STUDIES OF ACUTE TOXICITY AND ANTHELMINTIC ACTIVITY OF PYRETHRINS IN SHEEP AND RABBITS

By

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1990

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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J. M. Mbaria

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This thesis has been submitted for examination with our approval as University supervisors.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

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and

children Wanjiku and Mbaria

ABSTRACT

STUDIES OF ACUTE TOXICITY AND ANTHELMINTIC ACTIVITY OF PYRETHRINS IN ANIMALS

Pyrethrins are natural insecticides extracted from the dried flowers of *Chrysanthemum cinerariaefolium* plant. They are considered to be the safest of the insecticides available. Although they are widely used on farm animals for control of external parasites and many trials have been done in relation to their use in the control endoparasites, there is no information on toxicity of pyrethrins in farm animals. Acute toxicity studies of pyrethrins in laboratory animals have been reported, but similar studies have not been done in sheep and information on treatment of pyrethrin toxicity in farm animals is very scanty. If pyrethrins have to increasingly continue being used in the farm, veterinarians should have some idea on their toxicity in farm animals and how such toxicities can be treated. The main objective of the present study was to evaluate the acute toxicity of pyrethrins in sheep and rabbits. The second objective was to investigate *in vitro* and *in vivo* effects of pyrethrins on *Haemonchus contortus*. The final objective was to evaluate the effectiveness of diazepam and pentobarbitone sodium in treatment of pyrethrin induced poisoning in rabbits.

Fourteen adult female red masai sheep and fourty adult newzealand white rabbits were used. The sheep were purchased from a farmer in Kiserian and the rabbits were obtained from Ngong veterinary farm in Kajiado district.

The oral LD_{50}s and their 95% confidence intervals in rabbits were 1,300 (630 to 2,521) mg/kg and 1,500 (1,008 to 2,268) mg/kg b.wt for pyrethrins with piperonyl butoxide and pyrethrins alone respectively. Rabbits previously treated with phenobarbitone sodium, an enzyme inducer, were very resistant to pyrethrin poisoning. The oral LD_{50} was greater than 4,500 mg/kg b.wt. The oral LD_{50} for pyrethrins with piperonyl butoxide in sheep was 600 mg/kg b.wt.

The clinical signs of acute pyrethrins toxicity observed after oral administration in sheep and rabbits were hyperexcitation, tremors, convulsions, paralysis and death.

At postmortem there was extensive pulmonary congestion and oedema and ecchymotic haemorrhages in respiratory and cardiovascular systems. Death was probably due to failure of respiratory and cardiovascular systems in both species.

Haematological and biochemical parameters were evaluated in a control and two treatment groups of sheep using routine laboratory procedures. Administration of pyrethrins orally at 210 mg/kg did not affect haematological parameters in sheep. However, administration of pyrethrins at 420 mg/kg caused a significant decrease in white blood cell counts (WBC), red blood cell counts (RBC) and neutrophils and an increase in packed cell volume and lymphocytes ($p<0.05$). Eosinophils, haemoglobin concentration and mean corpuscular haemoglobin concentration were not affected by pyrethrins in sheep ($p>0.05$).

Administration of pyrethrins orally at 210 and 420 mg/kg did not cause any significant effects on the following

biochemical parameters assayed in serum; aspartate aminotranferase, glutamate dehydrogenase, creatinine and total proteins ($p>0.05$). However pyrethrins caused a significant rise in sorbitol dehydrogenase ($p< 0.05$). The mean serum levels of sorbitol dehydrogenase were 288 for the control, 412 in the group that had received 210 mg/kg b.wt and 473 sigma units for the group that received 420 mg/kg b.wt pyrethrins.

Externally pyrethrins caused slight to moderate ocular irritation in sheep characterised by hyperaemia of conjunctiva, chemosis and lacrimation. On the skin, pyrethrins caused moderate irritation characterised by erythema and oedema.

Trials on the effects of pyrethrins on *Haemonchus contortus* indicated that pyrethrins were lethal to the adult worms and their larvae *in vitro*, were not ovicidal and did not affect faecal egg counts when administered orally at a dose of 168 mg/kg b.wt.

Pyrethrins alone and with the synergist piperonyl butoxide were slightly toxic to both sheep and rabbits, while pyrethrins alone after prior treatment with phenobarbitone sodium, an enzyme inducer were practically non toxic to rabbits.

Although there were some effects on some haematological and biochemical parameters, these effects were not significant in terms of range of values or in overall pattern. Pyrethrins administered orally were not lethal to the worms *in vivo* and this can be explained as being due to rapid decomposition of pyrethrins in the gastrointestinal tract.

It was concluded that pyrethrins have potential anthelmintic activity in farm animals. Acute pyrethrins toxicity can be treated with pentobarbitone sodium and diazepam. These studies indicate that pyrethrins are mild irritants of the skin and eyes and are slightly toxic to mammals.

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1. 1 Introduction

The pyrethrum plant is a small perennial herb growing to a height of up to 75 cm and it becomes woody at the base. The flower heads are produced on branched leafy stems arising from a crown of foliage and are used for the production of the natural insecticides named pyrethrins. It is indigenous in the coastal area of Dalmatia (part of present-day Yugoslavia). The diploid chromosome number is eighteen. A species of pyrethrum was grown in Caucasus and used as the secret basis of the Persian insecticide powder in the early nineteenth century. Around 1840, *Chrysanthemum cinerariaefolium* Vis, the present pyrethrum was grown in Dalmatia and it spread gradually to France, the United States and Japan. Japan was for a long time the world's largest producer until it was displaced by East Africa during the second world war (Glynne Jones, 1962).

Pyrethrum was introduced in Kenya in 1928 where it appeared to be a very suitable crop for the Highlands. After its introduction pyrethrum industry developed rapidly until Kenya became the world's Largest Producer. At the moment, Kenya is still the world's leading producer. Pyrethrum is therefore a very important cash crop in Kenya. A large percentage of Kenya's agricultural population is dependent upon small scale subsistence farming based on traditional techniques. Kenya's development plans emphasize improved

productivity in the small scale farms encouraging production of high valued cash crops which could raise the incomes and also increase the national foreign exchange earning. Pyrethrum satisfies these conditions. It is a highly valued cash crop with good export prospects and it is mainly grown by small scale farmers. In some areas it is the only cash crop for the peasant farmer. Pyrethrum production is labour intensive and thus provides gainful employment to family labour in the pyrethrum growing areas of Kenya. While coffee and tea are Kenya's leading export crops with respect to foreign exchange earnings, pyrethrum and sisal have been competing for the third position.

In Kenya, pyrethrum is grown almost everywhere in the highlands provided the ecological requirements are met. These are deep well drained soils, enough, well distributed rainfall (at least 1,000 mm annually) and sufficient low temperatures (Muturi, *et al.*, 1969). The main pyrethrum growing areas in Kenya include Kisii, Nyandarua, Kiambu, Nakuru, Nyeri, Meru, Embu and Muran'ga districts. These regions can be divided into three;

Area I. Kisii district. Pyrethrum is grown here between 2,159 and 2,220 metres above the sea level. The annual rainfall is from 1,400 to 1,800 mm.

Area II. Dundori range, Mau Narok, Molo, Londiani, Timboroa and east Eldoret. These areas are between 2,159 and 2,930 metres above sea level north of the region Mau Narok - Gilgil - Ol kalou. The annual rainfall varies between 1,000 and 1,400 mm.

Area III. This comprises the foot hills of the Aberdares, the Kinangop area, the higher regions of Kiambu and the foot hills of mount Kenya. Pyrethrum is grown between 2,066 and 2,776 metres above sea level. The annual rainfall varies from 1,100 to 1,500 mm.

Figure 1 (p.4) shows the main pyrethrum growing areas in Kenya.

Pyrethrins are found in all parts of the plant but only the flowers have high content for economical extraction. Within the flowers pyrethrins are predominantly found in the achenes (Head, 1966). The pyrethrins contents of the flowers range from 0.5 to over 2.5% w/w depending on the flowering stage, genotype and weather. Pyrethrins for commercial use are extracted from the dried flowers. The natural insecticide has been accepted world wide for domestic purposes. Although pyrethrum is essentially grown for its insecticidal properties, a by-product of pyrethrins extraction, pyrethrum marc, is used as a cattle feed.

Pyrethrum extracts contain pyrethrins which are organic compounds with an insecticidal action. These active principles in the pyrethrum extract are six esters formed by the combination of three closely related keto-alcohols with two carboxylic acids. The esters are named as pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II.

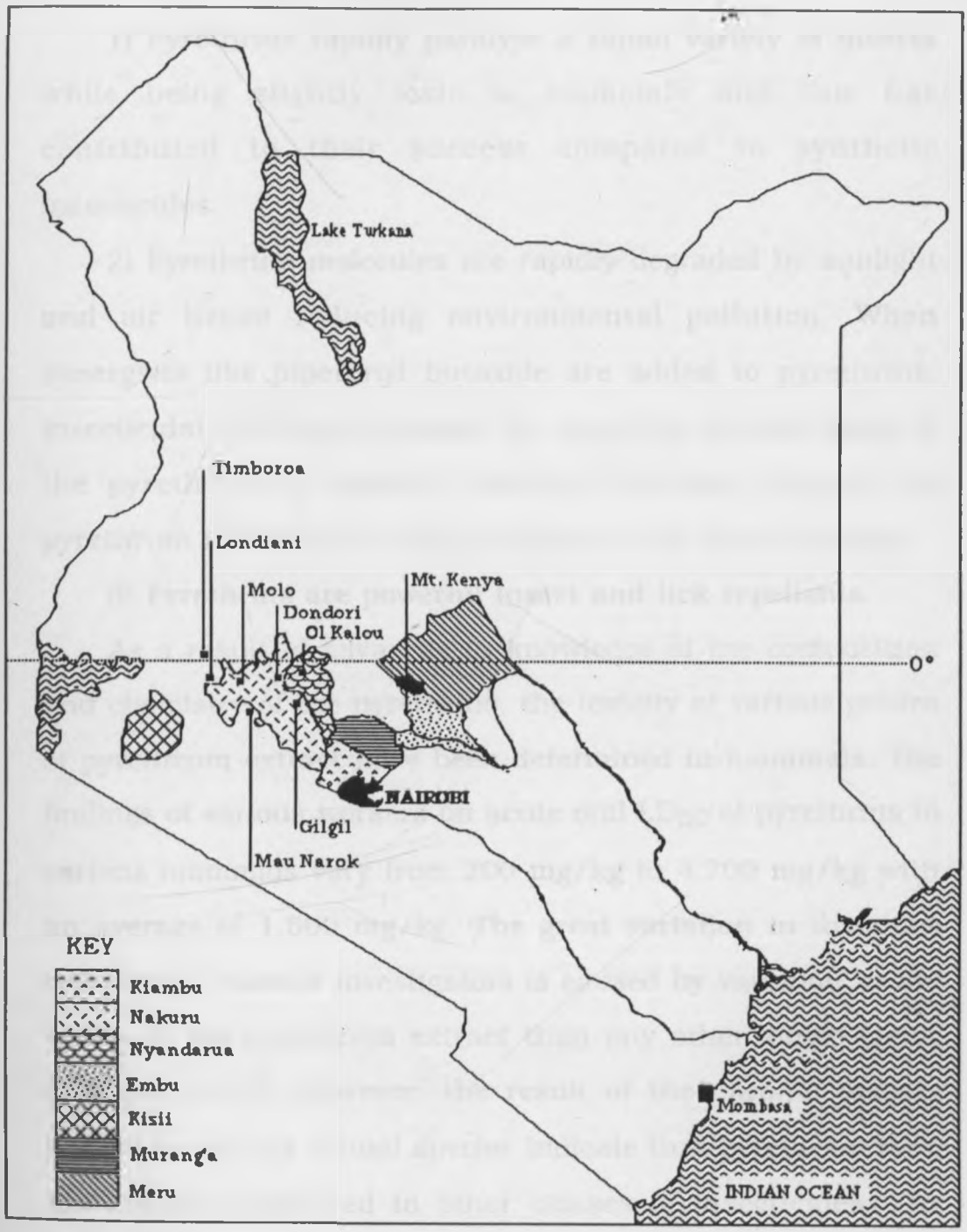


Figure 1: Map showing the main pyrethrum growing areas in Kenya

The outstanding qualities of pyrethrin-based insecticides over other insecticides are;

1) Pyrethrins rapidly paralyse a broad variety of insects while being slightly toxic to mammals and this has contributed to their success compared to synthetic insecticides.

2) Pyrethrins molecules are rapidly degraded by sunlight and air hence reducing environmental pollution. When synergists like piperonyl butoxide are added to pyrethrins, insecticidal effect is increased by retarding detoxification of the pyrethrins in insects. Another important property of pyrethrum is that little insect resistance has been recorded.

3) Pyrethrins are powerful insect and tick repellants.

As a result of advances in knowledge of the composition and chemistry of the pyrethrins, the toxicity of various grades of pyrethrum extract have been determined in mammals. The findings of various workers on acute oral LD_{50} of pyrethrins to various mammals vary from 200 mg/kg to 4,700 mg/kg with an average of 1,500 mg/kg. The great variation in toxicities reported by various investigators is caused by variation in the purity of the pyrethrum extract than any other single factor (William, 1973). However, the result of the chronic toxicity testing in various animal species indicate that pyrethrins have low toxicity compared to other classes of insecticides. The work done on the toxicity of pyrethrins in warm-blooded animals is mainly in rats, mice, rabbits and guinea pigs using various routes of administration. Some pyrethrins toxicity studies have been done in the dog and chicken. This work has shown that pyrethrins are virtually non-toxic to warm-blood

animals. Griffin (1973) noted tremors, ataxia, dyspnoea and salivation in beagle dogs fed pyrethrins at 130-200 mg/kg body weight over a 90-day period. Very little work has been done on the toxicity of pyrethrins in the ruminants. High parenteral fatality of pyrethrins have been demonstrated. In rats Kavlock *et al.*, (1979) found an LD_{50} of 4 mg decamethrin/kg body weight when given intravenously which emphasizes the toxicity of pyrethrins. The low oral toxicity in rodents is probably due to the rapid metabolism and detoxification in the gastrointestinal tract. Laboratory animals could be uniquely resistant to pyrethrins toxicity like many other substances which show different responses in different individuals and species due to inter individual and inter species variations. It is assumed that the low sensitivity of mammals to orally administered pyrethroids (synthetic pyrethrins) is partly attributed to rapid metabolic degradation and poor absorption from the gastrointestinal tract (Miyamoto, 1976; Soderlund and Casida, 1977 a, b). It is not clear at present whether other factors are involved. To conclude fully that pyrethrins are virtually non-toxic to mammals further studies should be conducted in a wide range of species such as laboratory animals, monogastric and ruminant animals. So the main objective of this study was to conduct controlled experiments on acute toxicity of pyrethrins in sheep since this has not been done before. Finally, the low toxicity of pyrethrins to mammals has stimulated research on the anthelmintic activity of pyrethrins but only isolated excerpts from literature are available and more research on this property is required.

1. 2 Objectives

The objectives of this study were;

1) To determine 24 hours oral median lethal dose (LD_{50}) of pyrethrins in rabbits and sheep.

