats. Nig. J. Nat. Prod. Med. 4: 48-52.

Naclero R.M., Baroody F.M., and Togias A.C. (1991): The role of leukotrienes in allergic rhinitis: a review. American Review of Respiratory Discase 143:591-595.

Nakano M., Kurokawa, M., Hozumi, T., Salto, A., Ida, M., Morohashi M., Namba. T. Shiraki, K., (1998): Suppression of recurrent genital herpes simplex virus type 2 infection by Rhus javanica in guinea pigs Anti-vival Research 59(1) 25-33.

Nair A., Bihide S.V., (1996): Antimicrobial properties of different parts of *Sernecarpus anacardium*. Indian Drugs 33:323-8.

Narayanan N. Thirugnasambantham. P., Vismanathan S., et al. (1998): Antinociceptive anti-inflammatory and antipyretic effects of ethanol extract of Clerodendren Serratum roots in experimental animals. *J. Ethnopharmacology* (accepted for publication).

Navarro, V., Rojas G., Delgado., G. Lozoya, X. (1998): Antimicrobial compounds detected in *Bocconia arborea* extracts a direct bioatographic method. Archives of medical Research 29(2) 191-194.

Nagappa, A. N., Thakurdesai, P. A., Venket Rao, N., Singh, J. (2003). Antidaibetic of *Terminalia catappa* Linn fruits. *Journal of Ethnopharmacol*. 88: 45 – 50.

Newal, C.A., Auderson L.A. and Philipson A.D. (1986): Berb medicine a guide for health care professionals. *Pharmaceutical Press*. 296.

Nwude N.(1986). Veterinary aspects of medical plant research in Nigeria. In: state of medicinal plant Res. Nig . Ed. Sofowora A. p. 197.

Nwude N., Ibrahim M. A. (1980). Plant used in traditional veterinary medicinal practice in Nigeria. *Journal Vet.Pharmacol. Therap*. 3:261.

Noomrio, M. H. and M. U. Dahot, (1996). Nutritive values of *Eugenia jambolana* leaf decoration on rat streptozotocin diabetes. *Braz J Med Biol Rs*. 34: 389 – 395.

Nossslin, B. (1960). The direct diazo reaction of bile pigments in serum. Scand J. Clin Lab Invest. (supp. 49) 12: 1 – 4.

Ohigashi H, Takanmana H., Koshitaizu H, Tekuda H., Ito, Y. (1986): Search for possible antitumor promoters by inhibition of 12-O-tetradecanoyiphorbol-13-acetate- induced. Epstein-Barr virus activation: ursolic acid and oleanolic acid from an anti-inflammatory Chinese medicinal plant, *Glechoma hederaceae* L., Cancer Lett. 30: 143-151.

Olajide O.A., Makinde, J.M., and Awe, S.O., (1999c): Effect of the aqueous extract of *Bridelia ferrugina* stem barkon Carrageenan-induced oedema and granuloma tissue formation in rats and mice, Journal of ethnopha macology 66:113-117.

Olajide O.A., (1998): Evaluation of Extracts and some Chromatographic Fractions of Morinda Lucida and *Khaya Grandiofoliola* for Pharmacological Properties M.Sc Thesis, University of Ibadan, Ibadan, Nigeria.

Olajide, O.A., Makinde J.M., and Awe, S.O. (1999c); Effect of the Aqueous extract of *Bridelia ferruginea* stem bark on carrageanan-induced oedema andgranuloma tissue formuation in rats and mice. Journal of Ethno-pharmacology 66:113-117.

Olajide, O.A., Makinde, J.M. Okpako, D.T., and Awe, S.O. (2000): Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of Bridelia Ferruginea stem bark. Journal of Ethonopharmacology 71:153:160.

Olajide, A.O, Makinde J.M. and Okpako D.T., (2000) Evalution of the anti-inflammatoryproperties of the extract of combretum micrenthum G.Don (Combaretaceae) *Inflammiopharmacology* Vol. H No3. Pp. 293-298.

Okpanyi S. N., Ezeuku G. C. (1981). Anti-inflammatory and antipyretic activities of *Azadirachta indica*. Plant Med. 41:34.

Oladunmoye, M. K. (2006). Comparative Evaluation of Antimicrobial Activities and Phytochemical Screening of Two Varieties of *Acalyphawilkesiana.*Trends in Applied science Research 1 (5):538-541, 2006.

Oladunmoye,M. K. (2006) .Effect of concentration on the rate of killing of some microorganisms and haemolytic activity of two variety of *Acalyphawilkesiana.* Reseach Journal of Microbiology 1 (5): 453-457, 2006.

Olugbenga Morebise, Fafunsho Michael A. Makinde Modupe J: Olumayokun A. Olajide and E.O. Awe (2002): Anti-inflammatory property of the leaves of Gongronema Latifolum. Phytotherapy Res. 16: 875-877.

Oluyemi A (1998): *Bridella ferrugenea*. Medicinal plants and their therapeutic uses in South-Western zone of Nigeria.*Technical report of Nigeria, 101.*

Oliver-Bever. B. (1986): Medicinal Plants in Tropical West Canbridge University Press, New York Pp. 82-83.

Olugbemiga Morebise, Fafunsho Michael A. Makinde Modupe J: Olumayokun A. Olajide and E.O. Awe (2002): Anti-inflammatory property of the leaves of *Gongronema Latifolum*. Phytotherapy Res. 16: 875-877.

Olugbenga Morebise, E.O., Awe, J., Modupe Makinde, Olumayokun A., Olajide (2000): Evaluation of the anti-inflammatory and analgesic properties of *Chasmanthera dependens* leaf methanol extract Filoterapia 7:197-502.

Okiemy-Andissa, N., Miguel, M. L., Etou, A. W., Ouamba, J. M., Gbeassor, M, and Abena, A. A. (2004). Analgesic Effects of Aqueous and Hydroalcoholic Extarcts of three Congolese Medicinal Plants: *Hyptis suaveolens, Nauclea latifolia* and *Ocimun gratissimum* Pakistan Journal of Biological Sciences (9): 1613 – 1615.

Okolo, C. O., P. B. Johnson and E. M. Abdurahman (1995). Analgesic Effect of I*rvingia gabonensis* stem bark extract. J Ethnopharmacol, 45: 125 – 129.

Omamota, H., Ucgigata, Y., Hiroskitchan, (1981). STZ and alloxan induced DNA strand breaks and poly (ADP ribose) synthatase in pancreatic islet. Nature. 298: 284 – 286.

Oyelami O. A., O. Onayemi, F. A. Oladimeji, A. O. Ogundaini, T. A. Olugbade and G. O. Onawunmi. 2003. Clinical evaluation of Acalypha ointment in the treatment of superficial fungal skin diseases. *Phytother. Res*. 17(5): 555–557.

Onyeyili RA, Egwu GO (1995). Chemotherapy of African trypanosomiasis: A historical review. *Protozoology Abstracts*. 5:229-243.

Osma AS, Jennings FW and Holmes PH (1992) The rapid development of drug-resistance by *T. evansi* in immunosuppressed mice. *Acta Tropica* 50: 249-255.

Oyewole I.O, Ibidapo CA, Moronkola DO, Oduola AO, Adeoye GO, Anyasor G.N and Obansa JA (2008) Anti-malarial and repellent activities of *Tithoniadiversifolia* (Hemsl.) leaf extracts. *Journal of Medicinal Plants Research* 2(8): 171-175.

Panter, K. E. and James, L. F. (1990). Natural Plant toxicants in milk: a review. J. Anim. Sci.68: 892 – 904.

Padmaja V., Thankamany V., Hara N *et al*., (1995): Biological activities of *Amona glabra. J. Ethnopharmacol*. 48:21-24.

Pal, B.C., Achari B., Oshinava, K., Arihara S., (1996): Saponins from Albizia lebbeek, Phytochem 38:287-91.

Pandarinnnathan Chithra,, Sajittalai G.B., Gowrl Chandrakasan (1998): Influence of vera on collegen turn over in healing of dermal wounds in rats. Indian journal of Experiment biology.

Pahor, M.(1995). Age and laxative use in hospitalized patients, Aging (Milan);7(2):128- 135.

Pascual, M.E. Slowing, K Carretero, E Sánchez Mata, D and Villar A(2001). Lippia: traditional uses, chemistry and pharmacology: a review. *J. Ethnopharmacol.* **76**, 201-214

Philipson, J.D., (1994): New products as drug. Transactions of the Royal Society of Tropical Medicine and Hygiene 88 (Suppl.t) 17-19.

Pini L.A., Vitale G., Ottani A., *et al* (1997): Naloxone reversible antinoceptive by paracetamol in rat. *J. pharmacol* Exp. Thera 286 (2) 934-940.

Plotnikov M.B., Koltuniv A. A., Aliev O.I., Kalinkina G.I., Berezovskaya T.P., Andreeva V.T.U., Tikhonova L.A. (1998): The haemorrheological properties of extracts from some plants containing flavonoids Rastitel'nye Resury 34: 87-91.

Popov, A.M., Malenovskaya, G.V., Uvarova, N.I., Antopkina, L.N., Loenko, Yu, N., Artyukov, A. A., Elyakov,G.B., (1998): Neuroprotective activity of triterpene glycosides and the preparation Ginsolaar developed on their basis Doklady Biochemistry 358 (1/6) 4-6.

Price, K.R . Johnson, I.T. and Fenwick G.R.(1987). The chemistry and biological significance of saponins in foods and feedingstuffs. 26(1)27-135

Pooley E. (1998). A Field Guide to Wild Flowers: Kwazulu-Natal and the eastern Region. Natal Flora Publications, Durban

Principe, P.P., (1989): The economic Significance of Plantsand their Constituents drugs. In: Economic and Medicinal plants Research, Vol 3 (Edited by Wagner, H., Hikino, H., and Farnsworth N.R) London Academic Press Pp. 1-17.

Rafathullah, G., Galac, A. M., Yhaya, M. A. and Al-Sadi, M. S. (1995). Gastric and duodenal anti-ulcer and cytoprotective effects of *Aframomum melegueta* in rats. International Journal of Pharmacognosy, 33 (4): 311 – 316.

Ral M.K., (1996): In vitro evaluation of medicinal plant extracts against pestacotiopsig Magiferae Hindustan Abtiblot Bull. 38:53-6

Rao, K.S., Mishra S.H., (1997): Anti-inflammatory and hepate protective activities of *sidarhom bifolia* Linn. Indian J. Pharmacol. 29:110-6.

