Trop. Vet. 17, 97-106 (1999)

The Effects of Single and Combined Repetitive Oral Administration of Common Pesticides and Alcohol on Rabbits

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Key words: Pesticides, Karate, alcohol, toxicity, mortality, anaemia, environment

Abstract

The toxicological effects of repetitive single or combined oral administration of a synthetic pyrethroid pesticide (Karate^{R}), nicotine and alcohol (ethanol) in rabbits were studied. Rabbits given daily oral doses of either Karate^{R}, nicotine or ethanol and a combination of either Karate^{R} and nicotine or alcohol exhibited various forms and severities of nervous signs, haematological and serum biochemical alterations, and lungs, liver, heart, kidney and brain damage. Single repetitive nicotine consumption proved most fatal with 75% mortality rate recorded within eight weeks, while combined Karate^{R} and alcohol consumption caused 100% mortality within ten weeks. Severe anaemia, panleucopenia, significant increase in serum gammaglutamyl transferase and hypocholesterolaemia were the hallmarks of severe toxicity with these agents. This study highlights the possible long-term effects of the indiscriminate use of pesticides, and some common environmental pollutants like alcohol on rabbits. The very serious threat to biodiversity conservation efforts in our environment that may result from this is discussed.

Introduction

The use of pesticides (insecticides and herbicides) and other disease-prevention and treatment drugs to grow, preserve and store crops is an age-long tradition in many countries of the world. Adesiyan (1986) reported that about 35% of the world's crops are lost to pests, diseases and weeds every year. The use of pesticides on crops worldwide has grown geometrically over the years because of the ever increasing human and livestock population to be fed. However, the repeated and often indiscriminate use and the extreme persistence of some of these pesticides have led to their accumulation in plants, soil, sediments, animal products and by-products such as milk, meat, egg and urine. Thus, there is widespread contamination of the environment and the eventual poisoning of man, himself.

The economic value and world importance of pesticides as tools for greater agricultural production are likely to persist and even

overshadow the long-term dangers inherent in their use. This is because their residues persist and accumulate in all segments of the biosphere and interact with other chemicals present within the animal (and man) tissue, and the environment, and also with other pesticides, drugs and medicants in plants thereby causing various toxicological effects in man and animals. The prediction of the toxicological consequences of chemical interactions has long been recognized as an important public health problem (Murphy, 1980; Calabrese, 1991).

A synthetic insecticidal pyrethroid, lambdacyhalothrin (Karate^{R}), was introduced into the Nigerian market by ICI in 1984 (ICI, 1984) and has since been enjoying a wide and sometimes indiscriminate usage. There has been little or no in-depth study on its toxic effects in man and/or animals, especially when in cumulative use either singly or in combination with common and equally abused chemical substances such as alcohol (ethanol) and nicotine. This study was carried out to investigate the toxicological effects of repetitive single or combined oral administration of Karate, ethanol and nicotine in rabbits and the implications with regards to environmental pollution and the ultimate effects on human and animal health in future.

Materials and Methods

Animals

Forty-eight (48) eight-week old *Chinchilla* rabbits weighing between 0.80-1.00 kg, consisting of twenty-four males and twenty-four females were used for this study. They were kept each in individual gauzed cages and fed once a day with a 100g commercial pelleted rabbit feed containing 18% crude protein. Clean tap water was provided *ad libitum*. The cages, drinking and feed troughs were cleaned and disinfected with detergent and soap twice weekly. The rabbits had no access to their faeces which usually dropped into regularly cleaned-out troughs below the cages.

Drugs

Lambdacyhalothrin (Karate^{R}) used for this study was purchased from Chemical and Allied Products Ltd. (CAPL), an affiliate of ICI Agrochemical, England. Reagent grade nicotine and ethanol were purchased from Analar (BDH Chemicals, Poole, England)

Experimental Design

The rabbits were randomly assigned to 6 feeding treatment groups (I, II, III, IV, V and VI) of four males and four females each after equilibration of their weights to 1kg/rabbit/group. The experiment lasted for twenty-four weeks. The feeding groups and quantity of feed given per rabbit/day are as follows:

Group I: 100g mashed ration/rabbit/day. These served as control rabbits.

Group II (Karate^{R}): 100g. mashed ration mixed with 50ml of 6,000ppm Karate.

Group III (Nicotine): 100g mashed ration mixed with 1.4g of nicotine.

Group IV (Alcohol): 10ml of 40% alcohol (ethanol) mixed with 100g mashed ration.