2) To observe the clinical signs, effects on haematology and biochemical parameters, and the postmortem lesions in red masai sheep fed pyrethrins orally.

3) To perform dermal and ocular irritation tests in sheep.

4) To investigate the effects of pyrethrins on eggs, larvae and adult *Haemonchus contortus*.

5) To test the effectiveness of pentobarbitone sodium and diazepam in the treatment of pyrethrin induced poisoning in rabbits.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background of the pyrethrum plant and the development of pyrethrum industry in Kenya

2.1.1 History of the pyrethrum plant

The use of pyrethrum flowers for insecticidal purposes apparently originated from Persia (Gnadinger, 1936). Great secrecy is said to have surrounded the early use and preparation of the material which no doubt accounts in part for the difficulty in fixing the date of the discovery of its insecticidal activity. Various writers agree that pyrethrum powder was introduced in Europe early in the 19th century by an American merchant who discovered the secret of its preparation while travelling in the Caucasus. A second version is that the secret was revealed to the Russians by military prisoners. Yet a third source is attributed to a woman in Dalmatia (part of present-day Yugoslavia) who picked the flowers for their beauty and after withering, she threw the flowers into a corner. Several weeks later she found that the flowers were surrounded by dead insects. She associated the death of the insects with the insecticidal properties of the flowers and embarked on the business of manufacturing pyrethrum powder. The flowers were indigenous along the coast of Yugoslavia.

From Dalmatia, the pyrethrum plant spread to France, Switzerland, United States and Japan (Hawkins, 1972). As a result of the 1914-1918 war, Japan outstripped Dalmatia as a

major producer of pyrethrum. However, when Japan entered the second world war, production declined heavily and Kenya became the world's largest producer of pyrethrum flowers.

2.1.2 Introduction and development of pyrethrum industry in Kenya

Pyrethrum was introduced in Kenya in 1928 by G. Walker, T. J. Anderson and V. A. Beckley (Beckley *et al.*, 1938; Chandler, 1948; Le Pelly, 1973). Walker attempted to grow it on commercial basis whereas Anderson and Beckley raised it for experimental purposes at the Scott Agricultural Laboratories (now the National Agricultural Laboratories) in Nairobi. Seeds produced at the Scott Laboratories were distributed to a number of farms ranging in altitude from 1,542 metres to 2,930 metres above sea level. By 1931, it became apparent that pyrethrum was suited for cooler districts of Kenya. By 1933, the pyrethrum industry had grown and required an organization to promote the marketing of the crop. In 1936, the Pyrethrum Board of Kenya was set up to regulate the industry, including compulsory registration of growers and grading of the product.

In the years preceding the second world war, Production increased steadily and the outbreak of the war created an even greater demand for the pyrethrum products (Mclaughlin, 1973). When the world war cut off pyrethrum supply from Japan, Kenyan pyrethrum which was superior in pyrethrins content replaced the Japanese product in the market. After the war, the demand for pyrethrum dropped drastically, with accompanying decline in production. The industry received a

further fatal blow after introduction of synthetic insecticides in the world market and accompanying aggressive sales propaganda and general discouragement of indiscriminate use of pesticides after publication of Rachel Carson's "The silent spring" (Maclaughlin, 1973). Production remained low for several years, till the "Mark Commission"- the commission that evaluated pesticides in relation to environmental health cautioned on the need to evaluate synthetic insecticides which were flooding the world market. Being a natural insecticide with minimal residue effects and practically non-toxic to mammals, pyrethrins gained acceptance. Kenya thus continued to expand production of the pyrethrum plant the source of pyrethrins.

Previously, the crop was mainly grown by European settlers but the Swynnerton plan of 1954 permitted Africans to grow the crop (Muturi, 1976). When land changed hands from the European settlers to Africans after independence, more Africans entered the industry. This change in land ownership retarded production for a time, but the industry soon gained momentum. In the years that followed, production in the small scale farming sector was intensified and accounted for more than 90% of the crop grown in the country which is the same situation today. At the moment, Kenya is still the world's leading producer of pyrethrum. Other significant world producers include Tanzania, Ecuador, Rwanda and Japan (Glynne-Jones, 1973). The main growing areas in Kenya include Kisii, Nyandarua, Kiambu, Nakuru, Nyeri, Meru, Embu and Muran'ga districts as shown in Figure 1 (p. 4).

2.2 Composition of pyrethrum extract

2.2.1 Non-pyrethrins ingredients

Compounds present in the unrefined pyrethrum extract (oleoresin) include sesquiterpenes, flavonoids, triterpenols, sterols, alkanes, fatty acids and carotenoids in addition to pyrethrins. These non-pyrethrins ingredients are progressively removed during the refining process.

Pyrethrosin (chrysanthin) was the first sesquiterpene identified in pyrethrum flowers in 1937. The other compounds include sesquiterpenoids lactones chrysanolide and sesamin. The relative proportion of these materials in the pyrethrum flowers is unknown but pyrethrosin predominates. Pyrethrosin has low solubility in light petroleum and only low levels are found in pyrethrum extract (Stafford, 1973).

The flavonoids detected in pyrethrum extract include the 7-glucosides and 7-glucuronides of apigenin, luteolin, quercetin and also the 3,6-dimethyl ether and 3,6,4-trimethyl ether of quercetagetin (Glennie and Harbone 1972).

The triterpenols and sterols that are present in pyrethrum include pyrethrol, β-amyrin and taraxasterol.

The alkane fraction from pyrethrum extract include all members of the series from n-C_{24} to n-C_{36}. The major components are n-heptacosane, n-nonacosane and n-hentriacontane. The total alkane content of pyrethrum oleoresin concentrate is about 4% w/w by weight and this is reduced to 0.2% w/w in refined extract.

Pyrethrum extract contains a number of free fatty acids. In a refined extract, the combined fatty acids are reduced to around 20% w/w of the extract composition, of which some 12% are non-hydroxy fatty acids.

The carotenoid content of the concentrate is between 0.8 and 0.9% w/w, expressed in terms of free xanthophyll or between 1.3 and 1.5% w/w, expressed as the naturally occurring esters. In the refined extract the carotenoid level is reduced to approximately 0.04% w/w. Table 1 shows the component of pyrethrum oleoresin and refined extract.

Table 1: The known components of pyrethrum oleoresin and refined extract

Component	Approximate percent composition, w/w	
	Oleoresin	Refined
Total pyrethrins	30-35	50-55
Non-hydroxy fatty acids	20	12
Hydroxy fatty acids	20	8
Alkanes	4	0.4
Carotenoids(as free xanthophylls)	0.8	0.04
Sterols and triterpenols	5	3
Sequiterpenes	trace	unknown
Flavonoids	trace	unknown
Chlorophylls	0.1	nil
Ceryl alcohols	unknown	unknown

2.2.2 Pyrethrins

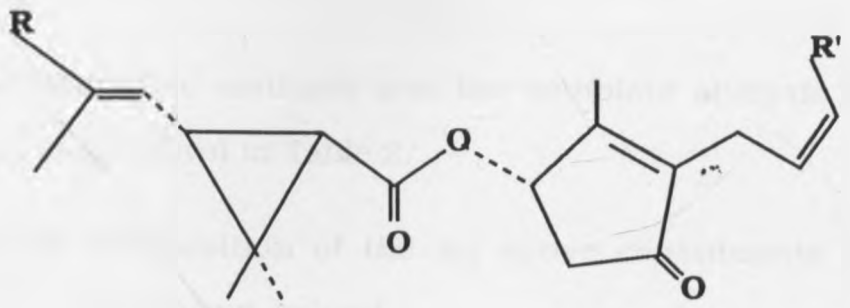
The insecticidal constituents of the pyrethrum flowers are collectively called pyrethrins (Harper, 1949; West, 1951; Head, 1973). There are two carboxylic acids (Chrysanthemic and pyrethric) and three keto alcohols (pyrethrolone, cinerolone and jasmolone) which can combine to give six organic esters. These are the pyrethrins. Six of such esters obtained by pairing the different acids and alcohols have been fully identified.

One set of esters known as the pyrethrin group consists of pyrethrin I, a combination of chrysanthemic acid with pyrethrolone and pyrethrin II, formed by the combination of pyrethric acid with pyrethrolone.

The second set of esters known as the cinerin group consists of cinerin I and cinerin II formed by the combination of cinerolone with chrysanthemic and pyrethric acid respectively.

A third set of esters discovered in 1964 known as jasmolin group consist of jasmolin I and jasmolin II formed by combination of jasmolone with chrysanthemic and pyrethric acid respectively (Godin *et al.*, 1964).

The constituents that have chrysanthemic acid (cinerin I, jasmolin I and pyrethrin I) are collectively termed pyrethrin I fraction whilst the esters with pyrethric acid (cinerin II, jasmolin II and pyrethrin II) are called the pyrethrin II fraction. Figure 2 (p. 14) shows the structural formulae of the pyrethrins.



	R	R'
Pyrethrin I	$-CH_3$	$-CH=CH_2$
Jasmolin I	$-CH_3$	$-CH2-CH_3$
Cinerin I	$-CH_3$	$-CH_3$
Pyrethrin II	$-COOCH_3$	$-CH=CH_2$
Jasmolin II	$-COOCH_3$	$-CH2-CH_3$
Cinerin II	$-COOCH_3$	$-CH_3$

Figure 2: The structural formulae of pyrethrins

There is a strong family relationship between various members of the pyrethrin, cinerin and jasmolin groups, but their insecticidal action varies from one compound to the another.

The commercially-accepted mercury reduction method for pyrethrins analysis rely on the estimation of the acidic portion of the pyrethrins. Therefore, the commercial analyses of pyrethrum extract are always expressed in terms of pyrethrin I and pyrethrin II. These six components together account for the kill and the knockdown properties of the pyrethrum extract.

The composition of the six active constituents in pyrethrum extract may be determined precisely by

chromatographic methods and the complete analysis of the extract is as shown in Table 2.

Table 2: Composition of the six active constituents in the pyrethrum extract

Pyrethrin I			Pyrethrin II			Total
% Py. I	% Cin. I	% Jas. I	% Py. II	% Cin. II	% Jas. II	% Pys
8.5	2.4	1.3	7.9	3.6	1.3	25

Py. pyrethrin, Cin. cinerin, Jas. jasmolin, Pys. pyrethrins

2.3 Pyrethroids

Pyrethroids (synthetic pyrethrins) are esters that have evolved from the natural product pyrethrin I. Both dihalovinyl substitution in the acid moiety and incorporation of 3-phenoxybenzyl alcohols have improved the stability of the molecules to breakdown and metabolism. The production of synthetic pyrethroid on commercial basis started with allethrin and cyclethrin around 1950 (O'Brien, 1967). These early compounds lacked sufficient stability and were less effective against many species of insects than the natural product. Later, there was development of more stable and highly active pyrethroids with more widespread application. Excellent reviews on the structural requirements of insecticidal active pyrethroids can be found elsewhere (Elliot, 1971; Elliot *et al.*, 1974 a ; 1976 a, b, ; 1977). Classical pyrethroids are esters of cyclopropane carboxylic acids with alkenylmethyl cyclopentenolone alcohols. Some important

synthetic pyrethroids include, allethrin, bioallethrin, barthrin, tetramethrin, prothrin, resmethrin, bioresmethrin.

2.4 Physical and chemical properties of pyrethrum pale extract

General information

(a) International union of physical and applied chemistry (IUPAC) nomenclature, pyrethrins

(b) Classification: insecticide, insect repellant, flushing agent.

Common name: pyrethrin I, pyrethrin II

Synonym: Pale extract

Colour: Light amber liquid, maximum specific absorbance of 1.3 for 1% w/v. pyrethrins (pale) equivalent to 12 on the Gardener scale for 25% pale extract.

Odour: Faint vegetable odour.

Specific gravity: 0.84 to 0.96 at 20°C depending on concentration. For formulations may be taken as 0.84 (partially refined 0.86, oleoresin 0.90)

Boiling point: Pyrethrin I, 170 °C at 0.1 mm Hg with decomposition and Pyrethrin II, 200 °C at 0.1 mm Hg with decomposition, 180 °C at sea level.

Flammability

Inflammable but non volatile. Flash point Min.50°C (122 °F) by Abel closed-cup method.

Solubility: Soluble in hydrocarbons and many other organic solvents. Fat solubility is undetermined. They are virtually insoluble in water at 20°C

Stability: Rapidly oxidized and inactivated in sunlight and ultraviolet light. Decomposed by exposure to light with loss of

insecticidal activity. Rapidly hydrolysed by pH above 8, stable at pH 1 to 8.

Thermostability: Relatively stable up to 100°C, moderate decomposition between 100-180°C, rapid decomposition at temperatures above 180°C.

Photostability: Degradable under exposure to light.

Volatility: non-volatile at ambient temperature.

Refractive index: 1.4865 at 20°C

Composition:

The constituents of the refined concentrate are: Pyrethrins-70.1% w/w. Fatty acids (mainly linoleic, palmitic and oleic acids)-25.5%. Sterols (mainly taraxasterol)-3.8%. Alkanes (mainly n- C_{29} H_{60} and n-C_{25} H_{52})-0.6%. For commercial purposes a paraffinic solvent is used to standardize the extract to 50% or 25% w/w pyrethrins.

Spectra data: pyrethrins absorb at 225 nm in the ultraviolet (UV) range and 8.7 nm in the infra-red (IR) range.

Stabilizing agent: Butylated-hydroxy-toluene (BHT) food grade at 3.7%.

Analytical methods: The methods used for qualitative and quantitative determination of pyrethrins include;

- Thin Layer Chromatography (TLC)
- Gas-Liquid Chromatography (GLC)
- High Performance Liquid Chromatography (HPLC)
- Mercury Reduction methods

2.5 Pyrethrins synergists

For many purposes it is useful and economical to include synergists in pyrethrins formulations. Commercial synergists

are synthetic chemicals which posses little or no insecticidal activity of their own but when used with pyrethrins increases insecticidal effectiveness of the whole formulation. The combination of a synergist which is comparatively cheap with the more expensive pyrethrins enables an equivalent insecticidal effect to be obtained at lower cost. Among the best known commercially available synergists are piperonyl butoxide, sulfoxide, Tropital, Bucarpolate and M.G.K 264. Besides increasing the toxicity of pyrethrins, synergists also increase the speed of knockdown and also the rate of activation, commonly referred to as the flushing action, since certain insects particularly cockroaches are driven from their hiding places. The synergists are believed to work by preventing the insect from destroying the pyrethrins which would otherwise kill it. Insects possess this ability to greater or lesser degree and therefore it is appropriate that more or less synergist is required for different species. The following is a summary of some of the commercially available synergists.

Piperonyl butoxide

Piperonyl butoxide is 1-(2,5,8-trioxadodecyl)-3, 4 methylenedioxy-6-(n-propyl) benzene or butyl-3:4-methlenedioxy-6-probenzyl-diethylene-glycol ether. The technical grade is a pale yellow oil containing 80% of the above and 20% of related compounds. The specific gravity at 20°/20°C is 1.04-1.07. It has a low volatility and is readily soluble in common organic solvents, namely petroleum fractions and liquified fluorochlorinated hydrocarbon propellants. It is also a solvent in its own right. Piperonyl

butoxide has a low toxicity to warm blooded animals. Acute oral LD_{50} for rats is 11,500 mg/kg and chronic oral LD_{50} for rats is 8,750 mg/kg.