Ray Sahelian, M.D. (2005). Antispasmodic saponins from bulbs of red onion, *Allium cepa* L. var. Tropea.J Agric Food Chem. 23;53(4):935-40.

Reichstein T. (2008). Encyclopaedia Britannica.*Ultimatereferencesuite.*Chicago: Encyclopaedia Britannica.

Richmond, N., (1973). Clinical Chemistry, 19: 1350-1356

Recio, M.C., Giner R.M., Menez S., Rois J.r. (1995): Structural requirements for the anti- inflammatory activity of natural tritepenoids, Plants Med. 62:182-185.

Rendal, J., (1984): American Health 3:37 cited in Cur. Sci 54:364.

Rec. GSCC (DGKC). 1972. Optimized standard colorimetri methods . J . Clin. Chem. Clin Biochem.10: 182.

Reitman, S.and Frankel, S. (1957). A colorimetric method of determination of serum glutamic oxaloacetic and glctamic pyruvic transaminases.Amer J Clin path 28: 52.

Rihoux, J.P., Mashah, J., Bereziat G., and Konig W., (1997): G proteins as biological targets for anti-allergic drugs. International Archives of Allergy and immunology 113:339-41.

Ringler, D.H.and Dabich, L. (1979). The laboratory Rat (H. J. Baker., J.R. Lindsey and S.H. Weisbroth eds).vol.1:105-123. *Academic press N.Y.*

Rungeler, P., Lyss, G, Ceastro, V., Mora., G., Pahl H.I., Merfort L., (1998): Study of three sesquiter pene Lactones from *Tithonia diversifolia* on their inflammatory activity using the transcription factor NF-KB and enzyme of arachidonic acid pathway as targets Planta Medica 64(7) 588-593.

Robinson, C.H., M.R. Lawler, W.L., Chenoweth and A.E. Cawick (1986). Diabetes mellitus In: Normal and therapeutic nutrition, seventh edition, 504-519, McMillian Publishing Co New York. U.S.A.

Rossen, G.M. Pou S., Ramos C.L., Cohen M.S., and Britigan B.E. (1995): Free radical and pnagocytic cells, FASEB, Journal 9:200-209.

Royal J.A., Kooy N.W., and Beckman J.S. (1995): Nitric oxide related oxidants in acute lung injury, New horizons 3:113-122.

Roth G.J., Majerus P.W., (1975): The mechanism of the effect of aspirin in human platelets 1: acetylation of a particulate fraction protein J. Chin. Invest 56:624-632.

Salemink, C.A. (1980) Problems involved in structure determination of active principles of plants used in traditional medicine: extraction, separation and determination of characteristics of active principles. *Journal of Ethnopharmacology* 2 (2): 135-43

Safayhi H., Sailer E.R., Ammon H.T., (1996): 5-lipoxygenase inhibition by acetyl-u- keto-beta-boswellic acid (AKBA) by a model mechanism. Phtomedicine 3:71-2.

Sahito, S.R., T.G., Kazi, G.H., Kazi M.A., Jakhranti and M.S. Shaikh (2001): Trace Elements in Two Varieties of Indigenous Medicinal Plant *Catharanthus Roseus*(Vinca rosea): The Sciences 1(2):74-77.

Samudralwar D.L., Garg A.N., (1996): Minor and trace elemental determination in the Indian herbal and other medicinal herbal and other medicinal preparations. Biol Trace Elem. Res. 54:113-21.

Sand Kuhler, and Gebhart, G.F., (1984): Characterization of inhibition of a spinal nociceptive reflex by stimulation medically and laterally in the mid-brain and medulla in the pentobarbitonal anaesthetized rat. Brain Res. 305:67-76.

Schmanass. C., Tammond D., Och J., Wtaksl T.L., (1983): Pharmacological antagonism of those antinociceptive effects of serotonin in hebat spinal cord Evr. Pharmacol 90:349-357.

Sharma, R.A., Janna, S.C. Jain R., Mittal C (1998): Antimicrobial activity of cassia species, Indian Journal ofs Pharmaceutical Sciences 60 (1) 29-32.

Saxena MJ (2001). Relevance of Herbs in improving index of livestock animals. An invited paper for the 38th Annual Congress State, Nigeria Veterinary Medical Association held in Topo Badagry.

Schrevel, J., Millieriouz, V., Sinov, V., Frappier, F., Santus, S., and Greither, P., (1996): New trends in chemotherapy of Human and Animal Blood Parasites Parasitol Res., 82(93):283-284.

Shoskes DA. (1998) Effect of bioflavonoids quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents. Transplantation 66:147–52.

Shutenko Z, Henry Y, Pinard E, (1999). Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron

paramagnetic resonance in rat brain during global ischemia and reperfusion. Biochem Pharmacol 57:199–208

Siedel, J.R., Hagele, E.O. Ziegenhom, J. (1983). Reagent for the enzymatic determination of serum cholesterol with improved lipolytic efficency.Clin. Chem. 29: 1075-1080.

Sikawar, R.L.S. (1994). Ethnoveterinary plant medicines in Morena district of M.P, India. *Ethnobiology in Human Welfare: Abstr. Fourth Intl. Congr. Ethnobioi*, Nat. Bot. Res. Institute, Lucknow, p.289

Singh R.K., and Paul B.L., (1996): Atni-inflammatory activity of seed extracts of *Pongamia Pinnata* in rat, Indian J. Physiol Pharmacol 40:355-8

Singh R.K., Nach G., Goel R.K., Bhaltacharya S.K., (1998): Pharmacological actions of *Abies pindow* Royle leaf Indian J. Expt. Biol. 36: 187-91

Singh R.K., Nalh G., Acharya S.B., *et al*. (1997): Pharmacological actions of *Pongamia pinnata* root in albino rats. *Indian J. Exp. Biol* 35:831-6.

Singh R.K., Pandy B.I. (1997): Further study of anti-inflammatory effects of *Abies pindow* Phytother Res. 11:535-7

Singh R.K., Joshi V.K., Goel R.K., et al (1996): Pharmacological actions of *Pongamia prata* seeds a preliminary study *Indian J. Exp. Biol.* 34:1204-7.

Singh V., Garg A.N., (1997): Availability of essential trace elements in Ayunvedic Indian medicinal herbs using instrumental neutron activation analysis Appl. Radiatized 48:50-101.

Schillhorn Van, Veen T. W. (1997).Sense or nonsense? Traditional methods of animal parasitic disease control. Vet. Parasitol. 71:177-194.

Schmidt, E., and Schmidt, F. W. (1963).Enzyme Biol. Clin.3:1.

Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. (2004). Saponins from edible legumes: chemistry, processing, and health benefits.J Med Food. Spring;7(1):67-78.

Sivaprakasam P., Viswama than S., Thrignanasambanthan P., Reddy M.K., vijaya Sekaran V., (1996).Pharmacological screening of *Ochna obtusata*. Filiterapia 67:117-20.

SPSS instruction manual. (1998) University of Waterloo, Department of statistic and actural science. Sept , 1998.

Stein E.A Myers, G.L. (1995). National Cholesterol Education Program Recommendations for Triglycerides Measurement: Executive summary. Clin Chem 41:1421-1426.

Subramoniam A., Pushpagacan P., Rajesekharan S., Laths P.G., (1995) Antipyretic activity of TBR-002 A herbal formation Ancient Science Life 15:7-14.

Sukumar, F., Hamsaeni G.R., Bhima R.R., (1995): Pharmacological actions of Cerpegin, a novel pyridine alkaloid from ceropega Juncea. Fitoteapia 66:403-6.

Suresh, B., Dhanasekaran, S., Kumar, R.V., Balasubramanian S. (1994). Ethnompharmacological studies on the medicinal plants of Nilgns Indian drugs 32:340-352.

Suresh B., Dhanasekaran S., Elango K. (1995): Anti-pyretic activity centella asiatia (L) *urban. J. Ethno pharmacol*, 48:53-7.

Takagi K., Park, E.H., Kato H., (1980): Anti-inflammatory activities of hederagenin and crude saponin isolated from Sapindus mukorossi Gacrtn chem.. Pharm Bull 28:1183-1180.

Terblanche F.C. and G. Kornelius (1996). Essential oil constituents of the genus *Lippia* (Verbenaceae): a literature review. *J. Essent. Oil Res.* **8**, 471-485

Teuscher, A. and Richterich; P Schweiz Med. Wschr. 1971:101:345-390.

Tietz N.W., (1995). Clinical Guide to Labouratory Tests.3rd Edition. WB Saunders Company. Philadelphia. PA pp 518-519.

Thalte U.M. Rao S.G., Dahanukar S.A. (1994): Tinospora Cordifolia induces Colony Stimulating activity in Serum. *J. Postgrad. Med*., 40:202-3.

Trivedi, N.A., Mazunder, B., Bhatt, J.D., Hemavathi K.G. (2004). Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. *Indian J. Pharamacol*. 36 (6): 373-376.

Turner I. Richens A (1978): Clinical Pharma day Vol. Churchil Livingstone E. Inberga London New York 8. 154.

Tona, I., Kambu, K, Ngimbi, N., Cimanga, K. and Vlictinck; A.J (1998); Antiamoebic and Phytochemical, Screening of some Congolese Medicinal plants. *Journal of Ethnopharmacology* 61 (1): 57-65

Tona,I., Kambu, K Mesia,K., Cimanga, K. Apers, S., De Bruyne, T.,Pieters L., Tottle. J, and Vlictinck A.J (1999); Biolgical Screening of traditional Preparations from some Medicinal Plants used as Antidiarrhoeal in Kinshasa, Congo; Phytomedicined, 6(1); 59-66.

Tona L, Mesia K, Ngimbi NP, Chrimwami B, Okond'akhoka A, Cimanga RK, DeBruyne T, Apers S, Hermons N, Totté J, Pieters L, Vlietinck AJ (2001) 'In vivo antimalarial activity of *Cassiaoccidentalis*,

Morindamorindiodes and *Phyllanthusniruri'Annals of Tropical Medicine and Parasitology*, 95, pp 47-57.

Ugochukwu N.H., Babdy, N.E., Cobourne, M and Gasset, S.R. (2003). The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J. Biosei 28:1-5.

Valdya A.B., (1997): The status and scope of India medicinal plants acting on central system Indian. J. Pharmacol 20: 530-3.