Group V (Karate^{R} + nicotine): A mixture of 1.4g nicotine and 6000ppm Karate^{R} in 100g of mashed ration

Group VI (Karate^{R} + alcohol): A mixture of 10ml alcohol and 6000ppm Karate in 100g mashed ration

The dose of nicotene was based on the assumption of a 70kg man smoking six to eight sticks of cigarette (containing 15g nicotine) per day, that is, about 4.76g/kg body weight. The rabbits were given 30% of human dose (approximately 1.4g nicotene/rabbit/day). Similarly, alcohol dosage was based on the assumption of a 70kg man consuming 70cl of Vodka per day, with each rabbit given 10ml/kg body weight of 40% alcohol.

Symptomatology, Haematology, Serum Biochemistry and Tissue Pathology

Symptomatological or behavioural observations were made daily both by distant watching for activities like grooming, physical activities such as body twitching, tremors, convulsions, paralysis, and reactions to stimuli such as sudden exposure to light, sound and touch. 2.5millilitres (ml) of blood per animal was collected into each of plain tubes and those containing the dipotassium salt of ethylene diamine tetraacetic acid (EDTA) through the ear veins weekly for biochemical and haematological studies, respectively. The haematological parameters carried out as described by Jain (1986) include PCV, haemoglobin (Hb) concentration, red blood cell (RBC), total and differential white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The serum biochemical parameters carried out as described by Ogunsanmi *et al.* (1994) include serum sodium (Na^{+}), potassium (K^{+}), Calcium (Ca^{2+}), chloride (Cl^{-}), bicarbonates (HCO_3^{-}), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT). Others include total protein, albumin, globulin, urea, creatinine, cholesterol and triglycerides (Ogunsanmi *et al.*, 1994). Means of group data were compared by the 2-way analysis of variance (ANOVA) (SAS, 1987) and Duncan's multiple range test (Duncan, 1959).

Dead rabbits were examined for gross lesions as soon as possible after death. Samples were collected from the brain, lungs, liver, heart and kidneys, fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 5µ, and stained with haematoxylin and eosin (H&E) for histopathology.

Results

Symptomatological Observations

Except for minor episodes of sneezing in one male rabbit, all the animals in Group I (control) were quite active and behaved normally throughout the experimental period. One male rabbit died of pneumonic lesions on the 38th day of experiment. Rabbits in Group

II (Karate^{R}) were quite active. Six of eight rabbits (75%) in this group developed alopecia of the forelimbs around the fetlock and pastern joints due to excessive grooming. The remaining two were dull when left undisturbed. Excitation of the rabbits usually led to frequent uncontrolled convulsions, paralysis of the hind limbs and twisted claws. These symptoms were observed more frequently in males than females. All the rabbits died within eighteen weeks of the experiment.

Rabbits in Group III (nicotine) were extremely vicious and aggressive, and exhibited voracious appetite. Four out of eight (50%) rabbits died within the first six weeks, usually due to self-mutilation, especially during or immediately after handling, while three (37.5%) died after a prolonged period of extreme dullness and lassitude in the 8th week. One female rabbit survived in this group. Rabbits in Group IV (ethanol) exhibited various degrees of hyperaesthesia, ataxia, incoordination and torticollis, usually starting after about 15 minutes of feeding, especially when disturbed. These symptoms became more severe with increased duration of the study. Six (75%) of eight rabbits died within twenty weeks of the experiment. Two female rabbits survived in this group. Rabbits in Group V (Karate^{R} + nicotine) showed varying degrees of anorexia, dullness and extreme docility. Death was usually precessed by anorexia, prostration and constriction of the pupils (myosis). All the rabbits died within sixteen weeks of the experiment. Rabbits in Group VI (Karate^{R} + alcohol) usually consumed more water than all the other groups, indicating persistent thirst. Most of the rabbits were initially very aggressive but later became dull, paraplegic and anorexic prior to death. Two rabbits showed exophthalmos (protruded eye balls), while another three developed epistaxis before death. All the rabbits in this group died within ten weeks of the experiment.

The haematology, serum biochemistry and organ pathology of the experimental rabbits are as shown on Tables 1, 2 and 3, respectively. The serum biochemical results are presented as the comparison of the values of each serum constituent analyzed in the experimental rabbits in Groups II to VI with their corresponding values in Group I (control) rabbits. The results are shown as no significant changes (-) ($P>0.05$), significant increase ($P<0.05$; up arrow) or decrease ($P<0.05$; down arrow) relative to those of the control rabbits. The gross and histologic lesions in the lungs, liver, heart, kidneys and brain were scored both on the basis of presence, consistency and severity.