Sulfoxide

Sulfoxide is 1,2-methylenedioxy-4-[2 (octylsulfynyl) propyl] benzene. It is also described as the n-octylsulfoxide of isosafrole. The technical material is a light yellow to amber liquid containing 88% of the above and 12% of related compounds. The specific gravity at 25°C/25°C is 1.06-1.09. It is soluble in most organic solvents, including Freon 11 propellants, but only partially soluble in insecticidal-based oil and Freon 12. The acute oral LD_{50} for rats is about 2,000 mg/kg. The compound has been fed to rats at 2,000 p.p.m in the diet for 15 months without ill effects apart from slight weight retardation.

Tropital

Tropital is piperonal bis [2-(2,-n-butoxyethoxy)ethyl] acetal. The technical material is a pale mobile oil with a faint bland odour. It contains a minimum of 90% of pure acetal with 10% of other related compounds. The specific gravity at 20°C is 1.075±0.015. Tropital is soluble in all common organic solvents, deodorised-based oils, pyrethrum extracts and flourochlorinated hydrocarbon propellants. The acute oral LD_{50} for rats is approximately 4.4 ml/kg.

Bucarpolate

Bucarpolate is the ester of piperonylic-acid and the mono-n-botyl ether of diethylene glycol. It is a straw-coloured liquid containing 80% as a minimum content of the above ester. The weight per ml at 20°C is 1.09 g. The material is soluble in most organic solvents including fluorochlorinated hydrocarbon propellants but sparingly soluble in liquid petroleum and kerosine.

MGK-264

MGK-264 is N-octyl bicycloheptenedicarboximide or N-(2-ethylhexyl)-bicyclo-(2:2:1)-5-heptene-2:3 dicarboximide. The technical material is 98±0.5% imide. It is nearly water-white, viscous liquid with specific gravity at 20°C of 1.05±0.01. It is miscible with kerosine, fluorochlorinated hydrocarbon propellants and most other organic solvents. The acute oral LD_{50} for rats is 2,800 mg/kg. It has been fed to rats at 5,000 p.p.m for 17 weeks without tissue damage.

2.6 Pharmacokinetics

Pyrethrins are absorbed from the gastro-intestinal tract and by the respiratory route. They are not absorbed to a significant degree through the skin, however, allergic reactions may result from this route of exposure.

The esters constituting pyrethrum extract mixtures are rapidly detoxified by hydrolysis in the gastro-intestinal tract and to some extent in the tissues of adult warm blooded animals (Lehman, 1954). The chrysanthemum monocarboxylic acid formed is excreted in urine (Audiffren, 1934). The

pyrethrins or their metabolites are not stored in the body or excreted in milk. These compounds exhibit little clinical effects in animals following repeated exposure to moderate doses partly because of their rapid excretion but the compounds or their metabolites lead to liver changes in rats (Kimbrough *et al.*, 1968).

Recent studies have elucidated the extensive metabolism that pyrethrins undergo mainly in the liver. This is not an easy task because of the complexity of the natural mixture, involving many interfering substances. It is already clear that the various active ingredients undergo significantly different biotransformation. For example, within 48 hours of oral administration of ^{14}C-pyrethrin II to rats, 53% of the ^{14}C was recovered as exhaled carbon dioxide whereas only 0.3% of ^{14}C-pyrethrin I was recovered in that form under the same circumstances. The corresponding proportions of ^{14}C recovered from the urine were 7 and 46% for pyrethrin II and pyrethrin I, respectively. Some of the oral administered material is excreted in the faeces, at least partially in metabolized form. Three compounds have been isolated from the urine and identified by NMR and mass spectra. All three compounds are produced by both pyrethrin I and II. The metabolites are due to oxidation of both the acid and alcoholic moieties leaving the main structure of the molecule intact (Elliot *at al.*, 1972 a, b). So far it has not been possible to identify the numerous compounds that result from hydrolysis of the esters.

Apparently the detoxication of pyrethrin esters by very young animals has not been studied. The fact that most severe

cases of poisoning are reported in infants suggest that young children are unable to hydrolyse the pyrethrin esters efficiently. In any event, mammals show approximately the same susceptibility to injected pyrethrins as do cold blooded animals including insects.

Some quantitative data is available on the metabolites of pyrethrins I and II excreted by rats administered at 3 mg/kg orally (Elliot *at al.*, 1972 b). There are three identified metabolites of pyrethrins excreted in rat urine which are formed by alterations of the acid part of the molecules. In addition to the identified compounds, 30% of the administered dose is excreted as unidentified polar metabolites in faeces.

2.7 Pharmacodynamics

It appears from the clinical signs that the chief action of pyrethrins in mammals is on the central nervous system. This could be consistent with the conclusion by Leonard (1942) that the diarrhoea produced by pyrethrins resulted from central vagus stimulation. He based the conclusion on the findings that hyperperistalsis caused by pyrethrins in the intact animal could be prevented by atropine and that, *in vitro* pyrethrins caused intestinal relaxation, not movement. The mechanism of action of pyrethrins in mammals is presumably the same as that studied very thoroughly in the invertebrates and lower vertebrates. Pyrethrins kill insects by severely disrupting nerve function for a sufficient period of time. In insects and in vertebrates pyrethrins and pyrethroids prolong the transient increase in sodium conductance in nerve

membrane leading to an induced repetitive activity. The effect on the sodium channel is to stabilize the sodium gate in the open position which leads to prolonged influxes of sodium lasting for milliseconds to seconds depending on the characteristics of the pyrethroid. There is evidence that sensory nerves are more susceptible than motor nerves to pyrethrins. Metabolism studies suggest that the reason why mammals are less sensitive to pyrethrin toxicity is due to rapid metabolism of these compounds by liver and plasma esterases and liver oxidases. There are no tissue lesions that are useful in establishing a diagnosis. Even after lifetime feeding the pathologic changes are minimal because of the rapid metabolism of the pyrethins. However, according to Camougis and Davis (1971), vertebrate nerve is inherently less sensitive than invertebrate nerve to pyrethrins. Specifically pyrethrins at concentrations of up to 2,000 ppm were inactive on rat peripheral nerve whereas at concentrations of 0.2 to 2 ppm they produced excitation followed by block in peripheral nerve of the crayfish with poor recovery of function at the higher concentration.

2.8 Products of pyrethrum flowers and their uses

The products obtained from pyrethrum flowers are extracts, powders and pyrethrum marc.

Extracts

These are liquid pyrethrins insecticide concentrates standardised to a specified pyrethrins contents on

weight/weight basis by addition of a solvent (odourless isoparaffin). They are produced in the following grades;

i) Oleo-resin (O.R) extract 25% w/w which is a crude material suitable for agricultural sprays and incorporation into mosquito coil formulation.

ii) Partially refined (P.R) extract 25% w/w which is a free flowing extract suitable for use in fly sprays and many other insecticidal formulations.

ii) Pale extract 25% w/w which is a high grade, fully refined and decolourised product which meets the most stringent requirements for insecticidal aerosol and sprays.

Pale extract is also available standardised to 50% w/w pyrethrin.

The extracts are packed in 25 or 100 kg steel drums coated internally with an epoxy lacquer.

Powders

The following grades of pyrethrum powders are produced by the Pyrethrum Board of Kenya;

i) Superfine powder containing 1.3% w/w pyrethrins for direct incorporation into insecticidal dusts and mosquito coil formulations, 95% passes bore size (BS) 100 mesh sieve.

ii) Mosquito coil powder No.1 (pyrethrum powder 0.06% w/w). This product is designed specially for the manufacture of mosquito coils, 95% passes BS 100 mesh sieve.

The powders are packed in polythene-lined jute bags containing 50 kg net.

Marc

Pyrethrum marc is the coarse flower materials which remain after extraction of pyrethins and is available in the following grades;

i) Pyrethrum marc which is the coarsely gristed flower material which remains after extraction of the bulk of pyrethrins. A trace of pyrethins (0.08% w/w) remains in the marc. normally. The product is used for feeding cattle and has the same value as green maize, bran or pollard. Unlined first grade jute bags containing 50 kg net are used for packing it.

ii) Pyrethrum fine marc which is pyrethrum marc finely ground to BS 100 mesh for use in mosquito coil formulations as an excellent filler with good burning properties and pleasant aroma. It is packed in 50 kg polythene-lined jute bags.

2.9 Toxicity of pyrethrins and pyrethroids to mammals

Pyrethrins are more toxic to invertebrates than mammals, the rapid metabolism of the pyrethrins in mammals account for this selective toxicity. This was established by Elliots *et al.*, (1972) in their report on the metabolic fate of pyrethrin I, and pyrethrin II, and allethrin. Many reviews (Ambrose and Robins, 1951; Lehman, 1952; Metcalf, 1955; Negherbon, 1959; Williams, 1973) on the toxicology of pyrethrins have appeared. Other reports include those of Leonard (1942), Carpenter *et al.*, (1950), Bond and DeFeo (1969), Kimbrough *et al.*, (1968), Malone and Brown (1968), Comaugis *et al.*, (1971), and Verschoyle and Barnes (1972).

Carpenter *et al.*, (1950) made an exhaustive study of the toxicity of pyrethrins and allethrin in rats, rabbits and dogs after inhalation, oral and skin exposure. He reported that the oral LD_{50} of the pyrethrum oleoresin was 820 mg/kg in male and female sherman strain white rats, whereas the LD_{50} of the purified 20% pyrethrins extract was 1870 mg/kg.

In a study of the acute toxicity of pyrethrins in rats, Lehman (1954) reported the oral LD_{50} as 200 mg/kg and the intravenous LD_{50} as 10.5 mg/kg. This shows the high intrinsic toxicity of pyrethrins, which is not realized in practise because it is readily broken down or metabolized to non-toxic metabolites.

Bond and DeFeo (1969) conducted a detailed study on the toxicity of pyrethrins using five groups of ten rats each and found an oral LD_{50} of 7200 mg/kg. As they were working with a pyrethrum concentrate containing 20% w/w pyrethrins, the LD_{50} of 7200 mg/kg must be divided by 5 if one wishes to have the oral LD_{50} for the 100% material. In 1951 Ambrose and Robbins reported that purified pyrethrins concentrate containing 86.2% total pyrethrins administered into the stomach of rats in doses of 2600 mg/kg produced no toxic reactions. Even doses of 1600 mg/kg administered subcutaneously produced no toxic effects. Extensive work have been done with pyrethrins determining the LD_{50} and the effect of chronic ingestion of pyrethrins on reproduction. In four rats that died after single oral doses of 450 mg/kg, given as 9% pyrethrins in peanut oil the following histopathological observations were made "brain normal, lungs intra-alveolar haemorrhage, heart normal", some evidence of liver

vacuolation was found in two rats. In another study six out of ten female rats dosed at 300 mg/kg pyrethrins using a 20% w/w concentrate died (William, 1973). In yet another study Kimbrough *et al.*, (1968) reported the combined effect of DDT, pyrethrins and piperonyl butoxide. At a dietary level of 1000 ppm pyrethrins, there was some enlargement of the liver but no death was reported. With respect to pathology, the author reported that pyrethrins caused liver changes similar to those caused by DDT namely uniform enlargement of the liver, smoother cytoplasm, many inclusion bodies and moderate vacuolation.

Weir (1966 a) studied the acute oral toxicity of pyrethrins in rats. He administered a 20% w/w pyrethrins solution in corn oil by a stomach tube. He found an acute LD_{50} for pyrethrins in male albino rats to be between 681 and 1000 mg/kg. In a second study repeated to obtain more data for statistical analysis, all animals given 562 and 826 mg/kg died. Therefore a third study was done with freshly prepared 20% w/w pyrethrins solution. In the third study the oral LD_{50} was found to be 710 mg/kg with confidence limits between 568 and 888 mg/kg. The animals that died showed depression, laboured or rapid breathing, ataxia, sprawling of limbs, tremors and weight loss. At necropsy the main findings were congestion of the lungs, liver, kidney and adrenals and slight gastric inflammation.

The pyrethrins show little evidence of causing chronic toxicity. Experimental animals can be fed a large percentage of the acute LD_{50} every day without harmful effects. Bond and DeFeo (1969) did not determine the chronic oral LD_{50},

whereas Lehman gave a chronic oral LD_{50} in rats of 250 mg/kg daily. His acute oral LD_{50} was 200 mg/kg for single doses. Table 3 (p. 29) summarises the LD_{50} values recorded for rats, mice, guinea pig, rabbits and cats using various routes of administration.

The impurities in pyrethrum extract are responsible for the asthma like symptoms in animals. Heavy inhalation dosages do not seem to produce harmful effects to animals.

Dermal toxicity of pyrethrins is negligible because the compounds are poorly absorbed through the intact skin. The consensus of reported values for the dermal LD_{50} of the pyrethrins range from 1350 mg/kg to 5000 mg/kg in various species.

There is little evidence of human toxicity from the use of pyrethrins. Long years of experience have produced no clear cut cases of human poisoning from the pyrethrins. However, some people experience dermal problems after exposure to pyrethrins. The dermatitis is an allergic manifestation and disappears rapidly after removal of the pyrethrins.

2.10 Treatment of pyrethrin toxicity

The treatment for various effects of pyrethrins in animals is symptomatic; antihistamines being the most commonly used. If sufficient pyrethrins have been ingested to cause nervous manifestation, pentobarbitone sodium should be used. The diarrhoea that occurs can be controlled with atropine sulphate. Experimental work in mice has shown that diazepam has some protective properties. However, the data is too limited at this stage for clinical use except as a last result. Atropine blocks salivation observed after pyrethrin poisoning but does not protect the animals from other clinical signs (Osweiler *et al.*, 1985).

Bradbury *et al.*, (1981) studied mephenesin as a treatment of pyrethroid intoxication in rats. They found that a continous i.v infusion of the muscle relaxant, mephenesin, (4-5 mg/kg/min) for 15 minutes prior to the i.v injection of a lethal dose of cimethrin or deltamethrin followed by mephenesin infusion at 2.5-10 mg/kg/min completely protected the rats from the neurotoxicity produced by the pyrethroids.

2.11 The anthelmintic activity of pyrethrins

Although pyrethrins have a reputation for possessing anthelmintic properties and isolated excerpts from literature are occasionally quoted in this regard, not much has been done on these properties of the pyrethrins. McLellan (1964) made a review of the work previously reported on the effectiveness of pyrethrum preparations against internal

parasites. More information can be obtained from his paper which has cited many references. In the review pyrethrins are claimed to be an effective treatment for *Enterobius vermicularis* , *Ascaris*, *Taenia* and *Trichuris* in man both adult and children, *Ascaris lumbricoides* of the pig and *Taenia* species of the dog. Liver flukes in contact with pyrethrins solution *in vitro* have also been reported to die rapidly.

McLellan (1964) reported an experiment which was done with the pyrethrins as a cure for anaemia caused by worms infestation in the horse. Successful treatment of 11 horses, heavily parasitized is reported. The principle parasites are Strongyles (*Strongylus vulgaris, S. equinus, S. edentus*), Cylicostomes (some 30 species) *Ascaris equorum* and *Oxyuris equi*..