Valecha N., Biaswa H., Badtuai V., (1994): Antimalarial activity of *Atemisin Japonica, Artemisia maritime and Artemisia nifegeria* India J. Pharmacol 26:144-6

Van Acker SA, Tromp MN, Haenen GR, van der Vijgh WJ, Bast A.(1995) Flavonoids as scavengers of nitric oxide radical. Biochem Biophys Res Commun 214:755–9.

Vane, J.R., (1971): Prostagladins and Aspirin like drugs to Maxwell and Acheson (Ecs) cellular mechanisma V. Karger Basel.

Vane J. R., and Pedling R.M. (1998): Mechanism of action of anti-inflammatory drugs International Journal of Tissue Reaction 20:3-15.

Vax, J., Kulkarni C., David J. Joseph T. (1998): Influence of caffeine on pharmacokinetic profile of sodium valproate and carbamazepine in normal lun in volunteers, Indian J. Exp. Biol. 36:112-4

Valentin, A., Pelissier, Y., Benoit, F., Marion, C., Kone, D., Mallie, M., Bastide, J.M., and Bessiere J.M., (1995): Composition and antimalarial activity in vitro Yolatile components of *Lippia multiflora (*Moldenke*)* phytochemistry. 40(5): 1429-1442.

Vessal, M., Hemmati, M and Vasei M (2003) Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 135C (3): 357-64

Vieira, R.F. Grayer, R.F. Paton, A and. Simon J.E (2001). Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. *Biochem. Syst. Ecol.* **29**, 287-304.

Vohora, S.B., (1986): Unani JOSHAANDAH Drugs for common Colds, Calarrh, Cough and associated Fevers, *J. Ethmopharmacology*, 16: 201.

Vyklichy, I., (1979): Techniques for the study of pain in animals in Bonica J.J. Liebeskind, J.C., Albefessard D.G., (Eds) Advances in pain Research and therapy vol, 3 Raven press, New York pp 727-745.

Ward P.A. and Let lenth A.B., (1999): The acute inflammatory response and its regulated Activity of surgery 124:666-669..

Wagner, H., Proksch, A., Vollmar, A., Kreutzkamp, B., Bauer., J. (1985). In vitro phagozytose-stimulierung durch isoherte pflanzenstoffe gemessen im phagozytose- chemilumineszenz-(CL)-modell, (In vitro

phagocytosi stmulation,by plant material in a chemaluminescence-phagocytosis model) Planta Med. 2: 139-144.

Wagner, W.L., Herbst, D.R., Sohmer, S.H. (1999). Manual of the flowering plants of Hawaii, Revised edition.Bernice P. Bishop Museum Special Publication.University of Hawaii Press.Honolulu pp1919.

Waterhouse, D.F.(1993). Prospects for biological of paddy weeds in Southeast Asia and some recent success in the biological control of aquatic weed.Camberra. Ext Bul. 366, Australia.

Watkins, L.R., Milligan, E.D and Maier, S.F. (2001).Glial activation, a driving force for pathological pain.Trends Neurosci. 24: 450-455.

Watt, J.M. and Bever-Brandwijik, M.C. (1963): The Medicinal and Poisonous Plants of Southern and Eastern Africa Livingstone, Edingburgh

Weaver, D.C., McDaniel, M.L., Naber, S.P., Barry, C.D and lacy, P.E (1978). Alloxan stimuation and inhibition of insulin release from isolated rat islets of Langerhans. Diabetes. 27: 1205-1214.

Welt, K., Weiss, J., Martin, R. Hermsdorf, T., Drews S., Fitzl, G. (2007).*Ginkgo biloba* extracts protects rat kidney ffrom diabetic and hypoxic damage. Phytomedicine. 14 (2-3): 196-203.

Wiebe, D.A., Bernert, J.T. (1984). Influence of incomplete cholestryl ester hydrolysis on eenzyme measurements of cholesterol. Clin Chem. 30: 352-356.

Winter, C.A.,E.A. Risley and G.W. Nuss. (1962). Carrageenan induced oedema in hind paw of rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol and Med. 111: 544-547

WHO (1995): The World health report, Briadging the gap. WHO Geneva L.P. 48.

WHO 1978 The Promotion and Development of Traditional Medicine. Technical Report Series 622.

WHO 1978 Alma Ata Declaration Primary Health Care.*Health for all series No 1.*

WHO (1995). Control and surveillance of African trypanosomiasis. Report of a WHO Expert Committee. World Health Organization Technical Report Series 881, I-VI, 1-114.

WHO 2001Legal status of Traditional Medicines and complementary/Alternative Medicine: A worldwide review. WHO publishing 1.

Willer, J.C., Board caur F, and Albe-fessard, D, (1979): Supraspinal influences on nociceptive flexion reflex and pain sensation in man Brain Res. 176:61-68.

Willoughby D., (1978): Inflammation Endeavour 2:57-65.

Winn. V.D., O'Banion, Young D.A. (1993): Anti-inflammatory action inhibition of gnPGHS, a new cyclooxygenase, Journal Lipid Medicators 6. 101.

Whittle B.J.R., Higge H.A., Eakins K.E., Moncada S, and Vane J.R., (1980): Selective inhibition of protagladin production in inflammatory excudates and gastric mucosa. Nature 284:271.

Wu. Tianshung; Chaa Yuyi; Maeijen; Fulwen, Shilishan, Chen Kutung (1997): Platelet aggregation inhitor from Murraya euchrestifolia in proceeding of the second international symposium on natural frugs, Marcitae Italy, 28 September-Oct 1, 1997 (Edited by capasso F; Basso F; Pasquale R., de; Evans F.J. Mascolo N.J. Phytomisapia 1998 12 (Supplement 1) S80-S82 XU, W., QiuX, C., How J.S., (1994): Serotonin receptor terminal system medicates antinociception D. Pharmacol.

Xian-Kang Fang, Jie Gao , Dan-Ni Zhu (2008). Kaempferol and quercetin isolated from *Euonymus alatus* improve glucose uptake of 3T3-L1 cells without adipogenesis activity. Life Sciences 82 : 615–622

Xing J.H.and Soffer E.E. (2001), Adverse effects of laxatives, Diseases of the Colon and Rectum. 44(8):1201-1209.

Xiao, S.H, and B.A. Catto, (1989): *In Vitro* and *invivo* studies of the effect of artemether on *Schistosoma mansoni* atimicrobia agents. Chemother 33:1557-1562.

Yamasaki, K, Nakanp M., Kawahata, T., Mori H., Otake, T, Ueba N., Osihi, I., Inami R., Yamane, M., Nakamura M., Murata, H., Nakanishi T., (1998): Anti-HIV I activity of herbs in Labiakae Bioloigical and Pharmaceutical Bulletin 21(8) 829-833.

Yesilada, E., Sezik, E. (1990). Screening of some Turkish medicinal plants used in the treatment of rheumatoid arthritis and inflammatory disease for their anti-inflammatory activities. Plana Med. 56: 659.

Yesilada, E., Ustun, O., Sezik, E., Takaishi, Y., Ono, Y., Honda, G. (1997). Inhibitory effects of Turkish folk remedies on inflammatory cytokines; interleukin-1α, interleukin-1β and tumor necrosis factor α. J. Ethnopharmacol. 58: 59-73.

Yoshiki K, and E. Hashimoto (1996): J. Med. Chemistry 30 1016-1017.

Yoxall, A.T. (1978): Pain in small animals Journal of small Animal practice 19-43.

Zaman, M.B. and M.K. Shariq (1995).Hundred drug plants of West Pakistan. Medicinal plant branch Pakistan Forest Institute Peshawar, 105.

Zemlan F.P., Corrigan, S.A. Pfaff D.W. (1980): Noradrenergic and Scrotonargic mediator of spinal analgesia mechanisms Euro J. Pharmacol 61:111-124.

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B., Grellier, P. (2005): In vitro antiplasmodial activity of 33 West African Plants used for treatment of Malaria Journal of Ethnopharmacology, 98, 281-285.

APPENDIX

SUMMARY OF THE TABLES FOR THE DIFFERENT PROTOCOLS

TABLE 1: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS.

(MEAN + STANDARD ERROR OF MEAN)

PLANTS	PCV	RBC	HB	MCV	MCH	MCHC	WBC	NEUT.	LYMP	MONO	BASO	EOSINO
Tithona diversifolia	29.0± 1.3	4.58± 0.2	9.72± 0.5	63.32± 0.2	21.18± 0.2	33.46± 0.4	6.44± 1.1*	26.0± 2.6	73.6± 2.7	0.20± 0.2	22.19± 22	0.20± 0.2
Acalypha wilkesiana (Mull. Arg)	28.4± 1.6	4.46± 0.3	9.54± 0.6	63.66± 0.6	21.36± 0.1	26.84± 6.6	3.94± 0.4	29.8± 4.5	69.6± 4.8	0.60± 0.4	0.00± 0.0	0.00± 0.0
Lippia multiflora (Moldenke)	23.8± 3.6	3.74± 0.6	7.94± 1.2	63.52± 0.3	21.24± 0.2*	20.33± 8.1	3.18+ 0.6	27.8± 1.0	71.6± 0.8	0.20± 0.2	0.00± 0.0	0.00± 0.0
Ocimum gratissimum	26.0± 1.3	4.18± 0.2	8.98± 0.4	63.62± 0.2	21.50± 0.6	33.76± 0.5	2.08± 0.3*	28.2± 3.6	71.2± 3.8	0.20± 0.2	0.00± 0.0	0.00± 0.0
Morinda morindoides (Baker) Milne-Redh	26.6± 0.6	4.16± 1.0	9.06± 0.2	63.94± 0.2	21.78± 0.7	34.08± 0.5	3.08± 0.4	26.6± 3.3	73.2± 3.2	0.00±0.0	0.00± 0.0	0.00± 0.0
Control	27.0+ 1.5	4.24+ 0.2	9.22+ 0.4	63.66+ 0.2	21.78+ 0.7	20.36+ 8.2	3.68+ 0.4	38.0+ 3.1	61.6+ 2.8	0.40+0.4	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume %), Hb = Haemoglobin Concentration (g/dl)

Rbc = Red blood cell ($x10^6/mm^3$), WBA= White blood cell ($x\ 10^3/mm^3$)

Lymp = Lymphocytes, NEUT= Neutrophils, MCV= Mean corpuscular volume (fl)

MCH = Mean Corpuscular Haemoglobin (pg)

MCHC = Mean Corpuscular Haemoglobin Concentration (%)

Note * Superscripted figures are statistically significant at P <0.05.