Discussion

The results obtained from this study revealed that while single continuous oral administration of lambdacyhalothrin (Karate^{R}), nicotine or alcohol could be lethal to rabbits, combined administration of Karate^{R} + nicotine or Karate^{R} + alcohol was highly fatal. Hyperexcitation of the central nervous system heralded the clinical manifestation of toxicity with either Karate^{R}, nicotine or alcohol. Severity increased with time, later yielding to terminal central nervous system depression and paralysis, and in 75-100% of the cases, death within eighteen to twenty weeks of the feeding trial. However,

Table 1: **Mean haematological parameters of rabbits with single or combined oral administration of Karate, nicotene or alcohol**

Parameter	Groups I	II	III	IV	V	VI
PCV (%)	28.5±1.5*[a]	28.5±1.5[a]	32.5±1.1[a]	30.5±0.7[a]	26.4±0.5[b]	23.5±1.1
Hb (mg/dl)	7.5±0.8[a]	7.5±0.6[a]	7.7±0.3[a]	8.4±0.9[a]	6.4±0.8[b]	6.1±0.8[b]
RBC ($1\upsilon^{12}$/ml)	5.3±0.50[a]	4.9±0.1[a]	5.7±0.7[a]	5.0±0.1[a]	4.0±0.5[b]	3.3±0.2[c]
MCV (fl)	53.8±1.5[c]	58.7±4.6[c]	57.5± 2.1[c]	61.6± 3.7[c]	65.5±1.3[b]	71.7±1.3[a]
MCH (pg)	14.2±1.2[a]	15.4±2.0[a]	13.6±1.1[a]	17.0± 0.9[a]	15.9±0.3[a]	18.6±2.1[a]
MCHC (%)	26.3±1.6[a]	26.3±1.7[a]	23.6±2.4[a]	27.6± 0.9[a]	24.3±2.0[a]	26.0±1.3[a]
Total WBC (& differentials; 10^9/ml) counts	12.0±1.6[ab]	13.1±1.5[ab]	17.4±0.6[a]	14.4±0.8[ab]	9.5±0.9[b]	8.1±1.0[b]
Lymphocytes	3.6±0.2[ab]	3.9±0.1[a]	4.3±0.3[a]	4.2±0.7[a]	2.1±0.6[b]	2.0±0.8[b]
Neutrophils	8.2±0.2[b]	7.9±0.4[b]	12.2±1.1[a]	9.9±0.1[b]	6.6±0.1[c]	5.1±0.3[d]
Eosinophils	0	0.8±0.1[a]	0	0	0.3±0.0[b]	0.6±0.3[a]
Monocytes	0.2±0.1[d]	0.5±0.0[b]	0.9±0.1[a]	0.3±0.0[d]	0.6±0.1[b]	0.4±0.1[c]
Basophils	0	0	0	0	0	0

*Mean ± standard error (n = 8)

Values on the same row with different superscripts differ significantly at $P<0.05$.

Table 2: Serum biochemical changes in rabbits with single or combined oral administration of Karate, nicotene or alcohol

Parameters	Group II	Group III	Group IV	Group V	Group VI
Na^+	↓*	↓	↑	↓	↑
K^+	↓	↑	↓	↓	↓
Ca^{++}	-	↑	↓	↓	↓
Cl^-	-	-	↑	↓	-
HCO_3^-	-	-	↑	-	-
ALP (IU/l)	↓	↑	↓	↓	↓
AST (IU/l)	-	↓	↑	-	↑
ALT (IU/l)	-	↓	↓	↑	↑
GGT (IU/l)	↑	↑	↑	-	↑
Total protein (g/dl)	-	-	↑	-	↑
Albumin (g/dl)	-	↑	-	-	↑
Globulin (g/dl)	↑	-	↑	-	↑
Urea (mg/dl)	-	↑	-	↓	-
Creatinine (mg/dl)	↑	↑	↑	↓	↑
Cholesterol (mg/dl)	↓	↓	↓	↓	↓
Triglyceride (mg/dl)	↓	-	↑	-	↑

* ↑, ↓ or - = Increase, decrease ($P<0.05$) or no significant change ($P>0.05$) in levels compared with corresponding levels in control (Group I) rabbits.

Table 3: **Tissue pathology scores in rabbits with single or combined oral administration of Karate, nicotene or alcohol.**

Organ lesions	Group I	Group II	Group III	Group IV	Group V	Group VI
Lungs						
Congestion	±	++	+++	+	+++	+++
Oedema	-	++	+++	+	+++	+++
Cellular infiltration	±	+	++	-	+++	++
Liver						
Congestion	±	+	+	-	++	++
Fatty change	-	++	++	++	++	+++
Necrosis	-	+++	+++	+	+++	++
Cellular infiltration	-	+++	++	+	+++	++
Fibrosis	-	+	-	+	-	+
Heart						
Congestion	-	++	-	++	+	+++
Haemorrhage	-	-	-	+	-	++
Hyaline degeneration	-	-	+	++	++	++
Kidney						
Congestion	±	-	++	+	+++	+
Nephrosis	-	+	+++	++	+++	++
Cellular infiltration	-	-	++	+	-	++
Fibrosis	-	-	-	++	-	-
Brain						
Meningeal congestion	-	++	+	+++	++	Rapid PM autolysis
Perivascular cuffing	-	+	++	+	++	
Neuronal degeneration	-	+	+	+++	+++	
Malacia	-	+	-	+++	++	
Gliosis	-	+	++	+++	++	

- = Absent; ± = Inconsistent; + = Moderate
++ = Severe; +++ = Very severe.

rabbits that received combined administration of Karate[R] + alcohol and Karate[R] + nicotine showed severe central nervous system depression characterized by extreme dullness, paralysis, and 100% mortality rate within ten and sixteen weeks of feeding, respectively.