Pyrethrins as a treatment for internal parasitism of sheep and poultry was investigated as reported by McLellan (1964). Experiments were done with a flock of sheep where heavy losses (60 to 70%) were caused annually by worm infestation. Postmortem examination showed massive parasitism of the small the intestines by tapeworm (*Moniezia*), of the caecum by *Trichuris* and *Charbertia*, and the abomasum and lungs by *Tricostrongyles*. The general condition of the flock improved following this treatment and mortality dropped to 12%.

All these investigations show that pyrethrins have a great potential of being developed into a useful anthelmintic in domestic animals.

Table 3 Acute LD_{50}s of pyrethrins.

Species	Route	LD_{50} (mg/kg)	Reference
rat	oral	820 (680-1,100)[a,b]	Carpenter *et al.*, 1950
rat	oral	1,870 (1,340-2,600)[a,b]	Carpenter *et al.*, 1950
rat	oral	200	Lehman, 1952
rat	oral	>2,600	Ambrose and Robins,1951
rat	oral	260-420[c]	Casida *et al.*, 1971
rat	oral	>600[d]	Casida *et al.*,1971
rat	oral	1,400	Bond *et al.*,1973
rat, m	oral	260-420[c]	Verschoyle and Barnes,1972
rat, m	oral	>600[d]	Verschoyle and Barnes,1972
rat	ip	130	Shimkin and Anderson,1936
rat	iv	10.5	Lehman, 1954
rat, f	iv	5	Verschoyle and Barnes,1972
rat, f	iv	5[c]	Verschoyle and Barnes,1972
mouse	oral	130	Fujimoto *et al.*,1973
mouse	ip	100	Shimkin and Anderson,1936
mouse	ip	<240[d]	Leonard,1942
g pig	oral	1,500	Shimkin and Anderson,1936
g pig	ip	110	Shimkin and Anderson,1936
rabbit	dermal	2,060 (110-3,680)[a]	Carpenter *et al.*, 1950
cat, f	iv	1[d]	Verschoyle and Barnes,1972

a. LD_{50} and confidence limits for purified extract. b. values for two preparations in the same laboratory. c. pyrethrin I. d. pyrethrin II e. "Lethal dose" m. male f. female g. guinea i.p. intraperitoneal i.v. intravenous.

CHAPTER THREE

STUDIES OF ACUTE TOXICITY OF PYRETHRINS IN SHEEP AND RABBITS

3.1 MATERIALS AND METHODS

3.1.1 Sheep

Adult female red masai sheep, aged 2 to 3 years were purchased in Kiserian town, Ewaso Kendong area, Ngong division of Kajiado district and transported to Kabete. They had no clinical history or evidence of disease. On arrival physical examination was done and vital parameters which included weight, temperature, pulse and respiratory rates were recorded. The vital parameters were in the normal ranges for the ovine species and their weights ranged from 15.5 to 19.5 kg. This was repeated twice every week. Blood and fecal samples were also taken to the laboratory for haematology, biochemistry and fecal egg counts. The animals infested with worms were dewormed with albendazole (Valbazen®) and a suspension containing 1.5 % levamisole and 80 % bithionol sulphoxide (wormicide plus®) given orally using a 20 ml dosing syringe. Haematological and biochemical parameters tested were in the normal ranges. Prior to introduction of the animals, the sheep pens were thoroughly washed and disinfected with kerol®. This was repeated five days later and one week allowed before introduction of the sheep. Good quality hay (Rhodes grass) was fed to the sheep ad libitum throughout the experimental period while wheat bran

was fed once daily. Salt in form of maclick blocks and clean water were available to the sheep always. The sheep were allowed one month acclimatization period before commencement of the experiments.

3.1.2 Rabbits

Albino New Zealand rabbits weighing from 1.60 to 2.34 kg were used. The rabbits were obtained from Ngong veterinary farm and were kept in cages and fed rabbit pellets. Clean water was supplied to the rabbits ad libitum. The animals were allowed one month to acclimatize before starting the experiments.

3.1.3 Pyrethrins samples

The pyrethrins samples were obtained from the pyrethrum Board of Kenya. Three samples were obtained as (i) pale extract containing 25% w/w pyrethrins alone. (ii) pale extract containing 25% w/w pyrethrins with synergist piperonyl butoxide. (iii) pale extract containing 50% w/w pyrethrins with piperonyl butoxide. In addition two other samples were obtained from the same source (i) Piperonyl butoxide alone. (ii) odourless isoparaffin which is the solvent for pyrethrins.

All the samples were stored in a refrigerator before use.

3.1.4 Preliminary studies

Seven rabbits were used for preliminary studies to estimate the oral toxic dose of the pyrethrins. Their weights ranged from 1.60 to 2.1 kg. Six rabbits were given the extract

orally at dosages of 84, 168, 336 and 672 mg/kg body weight using one rabbit per dosage for the first two dosages and 2 rabbits each for the next 2 dosage levels respectively. The pale extract containing 50% w/w pyrethrins with piperonyl butoxide was administered and clinical observations made. The rabbits recovered after showing various central nervous system signs such as, hyperesthesia, bending of the neck to one side, circling, incoordination and muscle tremors. Another rabbit was given a very high dose of pyrethrins (2,520 mg/kg) and it died later. This information was used as a guideline for estimating LD_{50} in rabbits and sheep since the values were estimated to be below 2,520 mg/kg but above 672 mg/kg b.w.t in rabbits for pyrethrins with piperonyl butoxide (synergist).

3.1.5 EXPERIMENT I: DETERMINATION OF THE ORAL MEDIAN LETHAL DOSE (LD_{50}) OF PYRETHRINS ALONE IN RABBITS

Eight rabbits were divided into 4 groups of 2 animals each corresponding to 4 dosage levels of pyrethrins. The pyrethrins were administered orally as a pale pyrethrum extract containing 25% w/w pyrethrins using a stomach tube introduced with the aid of a mouth gag. The dosage levels were spaced in a geometric progression or had a logarithmic relationship. Table 4 (p.35) shows the details of the experiment. The animals dying within 24 hours were recorded as shown in Table 4 for calculation of LD_{50} using the method of Wells (1952). The concentration of the extract used was 25%w/w pyrethrins and its specific gravity was 0.84. Calculations of the dosages were done as shown below.

Calculations

The extract used contained 25% w/w pyrethrins and its specific gravity was 0.84. Percent weight by weight (w/w) is the number of grams of pyrethrins in 100 grams of the extract,

i.e 25% w/w pyrethrins = 25 g of pyrethrins in 100 g of extract

1 ml of the extract = 0.84 grams

Example

If one wants 5 grams total pyrethrins, then the amount of extract (X grams) to contain these can be calculated as follows:

25 g pyrethrins are contained in 100 g of the extract

5 g pyrethrins are contained in X g of extract

$X = \frac{5 \times 100}{25}$ = 20 grams of the extract.

This is equivalent to 23.81 ml of the extract.

Table 4: Responses observed in New Zealand white rabbits after oral administration of a pale extract containing 25% w/w pyrethrins alone

Rabbit No.	Wt .of rabbit (Kg)	vol. of extract (ml)	Wt .of extract (g)	Total pyrethrins (mg)	Dosage (mg/kg)	Log Dose	Response
1	1.85	8.880	7.459	1865	1008	3.0035	survived
2	2.08	9.985	8.387	2097	1008	3.0035	survived
3	1.84	13.248	11.128	2782	1512	3.1796	died
4	1.95	14.040	11.794	2948	1512	3.1796	survived
5	1.80	19.440	16.330	4082	2268	3.3556	died
6	2.20	23.760	19.958	4990	2268	3.3556	died
7	2.30	37.250	31.298	7825	3402	3.5317	died
8	2.30	37.250	31.298	7825	3402	3.5317	died

3.1.6 EXPERIMENT II: DETERMINATION OF THE ORAL MEDIAN LETHAL DOSE (LD_{50}) OF PYRETHRINS WITH PIPERONYL BUTOXIDE IN RABBITS

Eight rabbits were divided into 4 groups of 2 animals each corresponding to 4 dosage levels of pyrethrins. The pyrethrins were administered orally as a pale extract containing 25% w/w pyrethrins with the synergist piperonyl butoxide using a stomach tube introduced with the aid of a mouth gag. The dosage levels were spaced in a geometric progression or had a logarithmic relationship. Table 5 shows the details of the experiment. The animals dying within 24 hours were recorded as shown in Table 5 for calculation of LD_{50} using the method of Wells (1952). The specific gravity of the extract was 0.84. Calculation of the dosages was done as in experiment I.

Table 5: Responses observed in New Zealand white rabbits after oral administration of a pale extract containing 25% w/w pyrethrins with piperonyl butoxide

Rabbit No.	Wt .of rabbit (Kg)	vol. of extract (ml)	Wt .of extract (g)	Total pyrethrins (mg)	Dosage (mg/kg)	Log Dose	Response
1	2.20	3.300	2.772	693	315	2.4983	survived
2	1.86	2.794	2.347	586	315	2.4983	survived
3	2.60	7.800	6.552	1638	630	2.7993	survived
4	2.05	6.150	5.166	1292	630	2.7993	survived
5	2.51	15.060	12.650	3163	1260	3.1004	died
6	1.70	10.200	8.568	2142	1260	3.1004	survived
7	1.90	22.800	19.152	4788	2520	3.4014	died
8	2.10	25.200	21.168	5292	2520	3.4014	died

3.1.7 EXPERIMENT III: DETERMINATION OF THE ORAL MEDIAN LETHAL DOSE (LD_{50}) OF PYRETHRINS ALONE IN RABBITS PRETREATED WITH PHENOBARBITONE SODIUM

Eight rabbits were injected with phenobarbitone sodium at 30 mg per kg intraperitonially two times daily for three days. The rabbits were then divided into 4 groups of 2 animals each corresponding to 4 dosage levels of pyrethrins. The pyrethrins were administered orally as a pale pyrethrum extract containing 25% w/w pyrethrins using a stomach tube introduced with the aid of a mouth gag. The dosage levels were spaced in a geometric progression or had a logarithmic relationship. Table 6 shows the details of the experiment. The responses observed within 24 hours were recorded as show in Table 6. The concentration of the extract used was 25% w/w pyrethrins and its specific gravity was 0.84. Calculation of the dosages was done as in Experiment I.

Table 6: Responses observed in New Zealand white rabbits after oral administration of a pale extract containing 25% w/w pyrethrins after pretreatment with phenobarbitone sodium

Rabbit No.	Wt .of rabbit (Kg)	vol. of extract (ml)	Wt .of extract (g)	Total pyrethrins (mg)	Dosage (mg/kg)	Log Dose	Response
1	2.18	13.952	11.720	2930	1344	3.1284	survived
2	2.64	16.896	14.193	3548	1344	3.1284	survived
3	2.33	22.368	18.789	4697	2016	3.3045	survived
4	1.94	18.624	15.644	3911	2016	3.3045	survived
5	2.50	36.000	30.240	7560	3024	3.4806	survived
6	2.40	34.560	29.030	7258	3024	3.4806	survived
7	2.34	50.544	42.457	10614	4536	3.6567	survived
8	1.76	38.015	31.933	7983	4536	3.6567	survived

3.1.8 EXPERIMENT IV: DETERMINATION OF THE ORAL MEDIAN LETHAL DOSE (LD_{50}) OF PYRETHRINS WITH PIPERONYL BUTOXIDE IN SHEEP

Eight sheep were divided into 4 groups of 2 animals each corresponding to 4 dosage levels of pyrethrins. The pyrethrins were administered as a pale extract containing 50% w/w pyrethrins as a drench using a 20 ml dosing syringe. The dosage levels were spaced in a geometric progression or had a logarithmic relationship. Table 7 (p.39) shows the details of the experiment. The animals dying within 24 hours were recorded as shown in Table 7 for calculation of LD_{50} using the method of Wells (1952). The concentration of the extract used was 50% w/w pyrethrins and its specific gravity was 0.84.

Calculations

The extract used contained 50% w/w pyrethrins and its specific gravity was 0.84. Percent weight by weight (w/w) is the number of grams of pyrethrins in 100 grams of the extract,

i.e 50% w/w pyrethrins = 50 g of pyrethrins in 100 g of extract

1 ml of the extract = 0.84 grams

This information was used for calculation of the dosages as in experiment I.

Table 7: Responses observed in red masai sheep after oral administration of a pale extract containing 50% w/w pyrethrins with piperonyl butoxide

Sheep No.	Wt .of sheep (Kg)	vol. of extract (ml)	Wt .of extract (g)	Total pyrethrins (mg)	Dosage (mg/kg)	Log Dose	Response
03	19.5	9.75	8.190	4095	210	2.3222	survived
06	18.0	9.00	7.560	3780	210	2.3222	survived
07	17.5	17.5	14.700	7350	420	2.6232	survived
11	18.0	18.0	15.120	7560	420	2.6232	survived
01	19.0	38.0	31.920	15960	840	2.9243	died
05	17.0	34.0	28.560	14280	840	2.9243	died
02	16.0	64.0	53.760	26880	1680	3.2253	died
09	18.0	72.0	60.480	30240	1680	3.2253	died

3.1.9 EXPERIMENT V: EVALUATION OF CLINICAL SIGNS, HAEMATOLOGICAL, BIOCHEMICAL AND POSTMORTEM CHANGES IN ACUTE PYRETHRIN TOXICITY IN SHEEP

All the animals in experiment IV were used in this experiment together with a control group. Evaluation of clinical signs and postmortem effects of acute pyrethrin toxicity in sheep was done in the sheep that showed clinical signs and died in experiment IV above. Haematological and biochemical effects of acute pyrethrin toxicity were evaluated in the animals that survived i.e those in 210 and 420 mg/kg dosage levels and a control group that was not treated.

3.1.9.1 Clinical signs and postmortem changes

Close observation of the sheep was made from the onset of giving the pyrethrins and postmortem examination performed in those that died. For the animals that survived and the controls, periodic examinations were made at one hour intervals for eight hours and the observations recorded. The parameters observed included rectal temperature, pulse and respiratory rates.

3.1.9.2 Blood for haematological and biochemical tests

Blood samples were taken from the sheep one day before treatment and daily for 14 days after treatment. The blood samples were collected always in the morning at 9.00 a.m from the jugular vein using a 10 ml syringe and 1 1/2" (3.81 cm) 18 gauge needle. Some of the blood was placed into bijou bottles with disodium ethylene diaminetetracetic acid (EDTA) as the anticoagulant. The rest was placed in universal bottles with no anticoagulant and allowed to clot at room temperature (20°C) before the serum was separated by centrifugation at 2,300 g for 10 minutes.