TABLE 2: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS	PCV	RBC	HB	MCV	MCH	MCHC	WBC	NEUT.	LYMP	MONO	BASO	EOSINO
Tithoina diversifolia	26.4± 1.5	4.22± 0.2	8.70± 0.6	63.35± 0.2	21.18± 0.2	25.01± 8.2	6.08± 0.4*	33.80± 1.7	66.00± 2.1	0.40± 0.4	0.00± 0.0	0.00± 0.0
Acalypha wilkesiana	28.4± 1.5	4.42± 0.2	9.45± 0.7	64.05± 0.2	21.20± 0.2	33.13± 0.3	3.64± 0.3	32.60± 4.3	68.20± 4.3	0.40± 0.4	0.00± 0.0	0.00± 0.0
Lippia multiflora	28.6± 0.5	4.50± 0.9	9.73± 0.2	63.53± 0.4	21.50± 0.1	25.41± 8.4	3.88± 1.3	30.60± 1.8	69.60± 1.6	0.20± 0.2	0.20± 0.2	0.00± 0.0
Ocimum grattissimum	24.0± 0.7	3.80± 0.1	8.20± 0.3	63.38± 0.3	21.43± 0.1	33.83± 0.1	2.72± 0.2	31.00± 4.3	71.00± 2.9	0.00± 0.0	0.00± 0.0	0.00± 0.0
Morinda morindiodes	29.2± 2.7	4.60± 0.4	9.90± 0.9	63.44± 0.2	21.50± 0.1	27.19± 6.7	3.76± 0.4	26.80± 3.5*	72.60± 3.3	0.20± 0.2	0.20± 0.2	0.20± 0.2
Control	27.0+ 1.5	4.24+ 0.2	9.22+ 0.4	63.66+ 0.2	21.78+ 0.7	20.36+ 8.2	3.68+ 0.4	38.0+ 3.1	61.60+ 2.8	0.40+ 0.4	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume %), Hb = Haemoglobin Concentration (g/dl)

Rbc = Red blood cell ($x10^6/mm^3$), WBA= White blood cell ($x\ 10^3/mm^3$)

Lymp = Lymphocytes, NEUT= Neutrophils, MCV= Mean corpuscular volume (fl)

MCH = Mean Corpuscular Haemoglobin (pg)

MCHC = Mean Corpuscular Haemoglobin Concentration (%)

Note * Superscripted figures are statistically significant at $P < 0.05$.

TABLE 3: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANT EXTRACTS ON THE HAEMATOLOGY OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS	PCV	RBC	HB	MCV	MCH	MCHC	WBC	NEUT.	LYMP	MONO	BASO	EOSINO
Tithoina diversifolia	24.40± 1.2	3.88± 0.2	8.56± 0.5	63.48± 0.1	21.42± 0.1	33.70± 0.1	4.94± 0.4*	36.40± 3.1	66.80± 2.3	0.20± 0.2	0.00± 0.0	0.00± 0.0
Acalypha wilkesiana	25.20± 1.0	3.96± 0.2	8.50± 0.4	63.88± 0.2	21.70± 0.0	33.68± 0.2	3.94± 0.3	29.40± 1.9*	71.60± 2.1*	0.00± 0.00	0.00± 0.0	0.00± 0.0
Lippia multiflora	27.80± 0.7	4.38± 0.1	9.36± 0.3	63.46± 0.2	21.38± 0.0*	33.68± 0.1	2.04± 0.3*	25.20± 2.9*	74.40*± 2.8	0.20± 0.2	0.20± 0.0	0.00± 0.0
Ocimum grattissimum	26.80± 1.7	4.20± 0.3	9.06± 0.5	63.78± 0.3	21.58± 0.1*	33.84± 0.2	1.96± 0.2*	24.80± 3.1*	75.00± 3.1*	0.20± 0.2	0.00± 0.0	0.00± 0.0
Morinda morindiodes	24.20± 1.8	3.78± 0.3	8.28± 0.6	64.08± 0.4	21.94± 0.1	34.20± 0.1	3.98± 0.1	31.20± 2.6	68.00± 2.1	0.60± 0.3	0.00± 0.0	0.40± 0.4
Control	27.00± .5	4.24± 0.2	9.22± 0.4	63.66± 0.2	21.78± 0.2	20.36± 8.2	3.68± 0.4	38.00± 3.1	61.60± 2.8	0.40± 0.4	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume %), Hb = Haemoglobin Concentration (g/dl)

Rbc = Red blood cell ($x10^6/mm^3$), WBA= White blood cell ($x\ 10^3/mm^3$)

Lymp = Lymphocytes, NEUT= Neutropils, MCV= Mean corpuscular volume (fl)

MCH = Mean Corpuscular Haemoglobin (pg)

MCHC = Mean Corpuscular Haemoglobin Concentration (%)

Note * Superscripted figures are statistically significant at $P < 0.05$.

TABLE 4A: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (A)

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T.PROTEIN (g/l)	UREA (mg/dl)	CREATININE (mg/dl)	ALBUMIN (g/l)	GLOBULIN (g/l)	AST (u/l)	ALP (u/l)	ALT (u/l)	TOTAL BILIRUBIN (mg/dl)	TOTAL BILIRUBIN (mg/dl)
Tithoina diversifolia	83.22± 4.9	49.15± 5.6	0.76± 0.07	44.43± 0.9	38.80± 4.6	103.08± 23.6*	70.05± 12.4*	141.10± 2.7*	0.78± 0.2	
Acalypha wilkesiana	74.55± 4.3*	38.41± 3.9	0.78± 0.12	44.61± 1.3	29.14± 5.3*	84.96± 9.7*	50.19± 4.6*	118.98± 7.6*	0.69± 0.3	
Lippia multiflora	94.69± 2.4*	46.50± 3.2	0.88+ 0.14	47.14± 1.3	47.65± 3.3	86.69± 18.5*	45.10± 8.3	137.18± 4.1*	0.78± 0.1	
Ocimum grattissimum	76.62± 3.4*	49.63± 6.7*	0.74± 0.13	45.79± 2.0	30.70± 4.3*	71.09± 9.8	52.21± 8.5*	125.22± 5.5*	1.26± 0.1	
Morinda morindoides	74.10± 4.8*	36.64± 3.8	1.10± 0.25	46.01± 2.3	28.08± 3.7*	69.22 ± 11.74	52.44± 1.8*	131.28± 5.9	0.77± 0.2	
Control	83.50± 4.9	36.23± 3.8	0.86± 010	40.97± 1.7	42.14± 5.7	37.27± 4.3	27.92± 9.5	124.00± 6.4	1.08± 0.3	

TABLE 4B: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (B)

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	Ka+ (mmol/l)	Na+ (mmol/l)
Tithoina diversifolia	80.50±3.4*	63.00±10.6	70.99±5.3	30.84±1.5	27.56±3.0	3.99±0.3	68.69±24.3
Acalypha wilkesiana	80.02±5.7*	67.55±3.2	86.08±2.1	50.23±5.0	22.52±3.3	5.32±0.5	70.66±15.7
Lippia multiflora	78.16±2.0*	66.48±8.6	87.29±9.0	49.31±4.2	24.68±4.7	4.56±1.5	117.12±19.9
Ocimum grattissimum	69.64±3.7*	77.04±7.0	106.97±29.3	62.15±28.4	29.43±2.0	4.53±0.4	87.48±8.9
Morinda morindoides	70.60±5.5*	74.44±3.9	108.37±8.1	66.77±10.5	26.69±52	5.67±0.9	104.9±20.3
Control	116.00±9.8	83.41±10.5	87.24±8.2	45.34±7.1	25.22±4.2	4.88±0.4	81.59±19.4

GLYC = Glucose, TRIG = Triglycerides, CHOL = Cholesterol, LDL = Low Density Lipoproteins, HDL = High Density Lipoprotein

Na+ = Sodium ions, K+ = Potassium ions

Note: * Superscripted items are statistically significant at $P<0.05$

TABLE 5A: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (A PART)

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T. PROTEIN (g/dl)	UREA (mg/dl)	CREATININE (mg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	AST (u/l)	ALP (u/l)	ALT (u/l)	T.BIL (mg/dl)
Tithoina diversifolia	81.11± 4.7	51.10± 4.6*	0.70± 0.1	46.62± 1.1	34.13± 4.7	57.08± 7.6*	50.38± 5.7	132.94± 6.0	0.52± 0.1
Acalypha wilkesiana	83.63± 4.3	32.78± 3.5	0.78± 0.1	42.41± 1.9	41.21± 5.1	76.07± 5.1*	45.12± 8.3	126.40± 5.1	0.75± 0.2
Lippia multiflora	76.20± 3.1	30.10± 3.7	0.64+ 0.1	43.70± 0.3	33.07± 3.0	47.70± 9.7	55.93± 3.4	116.66± 10.1	0.58± 0.2
Ocimum grattissimum	89.79± 3.5	38.29± 3.8	0.64± 0.0	42.68± 1.5	45.27± 3.1	59.01± 4.3*	48.04± 12.7	110.92± 7.4	0.39± 0.1
Morinda morindoides	74.22± 5.6	31.02± 3.9	0.58± 0.0*	45.09± 2.0	29.13± 4.9	45.85± 6.5	56.76± 6.5	127.32± 12.5	0.44± 0.1
Control	83.51± 4.9	36.23± 3.8	0.87± 0.1	40.97± 1.7	42.14± 5.7	37.27± 4.3	27.92± 9.5	124.00± 6.4	1.08± 0.3

TABLE 5B: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (B)

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	K^+ (mmol/l)	Na^+ (mmol/l)
Tithoina diversifolia	86.33± 5.6*	76.00± 9.0	99.49± 13.2	48.90± 6.4	35.53± 5.4	4.14± 0.4	101.88± 6.5
Acalypha wilkesiana	73.00± 6.6*	72.59± 12.6	89.57± 7.8	48.84± 5.0	26.22± 3.5	4.18± 0.2	91.04± 15.2
Lippia multiflora	77.58± 4.2*	53.94± 9.7	95.74± 8.7	47.57± 7.9	37.38 ± 4.0	2.95± 0.1*	96.01± 8.2
Ocimum grattissimum	76.45± 4.0*	70.83± 4.8	87.99± 6.2	68.11± 29.8	32.94± 8.2	3.73± 0.3*	67.02± 26.0
Morinda morindoides	94.62± 13.2*	64.44± 5.6	98.91± 13.7	50.37± 8.1	35.65± 3.4	3.76± 0.2*	103.10± 22.9
Control	116.00± 9.8	83.41± 10.5	87.24± 8.2	45.34± 7.1	25.22± 4.2	4.88± 0.4	81.59± 19.4