The hallmarks of Karate[R] toxicity in the rabbits were severe liver and moderately severe brain lesions, with little or no significant cardiac and renal pathology. The brain lesions led to the nervous signs observed clinically in the rabbits. The main serum enzyme affected was gamma-glutamyl transferase (GGT) which was significantly increased. Hyponatraemia, hypokalaemia and decreased serum lipids in these rabbits may have resulted from hepatotoxic and/or renal pathology (Zilva and Pannall, 1984).

Nicotine, an alkaloid obtained from the leaves of tobacco plants, *Nicotiana tabacum*, is a well known insecticide that is extremely dangerous because of the ease of its absorption even through intact skin (Clarke and Clarke, 1978). However, poisoning in animals, has been associated with the use of the 40% and 1.2% solutions of nicotine sulphate as an anthelminthic and a tsetse fly repellant, respectively (Clarke and Clarke, 1978). In this study, nicotine caused severe liver damage and considerable kidney, lungs and brain lesions. These lesions, especially those of liver and kidneys have resulted in increases in serum ALP and GGT, and decreases in AST and ALT. There was also azotaemia which was also due to renal dysfunction (Finco, 1989) as a result of renal pathology. Nicotine is known to cause tissue anoxia (Radeleff, 1978), hence death usually results from the paralysis of medullary centres in the brain and curare-like respiratory muscle paralysis.

Ethanol used in this study is one of the most abused drugs in our society. While alcohol toxicity in animals may be due to its use in alcohol-soluble medicants, accidental exposure to animals, especially pets, may assume a dangerous proportion. Chronic excessive consumption of alcohol has been shown to cause fatty liver, alcoholic hepatitis and cirrhosis (Robbins *et al*, 1985). In this study, while hepatic lesions were quite moderate, the most severe lesions were observed in the brain and less so in the kidneys and heart. Chronic liver damage is known to cause severe toxic changes, especially hepatic encephalopathy (choleric anger) and renal insufficiency as a result of the direct effect of undetoxified gut-derived endotoxins (Robbins *et al*, 1985) on the brain. The heart is a very dynamic and active organ that is very sensitive to anoxia and direct toxicants in blood. Hence, the cardiac lesions may not be unconnected with the liver, kidney and brain lesions. The changes in serum enzymes, especially increases in serum AST and GGT are indices of hepatic and cardiac damage (Ologhobo *et al.*, 1993).

The most severe tissue/organ damages were observed in rabbits given combined administration of Karate[R] + nicotine or Karate[R] + alcohol. Virtually all the organs were severely affected, with the liver, brain and kidneys showing the most severe lesions. Administration of Karate[R] + alcohol caused the most severe cardiac lesions, liver cirrhosis and rapid postmortem autolysis of the brain. Serum biochemical changes in Karate[R] + nicotine administration include hyponatremia, hypocalcaemia and hypochloridaemia, all of which are indicative of severe derangement of

homeostasis, due probably to severe and widespread damage to body organs (Finco, 1989).

Haematological changes observed in these rabbits are a reflection of tissue pathology, especially of the liver and/or the bone marrow (Jain, 1986). While severe macrocytic hypochromic anaemia was observed in rabbits given combination administration, lymphocytosis, neutrophilia, eosinophilia and monocytosis were observed in single administrations of Karate^{R}, nicotine and alcohol. These changes suggest a possible sub-clinical infection either as a result of organ damage or a compromised immune surveillance, against opportunistic infections such as ectoparasitism and helminthiasis (Obi and Anosa, 1980). Conversely, combined administration of Karate^{R} and nicotine or alcohol caused severe pancytopenia which may be due to bone marrow suppression resulting from toxaemia and/or immunosuppression (Anderson, 1985).

In conclusion, man, domestic and wild animals and birds may be exposed to various substances, either as part of their diets, vapours in their environment, or the substances may be applied to them directly or indirectly for the treatment or control of some diseases or pests. This study has revealed that cumulative consumption of otherwise innocuous or even known and popular pesticides and other substances that have polluted our environment could pose a very serious threat to biodiversity conservation, especially of fauna now and in the near future. Hence, there is a dare need to control the use of some of these substances through appropriate legislation.

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Accepted for publication July 20, 1998.