3.1.9.3 Determination of white blood cell (WBC), red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)

The electronic counting technique was employed using a coulter counter model Z M (coulter Electronics Inc., Hialeah, Florida). After mixing the blood thoroughly a dilution 1:500

was made using isoton for white blood cell count (W.B.C) and haemoglobin (Hb) determination. From this dilution a further double dilution at 1:50,000 dilution each was made for determination of red blood cells (R.B.C) which were expressed in million cells/ml. To the 1:500 dilution 6 drops of zap o globin were added and mixed to haemolyse the RBC to allow WBC counts expressed in thousands of cells/ml. The remaining portion was used to determine haemoglobin concentration (g/100ml) using a haemoglobinometer. Mean corpuscular volume (M.C.V) and mean corpuscular haemoglobin concentration (M.C.H.C) were calculated from the above measurements as;

$$M.C.V = \frac{\text{Haemactocrit(P.C.V)x10}}{\text{RBC counts in millions/mm}^3}$$

$$M.C.H.C = \frac{\text{Haemoglobin in grams/100ml x 100}}{\text{Packed cell volume, percent}}$$

3.1.9.4 Differential leucocyte counts

The cells were counted using the technique described by Dacie and Lewis (1968). Blood films were made normally and then fixed in methanol for 5 minutes and stained in Giemsa stain for 30 minutes. Then, a thorough examination in a strip running the whole width of the film and 100 leucocytes lying along this strip were counted. The number of lymphocytes, neutrophils, eosinophils e.t.c counted in this manner were then expressed as a percentage of cells counted.

3.1.9.5 Determination of microhaematocrit and total proteins

The microhaematocrit method described by Dacie and Lewis (1968) was used. Commercially available unheparinized

(plain) capillary tubes 75 mm in length and an internal diameter of 1.3-1.5 mm were used. The tubes were filled with uncoagulated blood until about three quarters of each tube was full of blood. The dry end of the tubes were then sealed using plasticin. The tubes were span at 9,300 g for 5 minutes in a microcentrifuge (Haemofuge®, Heraeus-christ, GmBH, West Germany). The percentage packed cell volume (microhaematocrit) was determined from the scale of Hawksley microhaematocrit reader. The buffy coat layer was not included in the reading. The plasma was used in the determination of the total proteins using a refractometer (Atago®, SPR-T2, Japan).

3.1.9.6 Determination of aspartate aminotransferase (ASAT)

Boehringer Mannheim GmBH West Germany enzyme diagnostic kits and Precinorm u® control sera were used. 2.00 ml of the reagent solution and 0.30 ml of serum were pipetted into a test tube mixed and incubated for 5-15 minutes at 37°C. Then 0.20 ml of the starter reagent was added. Mean absorbance was determined at a wavelength of 365 nm in a 1 cm light path cuvette at a temperature of 37°C. Measurement of the absorbance was done against air. The absorbance change per minute (ΔA/min.) obtained was used for the calculation. ASAT activity in the samples was obtained from the table of values or calculated as

$U/L\ (37°C) = 2451 \times \Delta A_{365\ nm}/min.$ The results were thus expressed in international units per litre.

3.1.9.7 Determination of glutamate dehydrogenase (GLDH)

Boehringer Mannheim GmBH West Germany enzyme diagnostic kits were used. 0.5 ml of sample was pipetted into reagent solution at 25 °C. Mixing was done and allowed to stand for 3 minutes at 25 °C, then poured into a cuvette (1 cm light path) and absorbance A_1 measured at Hg 365 nm wavelength. It was then allowed to stand at 25 °C for exactly 5 minutes and absorbance A_2 (non-creep reaction) read. 0.1 ml of solution 3 (α-oxoglutarate) was added, mixed and absorbance A_3 read. It was allowed to stand for exactly 5 minutes at 25 °C and absorbance A_4 read. The absorbance change (ΔA) was obtained as $(A_3 - A_4) - (A_1 - A_2) = \Delta A$. G.L.D.H activity in the samples in international units per litre was obtained from the table of values or calculated as U/l (25 °C) = $365 \times \Delta A_{365\ nm}$

3.1.9.8 Determination of sorbitol dehydrogenase (SDH)

Quantitative kinetic determination of sorbitol dehydrogenase in serum at 340 nm [procedure No.50-UV] method was used. Sigma diagnostic kit (Sigma chemical Co. Ltd.) were used. Into a 0.2 mg NADH vial (catalog No. 340-2), 2.0 ml TRIZMA® buffer (catalog No. 340-22) and 0.5 ml serum were pipetted and mixed gently by shaking. The contents were allowed to stand for 10 minutes for reactions of keto acids in serum and then the reaction was started by adding 0.5 ml fructose solution, (catalog No. 50-1) which had been prewarmed to reaction temperature (22°C). Mixing was then done by inversion and the solution immediately transfered to a

cuvette (1cm light path). The timer was started and absorbance read at 340 nm against potassium dichromate solution at 1 minute interval for 8 minutes and the average ΔA/min for the linear period determined. Sorbital dehydrogenase in sigma units/ml was calculated as SDH [sigma Units/ml] = ΔA/min x 58,000.

3.1.9.9 Determination of creatinine

Boehringer Mannheim GmBH West Germany enzyme diagnostic kits and Precinorm u® control sera were used. A standard was prepared by pipetting 2.0 ml of reagent mixture and 0.2 ml of solution 1 (standard creatinine) into a cuvette. Then 2.0 ml of reagent mixture and 0.2 ml of serum sample were pipetted into another cuvette. Mixing was done and stop watch started at the same time. After 30 seconds absorbance A_1 of the sample and the standard were read and exactly 2 minutes later absorbance A_2 of standard and sample were read. The readings were at 492 nm $A_2 - A_1 = A_{sample}$ or $A_{standard}$. creatinine concentration was calculated as $c = 2.0 \times A_{sample}/A_{standard}$ [mg /100 ml.]

3.1.9.10 Determination of total proteins

Biuret method using Boehringer Mannheim GmBH enzyme diagnostic kits was used. 0.1 ml serum and 5.0 ml solution 1 (Biuret reagent) were pipetted into a test tube and mixed. The mixture was incubated at 20-25 °C. Absorbance of sample (A_{sample}) was measured against solution 1 (reagent blank) at a wavelength of Hg 546 nm. Concentration of total

proteins was obtained from the table of values or calculated as: c = 19 x A sample [g/100ml].

3.1.9.11 Statistical data analysis

Statistical analysis of data was done by analysis of variance (ANOVA) (Daniel, 1983) using an IBM computer with a panacea statistical programme. Turkey's highest significant difference (HSD) test (Daniel, 1983) was used to determine if there was a significant difference in the group means at 5% level of significance. The test statistic used in this case was:

$$HSD^* = q_{\alpha,\kappa,N-\kappa}\sqrt{\frac{MSE}{n^*_j}}$$

where

α = chosen level of significance

k = number of group means

N= total number of observations

n= number of observations in a treatment

MSE = mean square error from the ANOVA table

q = obtained by entering a HSD statistic table

n^*_j = the smallest of the two sample sizes associated with the two sample means that are to be compared

3.1.10 EXPERIMENT VI: EVALUATION OF DERMAL AND OCULAR IRRITATION OF PYRETHRINS IN SHEEP

3.1.10.1 Ocular irritation test.

The method described by Draize *et al.*, (1944) was used. Eight female adult Red masai sheep were used. 0.1 ml. of a 50 % w/w pyrethrum extract was instilled into the left eye of

each sheep, the right eye remained untreated and served as control. In the case of the first three subjects, the treated eye remained unwashed. The remaining five subjects were divided into two groups. In the first group of two sheep, the eyes were instilled with the test substance and washed with 20 ml of lukewarm water (37°C) two seconds after instillation of the extract. In the second group of three sheep, the test eyes were washed with a similar amount of lukewarm water four seconds after instillation of the extract.

Ocular reactions were observed at 24, 48, and 72 hours, and at four and seven days after treatment. Readings were made on the cornea, iris and conjunctiva. The corneal reactions were evaluated on the basis of the density of the opacity and total area involved on a scale of 0-4. The iris was scored on the intensity or degree of inflammation exhibited on a scale of 0-2, while the palpebral and bulbar mucosae were scored on the extent of chemosis, redness and discharge. Each response was graded on a scale of 0-3 or 0-4 (chemosis).

3.1.10.2 Dermal irritation test

The method described by Draize *et al.*, (1944) was used. Eight female adult Red Masai sheep were used. On each sheep two patches were carefully shaved avoiding skin cuts. The skin on one patch in each sheep was then abraded using the tip of a syringe needle. Minor abrasions were made through the stratum corneum (not sufficiently deep to reach the dermis), in order to avoid bleeding. Then 0.5 ml. of a 50% w/w pyrethrum extract was introduced under a patch on the abraded and intact skin in the eight sheep. The patches were

secured in place by strips of adhesive tape. After 24 hours of exposure the patches were removed, and the resulting reactions were evaluated on the basis of scores. Effects on both intact and abraded skin were scored as erythma and scar formation and oedema formation on a scale of 0-4. Readings were also made after 72 hours, and the final score represented an average of the 24 and 72 hour readings.

The total erythema and edema scores were added in both the 24 and the 72 hour readings, and the averages of the scores for the intact and abraded skin were combined. This combined average was referred to as the primary irritation index.

3.1.11 EXPERIMENT VII: EVALUATION OF DIAZEPAM AND PENTOBARBITONE SODIUM IN TREATMENT OF PYRETHRIN POISONING IN RABBITS

A total of nine rabbits were used in this experiment the rabbit were randomly divided into 3 groups of three animals each. All the rabbits were then given a lethal dose of pyrethrum extract (25% w/w) orally (3,000 mg/kg b.w.t). Group 1 and 2 were treated with pentobarbitone sodium and diazepam respectively when the first signs of poisoning were seen. Pentobarbitone sodium was administered intravenously while diazepam was given as an intraperitoneal injection. Group 3 was not treated and served as the control.

3.2 RESULTS

3.2.1 The oral median lethal dose (LD_{50}) of pyrethrins alone in rabbits

The moving average method of calculating LD_{50} (Weil, 1952) was used in the present studies. The LD_{50} by this method is computed by interpolation involving $K + 1$ or more dosage levels by the use of respective arithmetic means of the log dose and a fraction responding critically for K successive points. K is the span and is obtained by substracting one from the number of dosage levels used. The method depends on the use of published tables which define the responses within each group, the number of groups and size of each group. The published tables provide for groups of 2, 3, 4, 5, 6 or 10 subjects per drug level with the stipulation that four or more dosage groups be used and each group be the same size. The LD_{50} and 95% confidence interval of the LD_{50} are obtained. The tables can be obtained from the original article by Weil, (1952).

Requirements for the use of the tables are :

a) Dose a constant number of animals on each dosage level (n=the number dosed per level). The most commonly used is $K = 3$ i.e 4 dosage levels

b) Space the dosage levels so that they are in a geometric progression. For example, if the geometric factor (R) is 2, with dosage level of 0.5, 1.0, 2.0 and 4.0 grams/kg then d=0.30103 d is the difference in logarithms of successive dosage levels hence it is a constant.

c) Dose the animals on at least $K+1$ level of dosage i.e 4 levels or more for $K = 3$.

When these requirements are followed we seek to obtain from the animals dosed at succeeding dosage levels a set of mortality data (r-value in the table) that match one of those in the table for given values of n and K to obtain values for f and σf. In any of the tables, the two middle numbers in the "r-value" column may be inverted without changing the other values in the row. The general formula for the calculation of m, the estimated LD_{50} may be reduced to :

$\log m \cong \log D_a + d \cdot (f + 1)$ for $K = 3$ d = logarithm of the constant ratio between dosage levels.

$\log D_a$ = logarithm of the lowest of the dosage levels used.

In estimation of a confidence interval that will encompass the LD_{50} 95 times in 100, we take that bounded by antilog $[\log m \pm 2 \cdot \sigma \log m]$. The following formula is used with the σf values from the table : $\sigma \log m \cong d \cdot \sigma f$

In Table 4 (p.35), the mortalities observed were, 0 rabbits of 2 dosed at 1,008 mg/kg, 0 of 2, 1 of 2 and 2 of 2 dead at dosages of 1,512, 2,268 and 3,402 mg/kg respectively. The variables in the formula were obtained from the tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use (Weil, 1952). $K = 3$, n = 2, r = 0, 1, 2, 2, $D_a = 1{,}008$, d = 0.17609, $f = 0.00000$ $\sigma f = 0.50000$

$$\log m \cong \log D_a + d \cdot (f + 1) \text{ i.e } \log LD_{50} \cong \log D_a + d \cdot (f + 1)$$

$$\log LD_{50} \cong 3.00346 + 0.17609\ (0.00000 + 1)$$

$$\cong 3.17955$$

LD_{50} = 1,512 mg/kg body weight (i.e LD_{50} is around 1500)

95 % confidence interval (C. I.) of the LD_{50} is given by;

antilog [log m ± 2 . σ log m].

The σf values are obtained from the table and $\sigma \log m \equiv d \cdot \sigma f$

antilog [log m ± 2 . σ log m]

95% C. I. is antilog [3.17955 ± 2 x 0.17609 x 0.50000

i.e antilog [3.17955 ± 0.17609] = 1,008 to 2,268 mg/kg

Therefore median lethal dose and its 95% C. I. is 1,500 (1,008 to 2,268) mg/kg b.wt

3.2.2 The oral median lethal dose (LD_{50}) of pyrethrins with piperonyl butoxide

In Table 5 (p.36), the mortalities observed were, 0 rabbits of 2 dosed at 315 mg/kg, 1 of 2, 2 of 2 and 2 of 2 dead at dosages of 630, 1,260 and 2,520 mg/kg respectively. The variables in the formula were obtained from the tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use (Weil, 1952). $K = 3$, n = 2, r = 0, 0, 1, 2, $D_a = 315$, d = 0.30103 $f = 1.00000$ and $\sigma f = 0.50000$

$\log m \equiv \log D_a + d \cdot (f + 1)$ i.e $\log LD_{50} \equiv \log D_a + d \cdot (f + 1)$

$\log LD_{50} \equiv 2.49831 + 0.30103\ (1.00000 + 1)$

$\equiv 3.10037$

LD_{50} = 1260 mg/kg body weight (i.e LD_{50} is around 1300)

95 % confidence interval (C. I.) of the LD_{50} is given by;

antilog [log m ± 2 . σ log m].

The σf values are obtained from the table and $\sigma \log m \equiv d \cdot \sigma f$

antilog [log m ± 2 . σ log m]

95% C. I. is antilog [3.10037 ± 2 x 0.30103 x 0.50000

i.e antilog [3.10037 ± 0.30103] = 630 to 2,520 mg/kg

Therefore median lethal dose and its 95% C. I. is 1,300 (630 to 2,520) mg/kg b.wt

3.2.3 The oral median lethal dose (LD_{50}) pyrethrins alone in rabbits pretreated with phenobarbitone sodium

The rabbits did not die at dosage levels of 1,344, 2,016, 3,024 and 4,536 mg/kg b.w.t in 24 within hours. However one rabbit dosed at 4,536 mg/kg died 48 hours after the administration. Therefore the 24 hour oral median lethal dose was above 4,500 mg/kg b.wt.