GLYC = Glucose, TRIG = Triglycerides, CHIL = Cholesterol, LDL = Low Density Lipoproteins, HDL = High Density Lipoprotein

Na+ = Sodium ions, K^+ = Potassium lows

Note: * Superscripted items are statistically significant at $P<0.05$

TABLE 6A: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OF RATS. (A PART)

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	T. PROTEIN (g/dl)	UREA (mg/dl)	CREATININE (mg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	AST (µ/l)	ALP (µ/l)	ALT (µ/l)	T.BIL (mg/dl)
Tithoina diversifolia	77.28±6.3	32.35±1.6	0.70±0.1	44.99±1.0	28.78±6.3	77.38±20.0*	59.41±5.2	129.26±7.2*	0.80±0.1
Acalypha wilkesiana (Mull. Arg)	76.94±2.9	48.80±2.7*	0.78±0.1	44.28±4.1	33.61±5.4	58.69±4.6*	42.96±4.9	131.72±4.0*	1.16±0.2
Lippia multiflora (Moldenke)	80.74±8.1	40.19±1.3	0.80±0.1	39.05±5.0	41.69±5.5	60.27±5.6*	40.19±8.6	139.42±3.4*	1.22±0.6
Ocimum gratissimum	71.11±7.2	34.86±3.6	0.62±0.0	44.00±1.2	26.94±7.6	49.69±5.0	44.11±2.8	123.20±7.3	0.45±0.1
Morinda morindoides (Baker) Milne-Redh	73.55±5.3	34.70±5.7	0.64±0.1	42.85±2.7	30.70±4.0	31.23±1.4	41.97±8.2	107.30±8.1	0.56±0.3
Control	83.51±4.9	36.23±3.8	0.87±0.1	40.97±1.7	42.14±5.7	37.27±4.3	27.92±9.5	124.00±6.4	1.08±0.3

T. Protein = Total protein, AST = Aspantale Amino transferase; T.BIL= Total Bilirubin

ALP = Alkaline Phosphataze, ALT = Alamine amino transferase

Note: Superscripted figures are statistically significant at P<0.05.

TABLE 6B: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM BIOCHEMISTRY OFRATS. (A PART)(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	GLUCOSE (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	LDL	HDL	K^+ (mmol/l)
Tithoina diversifolia	70.00±2.5*	65.17±9.5	79.48±6.2	38.56±8.7	27.89±5.3	4.28± 0.5
Acalypha wilkesiana	81.13±5.7*	77.55±11.9	103.87±2.7*	49.49±5.2	38.88±4.3	4.33±0.2
Lippia multiflora	84.74±5.4*	68.43±6.9	79.99±2.7	39.82±2.8	26.49±4.0	5.49±1.2
Ocimum grattissimum	62.00±1.5*	60.00±4.5	91.63±8.9	46.32±7.6	33.31±3.2	3.25±0.2
Morinda morindoides	73.64±3.3*	70.75±8.6	110.45±6.5*	60.79±4.5*	35.45±2.7	3.9±0.2
Control	116.00±9.8	83.41±10.5	87.24±8.2	45.34±7.1	25.22±4.2	4.88±0.4

Trig = Trighyceride, Chol = Cholestenol

Note: * Superscripted item and significant P <0.05

ANTI-DIABETETOGENIC STUDY

TABLE 7: EFFECTS OF 400MG/KG DOSE OF THE EXTRACTS OF EACH PLANT ON ALLOXAN INDUCED DIABETIC RATS.

(MEAN ± STANDARD ERROR OF MEAN)

PLANTS EXTRACT	BLOOD GLUCOSE "DAY O"	BLOOD GLUCOSE "Day 21"	SERUM CHOLESTEROL "Day 21"	SERUM TRIG " Day 21"	SERUM HDL "Day 21"	SERUM LDL "Day 21"
Tithoina diversifolia	584.40±10.6	102.95±2.6*	127.43±0.7*	101.98±5.8	52.42±0.6*	57.95±4.2*
Acalypha wilkesiana	168.00±2.8	82.72±3.9*	121.80±0.5*	84.23±2.6	42.95±1.0*	61.70±1.4*
Lippia multiflora	539.70±50.4	351.00±19.1	130.43±3.3*	83.45±2.0	37.25±0.7	78.15±3.2*
Ocimum grattissimum	567.68±49.6	195.15±42.5*	137.57±4.3*	82.75±2.8	41.27±2.0*	79.88±3.5*
Morinda morindoides	277.87±17.5	103.00±2.8*	133.48±1.1*	80.00±4.6	51.03±3.0*	66.38±2.5*
Glibenclamde	268.63±14.8	78.85±2.9*	134.13±1.5*	86.45±4.5	47.32±2.2*	68.70±3.5*
Untreated Diabetic (-VE control)	269.50±10.6	324.53±22.3	159.62±2.6	83.900±4.3	25.70±0.9	116.98±3.0
Non- Diabetic Control	113.18±2.3	108.00±3.1	128.82±4.1*	91.87±4.8	40.80±2.5	69.08±4.9*

* Superscripted figures are statistically significant at P <0.05

ANALGESIC STUDY

TABLE 8: EFFECTS OF 400MG/KG DOSE OF THE EXTRACTS OF EACH PLANT ON HOT PLATE AND ACETIC ACID INDUCED WRITING TEST IN MICE.

(MEAN ± STANDARD ERROR OF MEAN)

	HOT PLATE REACTION TIME (S)	NO OF WRITHINGS	% INHIBITION OF WRITHING
Tithoina diversifolia	25.18 ± 07*	26.17 ± 0.7*	43.92%
Acalypha milkesiana	30.00 ± 0.6*	25.83 ± 1.3*	44.65%
Lippia multiflora	26.26 ± 2.8*	27.00 ± 2.2*	42.15%
Ocimum grattissimum	21.55 ± 0.9*	28.67 ± 1.0*	38.57%
Morinda morindoides	25.73 ± 10.0*	26.00 ± 0.7*	44.29%
Morphine	31.8 ± 1.5*	-	-
Paracetamol	-	23.50 ± 1.3	49.65
Untreated Control	18.22 ± 0.3	46.67 ± 1.4	

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 7A: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM MORPHOLOGY OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

	HEADLESS TAIL	TAILESS HEAD	RVDIMENTALLY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithoina diversifolia	191.25± 62.3	143.75± 74.4	13.50± 4.8	2461.8± 249.7	1464.3± 748.4	2727.3± 288.3	2991.75± 261.1	11.00± 4.1	6.00± 5.0	401.3± 1.3
Acalypha wilkesiana	191.25± 62.3	118.75± 56.5	11.0± 4.1	3796.3± 274.9*	3519.00± 268.2*	2769.3± 323.8	3102.00± 260.9	18.50± 2.5	11.00± 4.1	406.3± 2.4
Lippia multiflora	163.75± 47.6	191.3± 62.3	11.0± 4.1	3471.3± 249.9*	3047.00± 239.7	3596.00± 242.8	3822.00± 258.8	8.50± 4.8	8.50± 4.8	401.3± 1.3
Ocimum grattissimum	239.00± 27.3	164.00± 47.7	11.0± 5.8	2766.5± 248.2*	4576.5± 248.2*	3746.3± 274.8	4101.8± 219.9	11.0± 4.1	6.00± 5.0	401.3± 1.3
Morinda morindoides	166.00± 48.4	239.50± 27.1	13.50± 4.8	41017.3± 236.5*	3903.8± 273.6*	4422.5± 57.0	3871.5± 263.6	11.0± 5.8	13.50± 4.8	402.5± 2.5
Control	116.25± 54.9	68.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75± 2.4

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 7B: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM CHARACTERISTIC OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

	SPERM MOLITY	LIVE/DEAD	SPERM VOLUME	SPERM COUNTS		
Tithoina diversifolia	90.00±0.0	95.8±0.8	5.18±0.0	97.25±2.1		
Acalypha wilkesiana	80.00±7.0	96.00±2.0	5.18±0.0	82.75±2.7		
Lippia multiflora	77.50±6.3	93.25±2.8	5.18±0.0	78.50±2.5		
Ocimum grattissimum	85.00±2.9	96.50±0.8	5.20±0.0	82.50±2.7		
Morinda morindoides	22.50±13.2*	86.3±2.4	5.15±0.0	45.75±5.1*		
Control	93.75±1.3	98.00±0.0	5.18±0.0	129.00±5.0		

* Superscripted items are significant at P <0.05.

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 8A: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM MORPHOLOGY OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

	HEADLESS TAIL	TAILESS HEAD	RVDIMENTALLY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithoina diversifolia	148.33± 63.7	147.67± 63.3	17.67± 3.3	3217.7± 3.3	2882.33± 329.7	3965.00± 367.00	3981.7± 330.3*	14.33± 3.3	1.00± 0.00	405.00 ± 2.9
Acalypha wilkesiana	164.00± 47.7	188.50± 60.6	16.00± 2.9	3546.00± 259.4	3517.25± 269.2*	3216.8± 408.1	3256.5± 28.6	16.00± 2.9	11.00 ± 5.8	403.75 ± 24
Lippia multiflora	266.00± 31.8	166.50± 48.6	11.00± 5.8	3721.5± 283.2	3227.00± 573.9	3822.0± 288.1	3763.00± 259.2	11.0± 5.8	6.00± 2.9	402.5± 1.4
Ocimum grattissimum	164.00± 47.7	238.75± 27.4	11.0± 4.1	4074.00± 251.0	4448.8± 45.5*	4099.00± 226.0	4096.5± 258.9*	8.50± 4.8	11.00 ± 4.1	401.3± 1.3
Morinda morindoides	239.00± 27.3	163.75± 47.6	16.00± 2.9	4629.00± 235.5	3822.3± 288.5*	4544.8± 226.9	4567.00± 220.3*	13.50± 4.8	11.00 ± 4.1	400.00 ± 0.0
Control	116.25± 54.9	68.75± 47.5	8.50± 4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75 ± 2.4

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 8B: EFFECTS OF 800MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM CHARACTERISTIC OF RATS.

(MEAN ± SEM)

	SPERM MOLITY	LIVE/DEAD	SPERM VOLUME	SPERM COUNTS
Tithoina diversifolia	70.00±11.6	98.00±0.0	5.15±0.0	77.0±1.7
Acalypha wilkesiana	75.00±2.9	94.50±1.7	5.18±0.0	76.50±3.8
Lippia multiflora	60.00±0.0*	96.50±0.9	5.20±0.0	60.50±1.4*
Ocimum grattissimum	87.50±2.5	96.50±0.9	5.15±0.0	74.00±2.8
Morinda morindoides	47.50±6.34*	90.00±2.0*	5.13±0.0	40.75±1.1*
Control	93.75±1.3	98.00±0.0	5.18±0.0	129.00±5.0

- Superscripted items are significant at P<0.05

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 9A: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM MORPHOLOGY OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

	HEADLESS TAIL	TAILESS HEAD	RVDIMENTALLY TAIL	BENT TAIL	CURVED TAIL	BENT MID PIECE	CURVED MID PIECE	LOOPED TAIL	COILED TAIL	TOTAL ABNORM
Tithoina diversifolia	321.5± 0.3*	171.00± 50.0*	13.50± 2.5	3766.0± 320.5*	4072.00± 811.3	4052.00± 280.00	3792.00± 526.7	18.50± 2.50	11.00± 5.8	400.00± 0.00
Acalypha wilkesiana	239.3± 27.3*	239.25± 27.6*	18.50± 2.5	4157.0± 475.4*	4099.3± 260.0*	4379.00± 388.0	4557.5± 259.3*	16.00± 2.9	11.00± 4.0	403.75± 2.4
Lippia multiflora	117.00 ±55.4	211.50± 0.3*	16.25± 4.7	3777.5± 320.1*	4269.5± 33.3*	3836.5± 221.9	4826.5± 285.5*	16.00± 2.9	16.00± 2.9	400.00± 0.00
Ocimum grattissimum	339.00 ±27.3*	116.0± 54.9	16.00± 2.9	3649.0± 262.0*	4452.8± 45.1*	4204.0± 258.1	4654.8± 228.9*	11.00± 5.8	16.00± 2.9	403.75± 2.4
Morinda morindoides	340.0± 27.0*	239.25± 27.3*	16.25± 4.7	5822.5± 225.5*	4433.00± 57.7*	4827.50± 286.7	5347.3± 25.3*	18.50± 2.5	8.50± 2.5	402.50± 1.4
Control	116.25 ±54.9	68.75± 47.5	8.50±4.8	2492.00± 279.0	2767.00± 290	3494.25± 240.0	2696.25± 300.6	13.50± 4.8	6.00± 2.9	403.75± 2.4

REPRODUCTIVE TOXICITY STUDY ON MALE RATS

TABLE 9B: EFFECTS OF 1600MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM CHARACTERISTIC OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

EXTRACT OF PLANT	SPERM MOLITY	LIVE/DEAD	SPERM VOLUME	SPERM COUNTS
Tithoina diversifolia	62.50±7.5*	97.25±0.1	5.20±0.0	69.75±0.5*
Acalypha wilkesiana	85.00±5.0	96.50±0.9	5.30±0.0	71.50±3.5*
Lippia multiflora	92.50±1.4	98.00±0.0	5.15±0.0	68.00±2.9*
Ocimum grattissimum	50.00±7.1*	92.50±1.4	5.18±0.0	68.25±3.8*
Morinda morindoides	12.50±7.5*	70.00±5.8	5.18±0.0	35.50±2.4*
Control	93.75±1.3	98.00±0.0	5.18±0.0	129.00±5.0

- Superscripted items are significant at P<0.05.

ANTITRYPANOSOMAL STUDY

TABLE 10: EFFECTS OF 400MG/KG DOSE OF THE PLANTS EXTRACTS ON TRYPANOSONAL PARASITAEMIA LEVEL AND PERIOD OF SURVIVAL OF THE RATS

(MEAN ± SEM) PERIOD OF SURVIVAL

	DAY 1	DAY 3	DAY 6	DAY 7	DAY 8	DAY 9	SURVIVAL PERIODS IN DAYS
Tithoina diversifolia	5.40±0.3	2.60±1.1	5.80±0.2	8.20±0.8	8.20±0.8	9.00±0.0	7.40±0.40
Acalypha wilkesiana	6.00±0.0	3.20±1.3	5.60±0.3	9.00±0.0	9.00±0.0	9.00±0.0	7.00±0.0
Lippia multiflora	5.60±0.3	5.80±0.8	6.20±0.7	8.20±0.8	8.20±0.8	9.00±0.0	6.60±1.0
Ocimum grattissimum	6.00±0.0	6.60±0.6	6.60±0.6	9.00±0.0	9.00±00	9.00±0.0	6.20±0.8
Morinda morindoides	6.00±0.0	3.80±0.7	6.00±0.0	7.80±0.7	7.80±0.7	7.80±0.7	8.20±0.7
Artesinate	5.60±0.3	5.40±0.3	5.20±0.2	9.00±0.0	8.40±0.6	8.40±0.0	7.40±0.4
Diminazene aceturate	6.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	15.00±0.0
Untreated control	5.20±0.2	6.00±0.0	6.00±0.0	9.00±0.0	9.00±0.0	9.00±0.0	7.40±0.4

ANTI-INFLAMMATORY STUDY

TABLE 13: EFFECTS OF 400MG/KG DOSE OF THE 5 PLANTS EXTRACTS ON SERUM CHARACTERISTIC OF RATS.

(MEAN ± STANDARD ERROR OF MEAN)

	CBF	**CAF**	**% INHIBITION OF SEIN P.VOL**
Tithoina diversifolia	2.82±0.1	3.03±0.1*	62.3±13.0%
Acalypha wilkesiana	2.83±0.2	2.98±0.0	74.1±10.0
Lippia multiflora	2.90±0.5	3.13±0.3	54.2±16%
Ocimum grattissimum	2.78±0.2	3.10±0.6	45.4±21.0
Morinda morindoides	2.82±0.7	3.02±0.7	65.5% ± 12.0
Aspirin	3.03±0.0	3.23±0.4	65.5%±12.0
Indomethacine	2.90±0.0	2.97±0.0	85.3±3.0%
Untreated control	2.82±0.0	3.38±0.0	0.00%

N.B

CBF: Circumference of the rat paw at "O Hr" of injection of Carrafenan

CAF: Circumference of the rat paw at "3Hrs" after carrafenana injection

% inhibition of increase in rat paw volume.

ORIGINAL UNPROCESSED DATA

MORPHOLOGY
DOSE: 400mg/kg

1. Control.

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	total
G1	21	21	21	2312	3212	3232	3221	21	11	410
G2	212	21	1	2212	3321	4212	2221	11	1	405
G3	21	212	11	3321	2323	3222	3211	21	1	400
G4	211	21	1	2123	2212	3311	2132	1	11	400
G5										
X										
SD										
Sem										

2. *Tithoniadiversifolia*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
A1	21	211	21	2213	3211	3221	3321	1	21	405
A2	211	21	11	3211	221	2214	3222	11	1	400
A3	212	322	1	2212	213	2222	2212	21	1	400
A4	321	21	21	2211	2212	3212	3212	11	1	400
A5										
X										
SD										
Sem										
't'										

3. *Acalphawilkesiana*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
B1	211	21	21	4321	4321	2213	3342	21	21	410
B2	212	21	11	3321	3221	3231	2322	11	1	410
B3	21	212	1	3322	3312	2212	3421	21	11	405
B4	321	221	11	4221	3222	3421	3323	21	11	400
B5										
X										
SD										
Sem										
't'										

4. *Lippiamultiflora*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
C1	21	212	21	3231	2332	3321	3323	11	21	405
C2	212	211	1	3221	3321	3421	4212	1	1	400
C3	211	321	11	3212	3213	3321	3431	21	11	400
C4	211	21	11	4221	3322	4321	4322	1	1	400
C5										
X										
SD										
Sem										
't'										

5. *O. grattissimum*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
D1	211	211	21	3221	4321	3222	3442	21	21	400
D2	212	212	1	2212	4332	3321	4321	11	1	405
D3	321	21	21	3421	5321	4221	4323	1	1	400
D4	212	212	1	2212	4332	4221	4321	11	1	400
D5										
X										
SD										
Sem										
't'										

5. *O. grattissimum*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
F1	211	213	21	4212	3431	4521	3415	1	21	400
F2	221	211	11	4332	4332	4324	4323	21	11	410
F3	211	213	21	4212	3431	4521	3415	1	21	400
F4	21	321	1	3313	4421	4323	4333	21	1	400
F5										
X										
SD										
Sem										
't'										

Dose 800mg/kg

1. *Tithoniadiversifoliab*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coi tail
A1	212	211	21	3221	3212	4332	4312	11	1
A2	21	211	21	3211	3212	4332	4312	11	1
A3	212	21	11	3221	2223	3231	3321	21	1
A4									
A5									
X									
SD									
Sem									
't'									

2. *Acalphawilkesiana*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
B1	211	21	21	4321	4321	2213	3342	21	21	410
B2	21	311	21	3221	3322	4212	3222	21	21	405
B3	212	211	11	3321	3213	3221	3231	11	1	400
B4	212	211	11	3321	3213	3221	3231	11	1	400
B5										
X										
SD										
Sem										
't'										

3. *Lippiamultiflora*

S/N	Headless tail	Tailless head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
C1	321	212	21	3231	2233	3323	4212	21	11	405
C2	321	212	21	3231	2233	3323	4212	21	11	405
C3	211	221	1	4212	4221	4321	3314	1	1	400
C4	211	21	1	4212	4221	4321	3314	1	1	400
C5										
X										
SD										
‘t’										

6. *O. grattissimum*

S/N	Headless tail	Tailless head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
D1	21	212	21	3321	4421	3421	4421	21	21	400
D2	212	211	11	4321	4521	4322	4321	1	11	405
D3	211	321	1	4333	4332	4331	3323	11	1	400
D4	212	211	11	4321	4521	4322	4321	1	11	400
D5										
X										
SD										
‘t’										

7. *M.morindoidess*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
F1	212	212	21	4341	4321	4432	4213	21	21	400
F2	321	211	21	4322	3322	4212	4412	11	1	400
F3	211	21	11	4532	4323	5212	4432	21	11	400
F4	212	211	11	5321	3323	4323	5211	1	11	400
F5										
X										
SD										
't'										

DOSE 1600mg/kg

1. *Tithoniadiversifolia*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
A1	321	21	11	3211	3212	4332	4312	11	1	400
A2	322	221	11	4321	5432	4332	4322	21	21	400
A3	322	221	11	4321	5432	4332	4322	21	21	400
A4	321	221	21	3211	2212	3212	2212	21	1	400
A5										
X										
SD										
Sem										
't'										