3.2.4 The oral median lethal dose (LD_{50}) of pyrethrins with piperonyl butoxide in sheep

The calculation of the median lethal dose was done using the method of Weils described above. In Table 7 (p. 39), the mortalities observed were, 0 sheep of two dosed at 210 mg/kg, 0 of 2, 2 of 2 and 2 of 2 dead at dosages of 420, 840 and 1,680 mg/kg respectively. The variables in the formula were obtained from the tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use (WEIL, 1952). $K = 3$, $n = 2$, $r = 0, 0, 2, 2$, $D_a = 210$ $d = 0.30103$ $f = 0.50000$ and $\sigma f = 0.00000$

$$\log m \cong \log D_a + d \cdot (f + 1) \text{ i.e } \log LD_{50} \cong \log D_a + d \cdot (f + 1)$$

$$\log LD_{50} \cong 2.3222 + 0.30103\ (0.50000 + 1)$$

$$\cong 2.77377$$

$LD_{50} = 594$ mg/kg b.w.t (i.e LD_{50} is around 600)

3.2.5 Clinical observations and postmortem changes

Signs of poisoning were seen within five minutes to one hour after oral administration of a pale pyrethrum extract containing 50% w/w pyethrins for sheep and 25% w/w for rabbits. The rabbits and sheep that died were showing signs of central nervous stimulation. The first signs were staring coat, imbalance, spasmodic and rapid respiration and shaking of the head. In rabbits, this was followed by twitching and jumping. In sheep there were tremors of the ears, neck, fore limbs and hind legs. With time the hind limbs then became splayed, fine tremors become more severe and progressed to fits. Some animals showed excess salivation accompanied by foaming of the mouth. The animals showed excess response to external stimuli (hyperaesthesia). As tremors became intense, there was difficulty in walking, prostration and finally recumbency. The animals continued paddling all the four limbs strongly and then died. In summary the toxic signs observed comprised, hyperaesthesia, muscle tremors, increased respiratory rate, incoordination, convulsions, recumbency and finally death or full recovery. Both sheep that received pyrethrins at 210 mg/kg did not show signs of poisoning. In the group of sheep that received 420 mg/kg pyrethrins, one recovered after the onset of the CNS signs while the other one did not show any signs of poisoning. All the sheep that recieved pyrethrins at 840 mg/kg and above died after showing the CNS signs. Over a period of 8 hours there was no difference in the temperature and respiratory rates between the control groups and the two experimental

groups that survived ($p>0.05$). Figure 3 shows the temperature, pulse and respiration variation in sheep dosed 210 and 420 mg/kg pyrethrins.

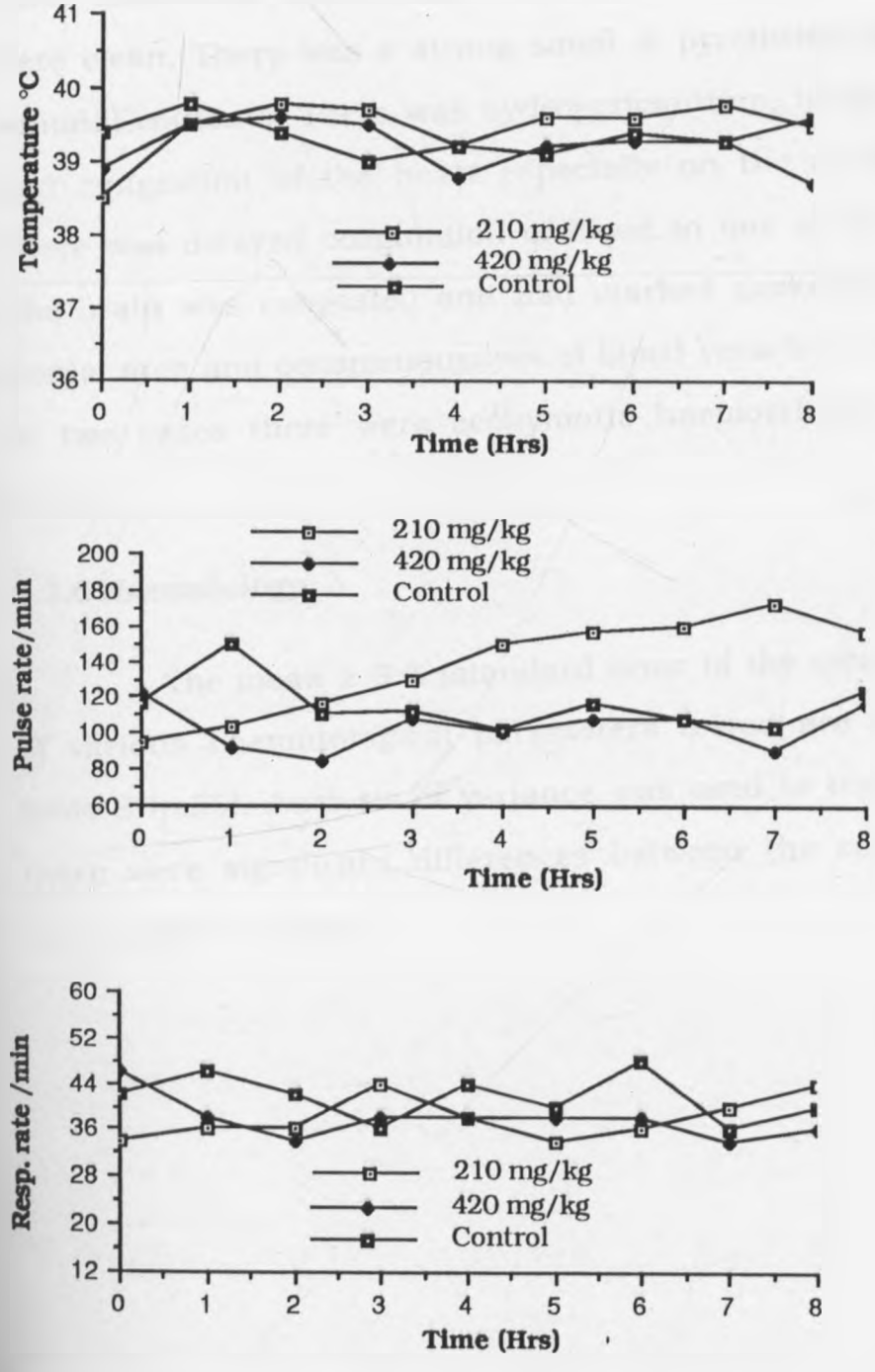


Figure 3: Effects of pyrethrins given orally as a single dose at 210 or 420 mg/kg on temperature, pulse and respiration in sheep

At postmortem, there were small volumes of fluid in the peritoneal cavity. The rumen were distended with gas. There was pulmonary oedema and congestion. The liver was congested with purplish colouration. Upper portions of the small intestines were congested while the large intestines were clean. There was a strong smell of pyrethrins from the ruminal contents. There was hydropericardium, haemorrhage and congestion of the heart especially on the epicardium. There was delayed coagulation of blood in one of the sheep. The brain was congested and had marked darkening of the frontal area and conspicuousness of blood vessels in all cases. In two cases there were ecchymotic haemorrhages in the kidney.

3.2.6 Haematology

The mean ± S.E (standard error of the mean) values of various haematological parameters tested are shown in table 8 (p.55). Analysis of variance was used to test whether there were significant differences between the control and the treatment groups.

Table 8: Effects of single oral doses of pyrethrins (210 and 420 mg/kg) on microhaematocrit, Hb, RBC, M.C.H.C, M.C.V, total and differential leucocyte counts in red masai sheep

	mean ± S.E		
	Control (n=2)	210 mg/kg (n=2)	420 mg/kg (n=2)
microhaematocrit (%)	27.7±0.70	27.0±0.45	29.3±0.57
Hb. (g/100 ml)	11.8±0.28	12.0±0.22	13.6±1.22
RBC count (x10^6)	9.3±0.32	9.48±0.37	8.1±0.14
M.C.H.C (%)	43.1±0.87	44.3±0.59	42.6±0.47
M.C.V (cu. microns)	30.1±0.87	29.3±0.96	36.3±0.50
Total WBC count,	15,478±1,835	12,690±511	9,619±427
Neutrophils (%)	53.7±4.20	29.3±2.47	36.3±1.79
Lymphocytes (%)	44.2±3.95	68.0±2.53	61.5±1.81
Eosinophils (%)	1.74±0.36	2.73±0.32	2.19±0.52

Abbreviations ; Hb, haemoglobin, RBC, red blood cells, MCHC, mean corpuscular haemoglobin concentration, MCV, mean corpuscular volume WBC, white blood cell, Hb, haemoglobin, MCT, microhaematocrit.

3.2.6.1 WBC counts and differential leucocyte counts

The white blood cell counts were in the normal range for the ovine species. (appendix 16 p.113). However, there was an overall significant difference between the control and the two treatment groups in WBC counts. The data showed that

administration of pyrethrins orally at 420 mg/kg caused a slight decrease in total WBC counts ($p<0.05$). Figure 4 shows the variations observed in WBC counts.

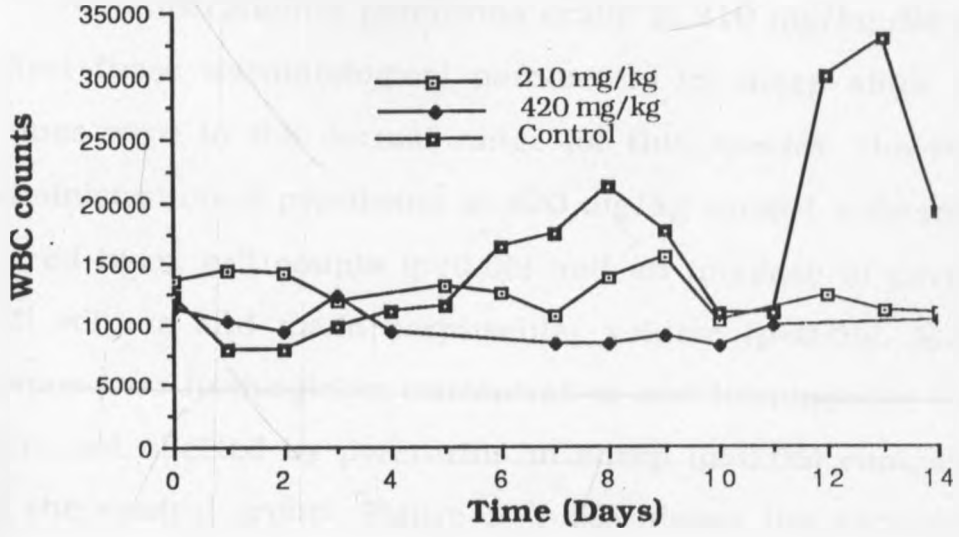


Figure 4: Effects of pyrethrins on WBC in Red Masai sheep

In differential leucocyte counts, there was overall significant difference in the percentage neutrophils and lymphocytes between the control and the two treatment groups. Pyrethrins at 210 mg/kg did not affect differential leucocyte count. However at 420 mg/kg pyrethrins caused an increase in lymphocytes, a decrease in neutrophils ($p<0.05$) and no effects on eosinophils ($p>0.05$). The control group showed significant increase in WBC counts between days 11 and 14 which was explained as being an artifact. It should be mentioned that with a group size of only 2 one should be careful when using sophisticated statistical methods, and should not rely blindly on the results of such calculations.

3.2.6.2 Red blood cell counts, haemoglobin, mean corpuscular volume and microhaematocrit

Administration of pyrethrins orally at 210 mg/kg did not affect these haematological parameters in sheep since the values were in the normal range for this species. However, administration of pyrethrins at 420 mg/kg caused a decrease in red blood cell counts ($p<0.05$) and an increase in packed cell volume and mean corpuscular volume ($p<0.05$). Mean corpuscular haemoglobin concentration and haemoglobin level were not affected by pyrethrins in sheep ($p>0.05$) compared to the control group. Figure 5 (p.58) shows the variations observed in red blood cell counts, haemoglobin concentration, mean corpuscular volume and packed cell volume (MCV).

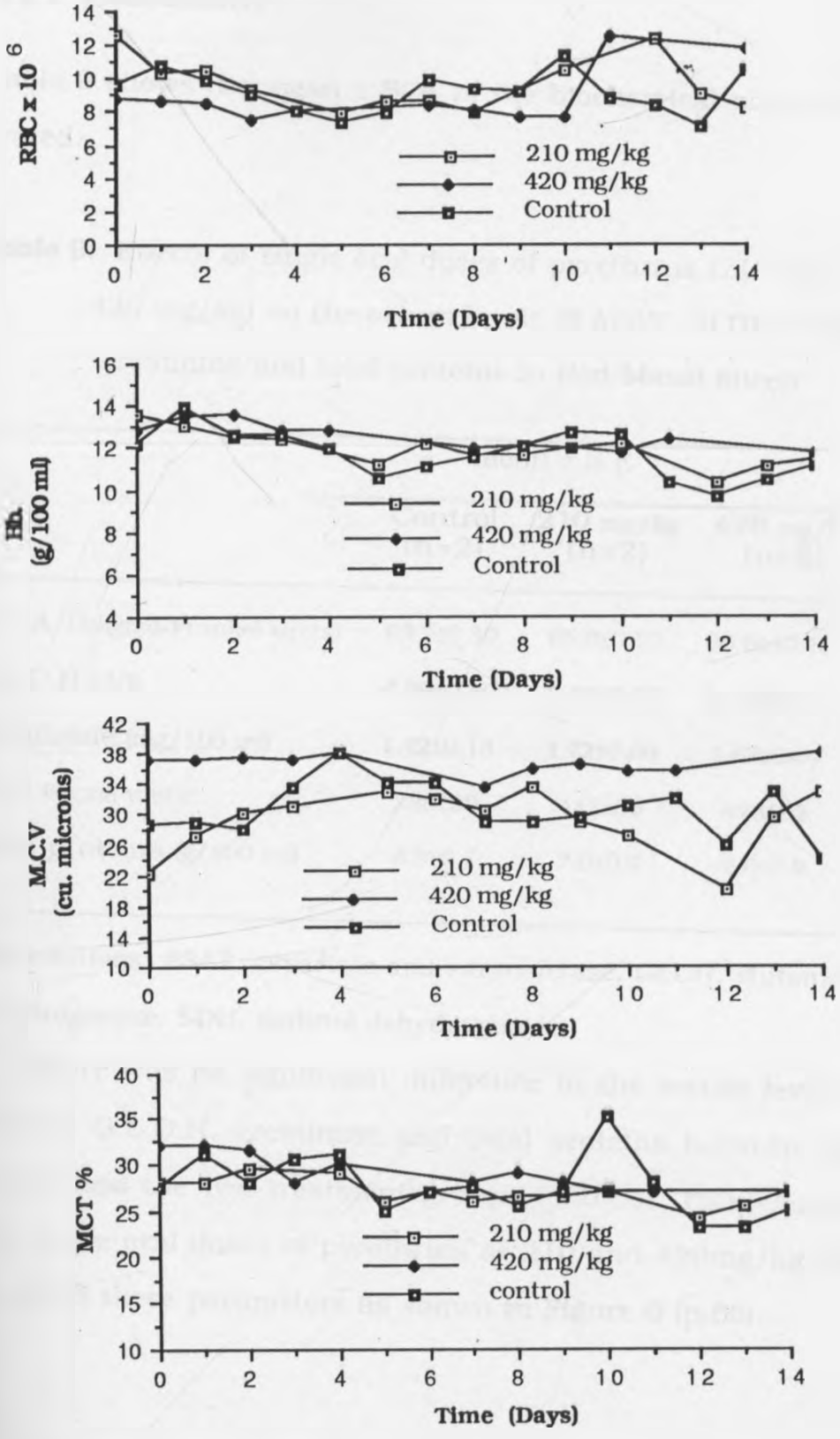


Figure 5: Effects of pyrethrins on RBC counts, haemoglobin concentration, MCV and MCT.