2. *Acalphawilkesiana*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
B1	212	211	21	3432	4321	4342	4323	21	11	400
B2	211	212	11	3431	4432	5321	4232	11	21	405
B3	321	322	21	5433	3323	4431	4343	11	1	400
B4	213	212	21	4332	4321	3422	5332	21	11	410
B5										
X										
SD										
Sem										
't'										

3. *Lippiamultiflora*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	total	
C1	21	211	21	4332	4212	4221	5321	21	21	400	
C2	213	212	2	3223	4322	3452	4332	11	11	400	
C3	21	212	21	3223	4212	4221	4332	11	11	400	
C4	213	211	21	4332	4332	3452	5321	21	21	400	
C5											
X											
SD											
Sem											
't'											

4. *V. grattissimum*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
D1	312	211	21	3421	4523	4521	4521	21	11	400
D2	421	21	11	3322	4431	3441	5332	1	21	400
D3	311	21	11	4432	4334	4333	4333	1	21	405
D4	312	211	21	3421	4523	4521	4433	21	11	410
D5										
X										
SD										
Sem										
't'										

5. *C. indica*

S/N	Headless tail	Tailess head	Rundmerty tail	Bent tail	Curved tail	Bent midpiece	Curved midpiece	Looped tail	Coiled tail	Total
F1	313	212	21	5432	4333	4332	5321	21	11	400
F2	421	321	21	5432	4533	5323	5422	21	1	400
F3	313	212	2	6213	4333	4332	5321	11	11	405
F4	313	212	21	6213	4533	5323	5321	21	11	405
F5										
X										
SD										
Sem										
't'										

CHARACTERISTICS
CONTROL

S/N	motility	Live/dead	volume	Count		
G1	95	98	5.2	125		
G2	95	98	5.2	132		
G3	95	98	5.1	118		
G4	90	98	5.2	141		
G5						
X						
SD						

400MG/KG OF PLANT A

S/N	motility	Live/dead	volume	Count		
A1	90	95	5.1	92		
A2	90	95	5.2	101		
A3	90	95	5.2	96		
A4	90	98	5.2	100		
G5						
X						
SD						
Sem						
‘t’						

800MG/KG

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
A1	50	98	5.2	74		
A2	90	98	5.1	80		
A3	90	98	5.2	74		
A4	50	98	5.1	80		
G5						
X						
SD						
Sem						
‘t’						

1600MG/KG OF A

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	50	98	5.2	70		
2	80	98	5.2	69		
3	70	98	5.2	69		
4	50	95	5.2	71		
5						
X						
SD						
Sem						
't'						

400MG/KG OF B

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	80	90	5.2	83		
2	90	98	5.2	90		
3	90	98	5.2	81		
4	60	98	5.1	77		
5						
X						
SD						
Sem						
't'						

800MG/KG OF B

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	70	95	5.2	70		
2	80	90	5.2	83		
3	80	98	5.1	83		
4	70	95	5.2	70		
5						
X						
SD						
Sem						
't'						

1600MG/KG OF B

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	70	95	5.2	65		
2	90	98	5.7	72		
3	90	95	5.2	68		
4	90	98	5.1	81		
5						
X						
SD						
Sem						
't'						

400MG/KG OF C

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	80	95	5.2	78		
2	80	98	5.2	80		
3	90	95	5.1	84		
4	60	85	5.2	72		
5						
X						
SD						
Sem						
't'						

800MG/KG OF C

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	60	95	5.2	63		
2	60	98	5.2	58		
3	60	95	5.2	63		
4	60	98	5.2	58		
5						
X						
SD						
Sem						
't'						

1600MG/KG OF C

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	90	98	5.2	63		
2	95	98	5.1	73		
3	90	98	5.2	63		
4	95	98	5.1	73		
5						
X						
SD						
Sem						
't'						

400MG/KG OF D

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	80	95	5.2	78		
2	90	98	5.2	85		
3	90	98	5.2	89		
4	80	95	5.2	78		
5						
X						
SD						
Sem						
't'						

800MG/KG OF D

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	90	98	5.2	70		
2	90	95	5.2	74		
3	80	95	5.1	82		
4	90	98	5.1	70		
5						
X						
SD						
Sem						
't'						

1600MG/KG OF D

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	40	90	5.2	64		
2	50	95	5.1	60		
3	40	90	5.2	73		
4	70	95	5.2	76		
5						
X						
SD						
Sem						
't'						

400MG/KG OF F

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	40	85	5.2	40		
2	0	80	5.1	44		
3	0	90	5.1	47		
4	50	90	5.2	52		
5						
X						
SD						
Sem						
't'						

800MG/KG OF F

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	50	90	5.2	42		
2	60	95	5.1	40		
3	30	90	5.1	38		
4	50	85	5.1	43		
5						
X						
SD						
Sem						
't'						

1600MG/KG OF F

S/N	MOTILITY	LIVE/DEAD	VOLUME	COUNT		
1	30	80	5.2	38		
2	20	80	5.2	41		
3	0	60	5.2	30		
4	0	60	5.1	33		
5						
X						
SD						
Sem						
't'						

ANTIDIABETIC STUDY
5OOMG/KG OF A

S/N	BLOOD GLUCOSE D1	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	612.0	111.6	130.3	113.1	54.2	53.5
2	608.4	108.0	125.2	76.0	51.4	78.6
3	579.6	100.8	126.7	108.6	53.0	52.0
4	586.8	104.4	128.4	110.5	52.8	53.6
5	579.6	99.0	127.5	94.5	52.9	55.6
6	540.0	93.9	126.5	109.2	50.2	54.4
SD						
Sem						
't'						

500MG/KG OF B

S/N	BLOOD GLUCOSE D1	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	162.0	77.4	121.8	73.1	40.1	67.1
2	165.6	97.2	121.2	91.4	45.6	57.3
3	171.0	90.0	122.4	84.2	42.8	62.6
4	167.4	75.6	120.3	82.3	44.1	59.7
5	162.0	72.0	123.6	87.8	45.2	60.4
6	180.0	84.1	121.5	86.6	39.9	63.1
SD						
Sem						
't'						

500MG/KG OF C

S/N	BLOOD GLUCOSE D1	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	289.8	351.0	116.7	87.4	35.1	64.4
2	594.0	345.6	136.0	74.3	36.6	84.5
3	597.6	397.8	137.7	84.0	36.0	84.9
4	601.2	345.6	126.4	86.5	38.3	81.4
5	597.6	396.4	136.8	86.9	40.1	79.1
6	558.0	269.6	129.0	81.6	37.4	74.6
SD						
Sem						
't'						

500MG/KG OF D

S/N	BLOOD GLUCOSE D0	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	612.0	129.6	129.2	86.9	35.0	77.1
2	615.6	322.2	155.2	74.3	48.1	92.1
3	655.2	336.6	140.3	88.2	36.6	86.1
4	579.6	129.6	142.0	87.4	43.2	81.8
5	619.2	133.4	130.0	85.5	39.7	73.3
6	324.5	119.5	128.7	74.2	45.0	68.9
SD						
Sem						
't'						

500MG/KG OF F

S/N	BLOOD GLUCOSE D0	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	243.0	111.6	137.5	87.4	57.1	62.9
2	325.8	97.2	130.3	61.1	41.4	76.7
3	291.6	108.0	133.9	91.4	48.3	67.3
4	327.6	97.2	131.4	87.9	44.3	69.5
5	243.4	108.1	135.0	78.7	56.9	62.3
6	235.8	95.9	132.8	73.5	58.2	59.6
SD						
Sem						
‘t’						

CONTROL 1

S/N	BLOOD GLUCOSE D0	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	236.6	73.8	132.8	100.0	44.6	68.2
2	259.2	81.0	137.7	76.0	48.0	74.0
3	252.0	75.6	130.4	99.6	57.0	53.4
4	288.0	72.0	136.3	86.4	42.2	76.8
5	333.0	79.2	129.8	80.5	48.0	65.8
6	243.0	91.5	137.8	76.2	44.1	74.0
SD						
Sem						
‘t’						

CONTROL 2

S/N	BLOOD GLUCOSE D0	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	277.2	430.2	170.7	76.9	26.9	127.7
2	288.0	293.4	161.6	95.4	23.4	119.2
3	279.0	311.4	154.3	98.6	28.6	106.0
4	295.2	329.4	153.6	75.2	25.2	113.3
5	252.0	277.2	161.4	83.0	23.0	121.8
6	225.6	305.6	156.1	74.3	27.1	113.9
SD						
Sem						
‘t’						

CONTROL 3

S/N	BLOOD GLUCOSE D0	BLOOD GLUCOSE D21	SERUM CHOLESTEROL	SERUM TRIGLYCERIDE	SERUM HDL	SERUM LDL
1	117.0	98.2	148.6	108.6	34.6	91.4
2	111.6	108.0	124.3	89.7	35.7	70.6
3	102.6	117.0	126.0	73.7	48.6	60.4
4	117.4	113.4	121.4	90.2	46.0	57.4
5	113.4	99.8	128.3	100.0	44.4	63.9
6	117.1	111.6	124.3	89.0	35.5	70.8
SD						
Sem						
‘t’						

SERUM
800MG/KG
Tithoniadiversifoliab

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^{3}$/ml	Neutro $x10^{3}$/ml	Mono $x10^{3}$/ml	Baso $x10^{3}$/ml	Eosino $x10^{3}$/ml	Hb	MCH	MCV	MCHC
A1	30	4.7	7.0	62	38	-	-	-	10.2	21.7	63.8	.34
A2	28	4.4	5.6	70	30	-	-	-	9.3	21.1	63.6	33.2
A3	24	3.8	5.3	60	38	2	-	-	7.9	20.8	63.2	32.9
A4	22	3.5	6.9	69	31	-	-	-	7.4	21.1	62.8	33.6
A5	28	4.7	5.6	69	32	-	-	-	-	-	-	-
X	26	4.22	6.08	66	33.8	0.4	0	0				
SD	3.28	0.54	0.80	4.63	3.89	0.89	0	0				
Sem												
't'	0.78	0.95	0.002 *	0.24	0.2	1	0	0				