3.2.7 Biochemistry

Table 9 shows the mean ± S. E of the biochemical parameters tested.

Table 9: Effects of single oral doses of pyrethrins (210 and 420 mg/kg) on the serum levels of ASAT, GLDH, S.D.H, creatinine and total proteins in Red Masai sheep

	mean ± S.E		
	Control (n=2)	210 mg/kg (n=2)	420 mg/kg (n=2)
A.S.A.T(Sigma-Frankel units)	83.3±6.39	85.9±6.65	96.6±10.18
G.L.D.H (U/l)	4.99±0.80	5.74±0.73	5.18±0.72
Creatinine (mg/100 ml)	1.42±0.13	1.72±0.09	1.67±0.07
SDH (sigma units)	288±39	412±36	473±62
Total proteins (g/100 ml)	6.9±0.1	7.0±0.2	7.0±0.2

Abbreviations; ASAT, aspartate aminotransferase, GLDH, glutamate dehydrogenase, SDH, sorbitol dehydrogenase

There was no significant difference in the serum level of A.S.A.T, G.L.D.H, creatinine and total proteins between the control and the two treatment groups ($p>0.05$). This showed that single oral doses of pyrethrins at 210 and 420mg/kg did not affect these parameters as shown in Figure 6 (p.60).

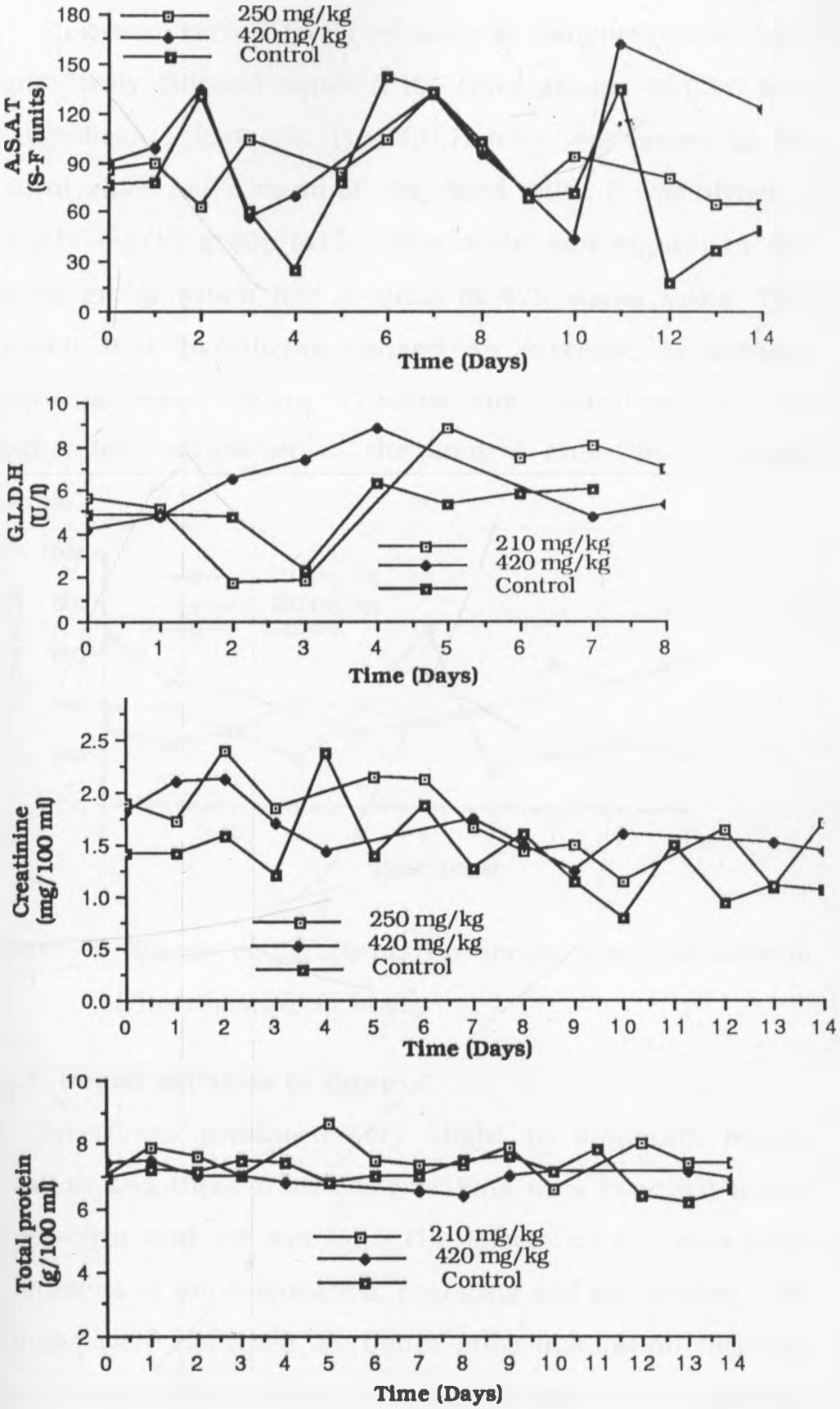


Figure 6: Effects of pyrethrins on serum levels of A.S.A.T, G.L.D.H, creatinine and total proteins in sheep

However, serum levels of sorbital dehydrogenase were significantly different between the three groups at 0.05 level of significance ($p<0.05$). The S.D.H level was lowest in the control which had a mean of 288 sigma units, it was higher in the 210 mg/kg group (412 sigma units) and highest in 420 mg/kg group which had a mean of 472 sigma units. This showed that pyrethrins caused an increase in sorbital dehydrogenase. Figure 7 show the variations in this biochemical parameter in the control and the treatment groups.

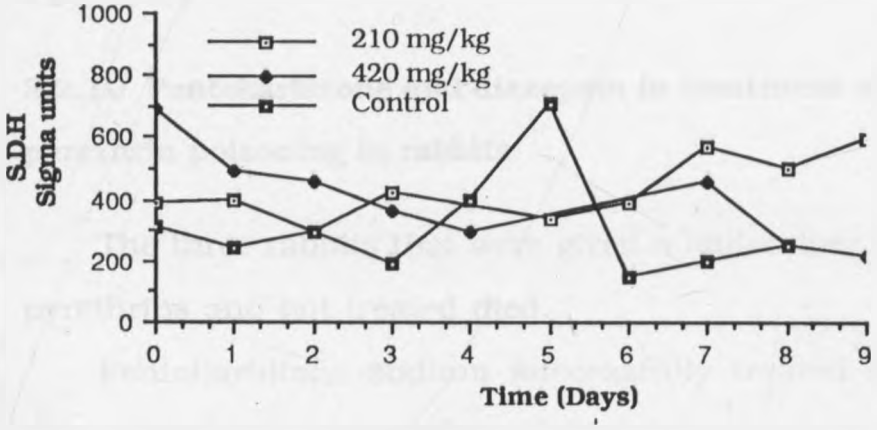


Figure 7: Effects of pyrethrins on serum levels of sorbitol dehydrogenase in sheep

3.2.8 Ocular irritation in sheep

Pyrethrins produced very slight to moderate ocular irritation and these transient reactions were confined to the conjunctiva and the eye lids. The effects on the eyes were hyperaemia of the conjunctiva, chemosis and lacrimation. The changes were observed 24 hours after instillation into the eyes, by four days the effects had almost subsided and at seven days the eyes were normal. These changes were the same in

washed and unwashed eyes indicating that washing had no effects on the eye irritation (Appendix 13 p.110)

3.2.9 Dermal irritation

Pyrethrum extract containing 50% w/w pyrethrins with piperonyl butoxide was found to cause moderate skin irritation in the sheep. The effects on the skin were erythema and edema formation of various degrees. A primary irritation index of five was obtained and according to the method the extract is said to cause a moderate skin irritation (Appendix 14 p. 111).

3.2.10 Pentobarbitone and diazepam in treatment of pyrethrin poisoning in rabbits

The three rabbits that were given a lethal dose of pyrethrins and not treated died.

Pentobarbitone sodium successfully treated two out of three rabbits that were given a lethal dose of pyrethrins.

Diazepam successfully treated one out of three rabbits that were given a lethal dose of pyrethrins.

CHAPTER FOUR

TRIALS ON THE ANTHELMINTIC ACTIVITY OF PYRETHRINS

4.1 MATERIALS AND METHODS

4.1.1 EXPERIMENT A: TRIALS ON EFFECTS OF PYRETHRINS ON *HAEMONCHUS CONTORTUS IN VITRO*

4.1.1.1 Effect of pyrethrins on the adult worms

Isolation of the worms

Abomasums of sheep were collected from Kiserian slaughter house in Kajiado district. The gut washings were strained through mesh sieves and adult worms were collected and identified as *Haemonchus contortus*.

Exposure to pyrethrins

The worms were placed in petri-dishes containing the pyrethrins solutions. Pale pyrethrum extracts containing 25% w/w and 50% w/w pyrethrins with piperonyl butoxide were used. The pyrethrins were diluted with odourless isoparaffin as the diluent to give pyrethrin concentrations of 12.5%, 6.25%, 3.125% and 1.56% w/w. Controls were set where worms were placed in piperonyl butoxide, physiological saline and odourless isoparaffin. The worms were observed after placing them in the petri dishes and the number of deaths occurring recorded.

4.1.1.2 Effects of pyrethrins on the eggs

Harvesting of the eggs

Adult worms were crushed using a pestle and mortar in order to obtain eggs which were later identified as strongyle eggs under the low power of the microscope.

Cultures

The eggs were cultured in sterile faeces in which pyrethrins were incorporated by adding 3 ml of 50 %, 25%, 12.5%, 6.25%, 3.125%, 1.56% and 0.78% w/w pyrethrins. Sterile faeces were broken up finely using a pestle and mortar to prepare the cultures. The eggs were then mixed with the faeces and damped with water to make a mixture that was moist and crumbly but not really wet. Wide-mouthed glass jars were filled with the mixture of eggs, faeces, water and pyrethrin solutions. Control cultures contained no pyrethrins. The lids of the glass jars were then replaced and incubated in a dark place for 7 days at 27°C after which the cultures were examined for hatchability of eggs.

4.1.1.3 Effects of pyrethrins on the larvae

Larvae were obtained by culturing eggs of the worm in sterile faeces for 7 days at 27 °C as described above. Water was added to the cultures until the jars were full to the brim. A standard petri dish was then inverted over the mouth of the jar and the contents turned upside down so that the inverted jar stood in the petri dish for the recovery of the larvae. 15ml

of water were poured into the petri dish and the jar and dish allowed to stand overnight for the larvae to move into the fluid. The fluid in the petri dish which contained larvae, was then pipetted into a measuring cylinder and allowed to stand so that the larvae settled at the bottom. The water was decanted and the larvae left at the bottom. The number of larvae in each drop of the sediment was estimated by placing a drop on a microscope slide and the larvae counted under low power of the microscope. This was repeated three times and the average obtained. Each drop was found to contain about 300 larvae. Serial dilutions of pyrethrins were prepared by diluting the extract with 50% w/w pyrethrins using odourless isoparaffin for diluting to obtain pyrethrins concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.56% and 0.78% w/w.

Three drops of each concentration were placed on different microscopic slides. Then a drop of the sediment with about 300 larvae was placed on the drop of pyrethrins and mixed. Controls were set where the larvae were placed in physiological saline, corn oil and piperonyl butoxide. The slides were observed under the low power of the microscope and death of the larvae recorded.

4.1.2 EXPERIMENT B: TRIALS ON THE ANTHELMINTIC ACTIVITY OF PYRETHRINS IN SHEEP

4.1.2.1 Sheep

Four naturally infected sheep having total egg counts of between 300-5300 eggs per gram of faeces were used. The details of the sheep and housing are described in chapter 3

section 3.1.1. The sheep were naturally infested with helminths at the time of purchase.

4.1.2.2 Administration of pyrethrins and determination of fecal eggs counts

The egg counts with modified McMaster technique were done daily for 5 days before treatment and then for 21 days after treatment with a 50% w/w pyrethrins with piperonyl butoxide as a synergist. The extract was given orally as a single dose of 168 mg per kg body weight. The effect of the extract was ascertained on the basis of reduction of total egg counts.

4.2 RESULTS

4.2.1 Effects of pyrethrins on the adult worms *in vitro*

It was found that when in contact with pyrethrins the adult worms died. The percentage of worms that died increased as concentrations of the pyrethrins increased. Table 10 (p. 67) shows the percentages of worms that died at different pyethrins concentrations for five hours after administration. The deaths in the controls where the worms were placed in physiological saline or in odourless isoparaffin were negligible. At least 75% of the worms that were placed in pyrethrins solutions died within five hours of exposure. Piperonyl butoxide alone was also found to kill worms because more than 70% of the worms in the solvent died. The death of the worms was attributed to *in vitro* anthelmintic activity of the pyrethrins and piperonyl butoxide.

Table 10: Effects of pyrethrins on adult *Haemonchus contortus*

Test Solution	Number exposed	Percentage deaths in 5 hrs				
		1hr	2hrs	3hrs	4hrs	5hrs
50% pys	39	92.3(36)	92.3(36)	100(39)	100(39)	100(39)
25% pys	30	30 (9)	60 (18)	100(30)	100(30)	100(30)
6.25 pys	48	0 (0)	48 (23)	50 (24)	85 (41)	100 (48)
12.5% pys	50	0 (0)	40 (20)	40 (20)	60 (30)	100(50)
3.125%pys	54	0 (0)	14.8(8)	14.8(8)	85.2(46)	85.2(46)
1.56% pys	40	0 (0)	0 (0)	0 (0)	62.5(25)	75 (30)
Pbo	38	57.9(22)	71.1(27)	71.1(27)	71.1(27)	71.1(27)
Isoparaffin	48	0 (0)	8.33(4)	8.33 (4)	8.33(4)	8.33 (4)
Ps	45	0 (0)	0 (0)	0 (0)	0.6 (3)	0.6 (3)

Pys. pyrethrins, Pbo. piperonyl butoxide, Ps. physiological saline solution

The figures in brackets are the actual number of worms that died out of the total number exposed.

4.2.2 Effects of pyrethrins on eggs

Larvae were harvested from all the cultures. The concentration of the larvae per ml was roughly the same for all experimental and control cultures.

4.2.3 Effects of pyrethrins on the larvae

The effects of pyrethrins on the larvae followed a similar pattern as in the adult worms. When in contact with solutions containing pyrethrins, larvae died. It was observed

that the percentage deaths were directly proportional to the pyrethrins concentrations. The scores were subjective and consisted of checking on the proportion of the larvae that were paralysed.