4. *Acalphawilkesiana*

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^{3}$/ml	Neutro $x10^{3}$/ml	Mono $x10^{3}$/ml	Baso $x10^{3}$/ml	Eosino $x10^{3}$/ml	Hb	MCH	MCV	MCHC
B1	27	4.2	4.5	80	20	0	0	0	9.1	21.6	64.3	33.7
B2	34	5.3	2.6	53	44	2	0	0	11.3	21.3	64.2	33.2
B3	28	4.4	3.9	70	30	0	0	0	9.3	21.1	63.6	33.2
B4	25	3.9	3.8	70	29	0	0	0	8.1	20.8	64.1	32.4
B5	28	4.3	3.4	68	40	0	0	0	-	-	-	-
X	28.4	4.42	3.62	68.2	32.6	0.4	0	0				
SD	3.00	0.47	0.62	8.68	8.52	0.8	0	0				
Sem												
't'	0.52	0.59	0.83	0.24	0.33	1	0	0				

5. *Lippiamultiflora*

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^{3}$/ml	Neutro $x10^{3}$/ml	Mono $x10^{3}$/ml	Baso $x10^{3}$/ml	Eosino $x10^{3}$/ml	Hb	MCH	MCV	MCHC
C1	30	4.7	9	72	28	0	0	0	10.2	21.7	63.8	.34
C2	29	4.6	2.8	64	36	0	0	0	9.8	21.3	63.0	33.8
C3	27	4.2	2.5	73	27	0	0	0	9.1	21.7	64.3	33.7
C4	29	4.6	2.5	70	28	1	1	0	9.8	21.3	63.0	33.8
C5	28	4.4	2.6	69	34	0	0	0	-	-	-	-
X	28.6	4.5	3.88	69.6	30.6	0.2	0.2	0				
SD	1.01	0.18	2.56	3.14	3.67	0.4	0.4	0				
't'	0.35	0.33	0.89	0.05*	0.08	0.67	0.37	0				

8. *V. grattissimum*

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^{3}$/ml	Neutro $x10^{3}$/ml	Mono $x10^{3}$/ml	Baso $x10^{3}$/ml	Eosino $x10^{3}$/ml	Hb	MCH	MCV	MCHC
D1	26	4.1	3.4	65	35	0	0	0	8.8	21.5	63.4	33.8
D2	22	3.5	2.4	80	20	0	0	0	7.5	21.4	62.8	34.1
D3	25	3.9	2.7	65	45	0	0	0	8.4	21.5	64.1	33.6
D4	24	3.8	2.5	75	25	0	0	0	8.1	21.3	63.2	33.8
D5	23	3.7	2.6	70	30	0	0	0	-	-	-	-
X	24	3.8	2.72	71	31	0	0	0				
SD	1.41	0.2	0.35	5.83	8.60	0	0	0				
't'	0.12	0.13	0.89	0.05*	0.23	0.37	0	0				

9. *Tithoniadiversifolia*

S/N	PCV (%)	RBC (x10 ^{12}g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCV	MCH	MCHC
E1	35	5.5	4.2	81	19	0	0	0	11.8	21.5	63.6	33.7
E2	29	4.6	3	65	25	0	0	0	9.8	21.3	63.0	33.8
E3	34	5.3	4.3	82	18	0	0	0	11.5	21.7	64.2	33.8
E4	26	4.1	4	70	30	0	0	0	8.8	21.5	63.4	33.8
E5	35	5.5	4.1	80	20	0	0	0	11.7	21.3	63.6	33.4
X	31.8	5	3.92	75.6	22.4	0	0	0				
SD	3.66	0.56	0.47	6.82	4.49	0	0	0				
‘t’	0.08	0.07	0.63	0.01*	0.004*	0.37	0	0				

8. *C. indica*

S/N	PCV (%)	RBC (x10 ^{12}g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCH	MCV	MCHC
F1	24	3.8	4.3	78	22	0	0	0	8.2	21.6	63.2	34.2
F2	25	3.9	3.7	60	40	0	0	0	8.5	21.7	64.1	.34
F3	29	4.6	5.1	75	25	0	0	0	9.8	21.3	63	33.8
F4	29	4.6	3.1	77	20	1	1	1	9.8	21.3	63	33.8
F5	39	6.1	2.6	73	27	0	0	0	13.2	21.6	63.9	33.8
X	29.2	4.6	3.76	72.6	26.8	0.2	0.2	0.2				
SD	5.31	0.82	0.88	6.53	7.03	0.4	0.4	0.4				
‘t’	0.49	0.47	0.89	0.03*	0.05*	0.67	0.37	0.37				

DOSE 1600mg/kg

6. Control S

S/N	PCV (%)	RBC (x10^{12}g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCH	MCV	MCHC
G1	27	4.2	3.7	57	43	0	0	0	9.2	21.9	64.3	34.1
G2	24	3.8	5.2	60	40	0	0	0	8.4	22.1	63.2	0.35
G3	28	4.4	2.9	70	28	2	0	0	9.5	21.6	63.6	33.9
G4	24	3.8	3	66	34	0	0	0	8.4	22.1	63.2	0.35
G5	32	5	3.6	55	45	0	0	0	10.6	21.2	64.0	33.1
X	27	4.24	3.68	61.6	38	0.4	0	0				
SD	2.97	0.45	0.82	5.60	6.22	0.8	0	0				
Sem												

2.Tithoniadiversifolia

S/N	PCV (%)	RBC (x10^{12} g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCH	MCV	MCHC
A1	21	3.3	4.8	68	42	0	0	0	7.1	21.5	63.6	33.8
A2	26	4.1	5.5	60	40	0	0	0	8.8	21.5	63.4	33.8
A3	28	4.4	6.1	74	25	1	0	0	9.3	21.1	63.6	33.2
A4	23	3.6	3.8	65	35	0	0	0	7.8	21.7	63.8	33.9
A5	24	4	4.5	67	40	0	0	0	-			
X	24.4	3.88	4.94	66.8	36.4	0.2	0	0				
SD	2.42	0.39	0.80	4.53	6.15	0.4	0	0				
Sem												
't'	0.21	0.26	0.05*	0.19	0.72	0.67	0	0				

3.Acalphawilkesiana

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^{3}$/ml	Neutro $x10^{3}$/ml	Mono $x10^{3}$/ml	Baso $x10^{3}$/ml	Eosino $x10^{3}$/ml	Hb	MCH	MCV	MCHC
B1	23	3.6	4.7	77	23	0	0	0	7.8	21.7	63.8	33.9
B2	27	4.2	4.3	66	34	0	0	0	9.1	21.7	64.3	33.7
B3	23	3.6	3.7	68	32	0	0	0	7.8	21.7	63.8	33.9
B4	28	4.4	3.1	72	28	0	0	0	9.3	21.7	63.6	33.2
B5	25	4	3.9	75	30	0	0	0				
X	25.2	3.96	3.94	71.6	29.4	0	0	0				
SD	2.04	0.32	0.54	4.13	3.77	0	0	0				
Sem												
't'	0.35	0.34	0.61	0.02*	0.05*	0	0	0				

4. *Lippiamultiflora*

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^3$/ml	Neutro $x10^3$/ml	Mono $x10^3$/ml	Baso $x10^3$/ml	Eosino $x10^3$/ml	Hb	MCH	MCV	MCHC
C1	29	4.6	1.7	80	20	0	0	0	9.8	21.3	63	33.8
C2	25	3.9	2.1	65	35	0	0	0	8.4	21.5	64.1	33.6
C3	29	4.6	3.1	75	23	1	1	0	9.8	21.3	63	33.8
C4	28	4.4	1.6	72	28	0	0	0	9.4	21.4	63.6	33.6
C5	28	4.4	1.7	80	20	0	0	0	9.4	21.4	63.6	33.6
X	27.8	4.38	2.04	74.4	25.2	0.2	0.2	0				
SD	1.47	0.26	0.56	5.61	5.71	0.4	0.4	0				
Sem												
't'	0.65	0.60	0.01*	0.01*	0.02*	0.67	0.37	0				

5. *V. grattissimum*

S/N	PCV (%)	RBC (x10^{12}g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCH	MCV	MCHC
D1	27	4.2	1.8	75	25	0	0	0	9.1	21.7	64.3	33.7
D2	32	5	2	82	18	0	0	0	10.8	21.6	64	33.8
D3	25	3.9	2.5	74	25	1	0	0	8.4	21.5	64.1	33.6
D4	28	4.4	2.2	80	20	0	0	0	9.4	21.4	63.6	33.6
D5	22	3.5	1.3	64	36	0	0	0	7.6	21.7	62.9	34.5
X	26.8	4.2	1.96	75	24	0.2	0	0				
SD	3.31	0.50	0.40	6.26	6.25	0.4	0	0				
Sem												
't'	0.93	0.91	0.01*	0.01*	0.02*	0.67	0	0				

6. *Tithoniadiversifolia*

S/N	PCV (%)	RBC (x10^{12} g/l)	WBC (x10^{9}/l)	Lymph x10^{3}/ml	Neutro x10^{3}/ml	Mono x10^{3}/ml	Baso x10^{3}/ml	Eosino x10^{3}/ml	Hb	MCH	MCV	MCHC
E1	25	3.9	4	75	25	0	0	0	8.5	21.8	64.1	.34
E2	25	3.9	2.7	80	20	0	0	0	8.6	22.1	64.1	34.4
E3	25	3.9	2.9	74	26	0	0	0	8.4	21.5	64.1	33.6
E4	32	5	3	71	29	0	0	0	10.8	21.6	64	33.8
E5	30	4.2	2.6	78	27	0	0	0				
X	27.4	4.18	3.04	75.6	25.4	0	0	0				
SD	3.01	0.43	0.50	3.13	3.00	0	0	0				
Sem												
't'	0.85	0.85	0.22	0.004*	0.01*	0.37	0	0				

7. *C. indica*

S/N	PCV (%)	RBC ($x10^{12}$g/l)	WBC ($x10^{9}$/l)	Lymph $x10^3$/ml	Neutro $x10^3$/ml	Mono $x10^3$/ml	Baso $x10^3$/ml	Eosino $x10^3$/ml	Hb	MCH	MCV	MCHC
F1	23	3.6	3.6	63	37	1	0	0	7.8	21.7	63.8	33.9
F2	25	3.9	4.1	65	35	0	0	0	8.6	22.1	64.1	34.4
F3	30	4.7	4.4	67	32	1	0	0	10.3	21.9	63.8	34.3
F4	19	2.9	4	70	30	0	0	0	6.5	22.4	65.5	34.2
F5	24	3.8	3.8	75	22	1	0	2	8.2	21.6	63.2	34.2
X	24.2	3.78	3.98	68	31.2	0.6	0	0.4				
SD	3.54	0.57	0.27	4.20	5.19	0.48	0	0.8				
Sem												
't'	0.26	0.24	0.52	0.11	0.13	0.68	0	0.37				