4.2.4 Effects of pyrethrins on fecal egg counts in sheep

Using analysis of variance there was no significant difference in fecal egg counts per gram of faeces between the pre-treatment and post treatment period ($p>0.05$). The actual raw data is show in Appendix 15 (p.112). When the animals were later treated with a suspension containing 1.5% levamisole and 80% bithionol sulphoxide (wormicide plus®), fecal egg counts dropped drastically.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Discussion

There are four methods used frequently for determination of LD_{50} and a measure of variability; namely Reed and Muench (1938), Miller and Tainter (1944), Litchfield and Wilcoxon (1949) and Weil (1952). The Miller and Tainter method is fairly simple and requires logarithmic-probit paper. The LD_{50} is obtained as well as the standard error of the LD_{50}. The method of Litchfield and Wilcoxon is a graphic method but is more complex in the use of nomographs which are designed to avoid use of probits and logarithms. The method yields the LD_{50}, slope of the dose response function and confidence limits. The method of Weils which was used in the present study requires more calculations than the Miller and Tainter method, but it is straight forward.

The oral LD_{50} s and their 95% confidence intervals in rabbits were 1260 (630 to 2,521) mg/kg and 1,512 (1,008 to 2,268) mg/kg for pyrethrins with piperonyl butoxide and pyrethrins alone respectively. Rabbits previously treated with phenobarbitone sodium, an enzyme inducer, were very resistant to pyrethrins poisoning. The oral LD_{50} was greater than 4,536 mg/kg. The oral LD_{50} in sheep was 594 mg/kg b.w.t for pyrethrins with piperonyl butoxide. In pharmacology, there are two types of responses that are observed when a drug is administered to a biological system. These are the graded dose effect relationship, and the quantal or the all-or-

none response (Levine, 1973). In the graded dose effect relationship, it is assumed that individual units in the biological system increases in their responses as the dose of the drug is increased upto a maximum when the drug has occupied all the receptors. In the quantal type of response, the assumption is that the individual units of the system respond to their maximum capacity or they do not (Beckman, 1961). Thus in toxicity studies, one criterion could be whether the animal dies or lives. The dose of a drug or chemical required to kill 50% of a population of animals is called the median lethal dose (LD_{50}) (Loomis, 1974). According to Loomis (1974), the following is a practical classification of toxicities which can serve as a useful guide;

Classification	LD_{50}
1. Extremely toxic	1 mg/kg or less
2. Highly toxic	1 to 50 mg/kg
3. Moderately toxic	50 to 500 mg/kg
4. Slightly toxic	0.5 to 5 g/kg
5. Practically non toxic	5 to 15 g/kg
6. Relatively harmless	more than 15g/kg

Basing the conclusions on the above classification, it was concluded that pyrethrins alone and with piperonyl butoxide are slightly toxic to both sheep and rabbits, while pyrethrins alone after prior treatment with phenobarbitone sodium, an enzyme inducer were practically non toxic to rabbits.

The LD_{50}s obtained in the present studies are in good agreement with the findings of various workers with respect to acute oral LD_{50} of pyrethrins in mammals. The oral LD_{50}

reported by various workers vary from 200 mg/kg to 4,700 mg/kg, the average for all mammals tested being 1,500 mg/kg. Table 3 (p.29) summarises the LD_{50} values recorded for rats, mice, guinea pig, rabbits and cats using various routes of administration.

The resistance to pyrethrin toxicity by rabbits which had been pretreated with phenobarbitone suggested that the pretreatment resulted in an increase in the rate of pyrethrins metabolism. Phenobarbitone stimulates increased microsomal enzymes activity. Part of the relative nontoxicity of pyrethrins to mammals appear to result from rapid metabolism (Soderlund and Casida, 1977a, b). Therefore resistance of pyrethrin induced toxicity in the rabbits preatreated with phenobarbitone sodium in present studies was explained as being due to increased metabolism due to increased microsomal enzyme production. On the other hand the increased susceptibility of rabbits poisoned by pyrethrins with piperonyl butoxide which is an enzyme inhibitor was probably due to microsomal enzyme inhibition.

Temperature, pulse and respiration varied in the eight hour period as shown in Figure 3 (p.53). However, these variations were in the normal ranges for sheep and can be accounted for by inter individual variations rather than being due to the effects of pyrethrins.

The toxic signs observed comprised hyperaesthesia, muscle tremors, increased respiratory rate, incoordination, convulsions, recumbency and finally death. Pyrethrins and many pyrethroids cause several signs of poisoning including hyperactivity, ataxia, convulsions and eventual paralysis (Blum

and Kearns, 1956, Burt and Goodchild, 1971 a, b; Camougis and Davis, 1971, Clements and May, 1977; Narahashi, 1971). With many of the newer synthetic compounds, in particular the α-cyano-3-phenoxybenzyl esters (for example, deltamethrin, cypermethrin and fenvalerate), clinical signs of excitation are absent. Instead, ataxia and a lethargic state develop, leading to flaccid paralysis (Adams and Miller, 1980). Gombe and Oduor-Okelo (1983), reported sign of acute poisoning in German Shepherd dogs receiving pyrethrins at 100 mg/kg body weight orally. They reported that within 10 minutes of feeding, the animals became quiet and tranquil. Other signs were, restlessness, mild jaw snapping and sternal recumbency. They also observed occasional clonic convulsions, violent shivering and jaw snapping among other clinical observations. In the present studies, similar signs of poisoning were observed but the dosage required was much higher. This indicated that sheep and rabbits are more resistant to pyrethrin poisoning than dogs. This can be explained by faster degradation of pyrethrins in the gastrointestinal tracts and livers in the sheep and rabbits which have more complex gastrointestinal tracts than the dog.

Pyrethrins are very similar to pyrethroids in many aspects including clinical signs of toxicity and mechanisms of action. The action of pyrethroid insecticides have been divided into two types (I and II) in mammals (Verschoyle and Aldridge, 1980; Lawrence and Casida, 1982). Type I action is prolongation of the sodium current in nerve axons leading to repetitive firing (Gammon, 1977, 1978). The type I syndrome

in the mouse involves marked increase in hypersensitivity and hyperactivity followed quickly by whole body tremors accompanied by clonic seizures. The animals become prostrate 5 to 20 minutes after initial onset of signs with profound body tremors persisting until death, usually 5 to 30 minutes later. Pyrethroids with a cyanophenoxybenzyl moiety produce the type II syndrome and do not cause repetitive firing in a variety of nerve axons (Duclohier and Georgescauld, 1979 ; Gammon, 1980 ; Gammon *et al.*, 1981). The type II pyrethroids signs are initially similar to type I signs but rapidly progress to include sinous writhing (Choreathetosis) and profuse salivation often with tonic seizures shortly before death in 15 to 45 minutes. Possible mechanism of action for the type II pyrethroid syndrome include action on GABA receptor complex or a closely linked class of neuroreceptors, or through depolarization of nerve terminals. In the present studies pyrethrins were found to cause a nervous syndrome consisting of clinical signs similar to the one described above, mainly type I syndrome .

In the present studies, it was found that acute pyrethrins toxicity can be treated with pentobarbitone sodium and diazepam. It has been suggested that pyrethroids may interact with binding sites in the brain for dihydropicrotoxinin(DHP) (Leeb-Lundberg and Oslen, 1980) or kainic acid (KA) (Staatz *et al.*, 1981). Diazepam reduces the severity of convulsions induced in rats or mice by permethrin (Staatz *et el.*,1980) or picrotoxinin (PTX) (Costa *et al.*, 1975) and KA (Ben-Ari *et al.*, 1979). In the present studies diazepam was found to control convulsions induced by pyrethrins toxicity in one out of three

rabbits. Pentobarbitone is a GABA-mimetic i.e, it interacts with GABA receptors to produce an increase neuronal chloride conductance (Booth, 1988). In treatment of pyrethrins poisoning in rabbits pentobarbitone could have worked through the same mechanisms.

The number of the various circulating blood cells vary with normal physiological states as well as with pathological conditions. The considerable variations that normally exist among a given population are attributed to sex, age, nutrition, ambient temperature, diurnal and sexual cycles. Therefore, the normal values listed are usually considered as guide lines rather than rigid criteria. The mean total white blood cell counts were in the normal range for the ovine species as shown in Appendix 16 (p. 113). However, there was an overall significant difference between the three groups in total WBC ($p<0.05$). The data indicated that oral administration of pyrethrins caused a slight decrease in total WBC counts. For the differential leucocyte counts, there was overall significant difference in the percentage neutrophils and lymphocytes between the three groups ($p<0.05$). Pyrethrins caused a decrease in neutrophils and an increase in lymphocytes. There was no overall significant differences in the percentage of the eosinophils between the control and the treatment groups ($p>0.05$). Depression of leucopoiesis is the most common toxic action on leucocytes, but a rise in the number of circulating neutrophils may occur, as has been reported with a variety of drugs e.g salicylates, quinine, phenylhydrazine, antipyrine, phenacetin, salvarsan and benzol derivatives.

Administration of pyrethrins orally at 210 mg/kg did not affect these haematological parameters in sheep since the values were in the normal range for this species. However, administration of pyrethrins at 420 mg/kg caused a decrease in red blood cell counts ($p<0.05$) and an increase in packed cell volume and mean corpuscular volume ($p<0.05$). The increase in PCV was explained as being due to oedema. At postmortem there was extensive congestion and oedema of the respiratory and cardiovascular systems. Mean corpuscular haemoglobin concentration and haemoglobin level were not affected by pyrethrins in sheep ($p>0.05$) compared to the control group. This is an indication that pyrethrins did not cause anaemia in sheep. Anaemia implies a fall in the haemoglobin level in blood, it can also occur due to a fall in red blood cell number. Changes in packed cell volume occurs due to alterations in the number of cell and/or their sizes. So the determination of red blood cell and their indices was designed to test for effects of pyrethrins on the erythropoetic system. Results of the present studies indicate that pyrethrins at 210 and 420 mg/kg as single oral doses do not affect the blood parameters significantly in terms of range of values or in overall pattern. This is in good agreement with various researchers who have reported that pyrethrins cause little or no effects on haematological parameters in normal animals. Gombe and Oduor-Okello (1983) in their chronic (90 days) toxicity of pyrethrins in the German shepherd dogs noted an immediate elevation in PCV, RBC and Hb in the 100 mg/kg group which gave the appearance of polycythaemia. However, this was not accompanied by reticulocytes or normoblasts and

other early forms of blood cells and was probably due to splenic contraction as there was a concomitant clinical appearance of anaemia. Other RBC parameters: MCH, MCV, MCHC, remained virtually unaltered during the 90 day experimental period.

Lorber (1972) compared the effects of pyrethrins on intact and splenectomized dogs. He reported no effect in normal dogs but observed erythroid hyperplasia in the bone marrow and an increase in reticulocytes in peripheral blood in two recently splenectomized dogs using the same dosage in all the dogs. These findings indicate that splenectomy permitted some of the pyrethrins, which would otherwise be metabolized in the liver, to reach the bone marrow which interfered with haematopoiesis.

Carpenter *et al.*, (1950), conducted detailed work on inhalation studies with pyrethrins aerosols in white rats and reported that there was no significant differences between the treated and the control in haematological parameters.

Bond *et al.*, (1973), studied the toxicity of pyrethrins in albino rats when administered daily for 90 days. Using the blood obtained from these animals on days 13, 30, 59 and 91, they determined the haematocrit value, haemoglobin content, white and red blood cell counts and differential leucocyte counts. They found that although some changes occurred in blood, these changes did not appear significant.

The present studies indicate that pyrethrins do not cause significant differences in the serum levels of A.S.A.T, G.L.D.H, creatinine and total proteins between the control and the treatment groups ($p>0.05$). This is an indication that

pyrethrins did not affect the levels of these biochemical values which are mainly used for detection of pathological changes in the liver and the kidney. This is in contrast with what was observed in the German Shepherd dogs where pyrethrins caused a 5-10 fold increase in plasma A.S.A.T in the male dogs receiving 100 gm/kg in the fifth and sixth week (Gombe and Oduor-Okelo, 1983). Serum levels of sorbital dehydrogenase were significantly different between the three groups ($p>0.05$). The S.D.H level was lowest in the control which had a mean value of 288 sigma units, it was higher in the 210 mg/kg group (412 sigma units) and highest in 420 mg/kg group which had a mean of 472 sigma units. This is an indication that treatment with the pyrethrum extract caused a rise in the serum levels of sorbital dehydrogenase. However the levels were still in the normal range for sheep.

The biochemical observations indicate that single oral doses of pyrethrins at 210 or 420 mg/kg cause very little changes in liver enzymes. It was only sorbital dehydrogenase that was significantly high in the treated group as compared with the control. Sorbital dehydrogenase is used in liver function test in ruminants and its rise implied that there were some pathological changes in the liver. Pyrethrins have been shown to cause some hepatotoxicity in rodents, in examining the combined effect of DDT, pyrethrins and piperonyl butoxide, Kimbrough *et al.*, (1968) found that synergized pyrethrins produced liver enlargement and cellular changes including margination and cytoplasmic inclusions. However, these studies were concerned with morphological changes and did not include biochemical

observations. Hepatotoxicity due to administration of drugs with varying chemical and biological characteristics is of value in diagnosis of toxicity.

Springfield *et al.*, (1971) showed that pyrethrins cause significant liver enlargement and increased microsomal enzyme activity in rats. Their results indicated that liver enlargement began within four days following daily administration of pyrethrins. The liver remained enlarged throughout the treatment period but returned to control values within 7 days when treatment stopped. The results are inconsistent with the findings of other researchers and again this raises the question about the purity of the materials these investigators used.

Serum creatinine level is used as a kidney function test in mammals. In most species, serum concentrations of both urea and creatinine are crude estimates of glomerular filtration rate (GFR). Creatinine is a better index of renal efficiency than urea which is influenced by other factors. Although blood urea levels may be considered to be an index of renal efficiency, it must be appreciated that blood non-protein nitrogen levels is influenced by the proportion of proteins in the diet, the volume of urine, and the occurrence and rate of endogenous protein catabolism. Delmar (1980) repoted that on the basis of mechanism of renal excretion of the two compounds, creatinine is a better indicator of GFR. There was no significant differences in serum creatinine levels indicating that the extract does not affect glomerular filtration rate in sheep.

The biochemical results indicate that single oral doses of pyrethrins at 210 or 420 mg/kg do not cause hepatotoxicity in sheep. These enzymes have been used by other workers in determination of liver toxicity. The biochemical parameters tested were designed to test whether pyrethrins affects the liver and/or the kidneys. Many liver function tests which are of considerable value in diagnosis of liver diseases in small animals are of limited value in ruminants. For example, in cattle and sheep total bilirubin levels rarely rise above 2 mg/100 ml even when obstruction or hepato-cellular damage is present (Doxey, 1971) and thus serum bilirubin levels are of little diagnostic value except in severe cases of liver damage. Plasma levels of aspartate aminotransferase (A.S.A.T) are widely used in diagnosis and prognosis of liver damage in all species. Since A.S.A.T is present in high concentration in both skeletal and cardiac muscles, elevation in enzyme levels may indicate muscle rather than liver damage. Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma allows leakage of large amounts of this enzyme in the blood. Alanine aminotrasferase (S.G.P.T) is a liver specific enzyme and is used in diagnosis of liver conditions in dogs (Hoe and O'shea, 1965). S.G.P.T concentrations are low in the livers of large animals (Cornelius, 1963) and thus little elevation in the serum activity is observed in hepatocellular necrosis in these species.

The enzyme sorbital dehydrogenase (S.D.H) occurs in high concentrations in livers of man (Gerlach, 1963), dogs (Zinkel *et al.*, 1971), calves and sheep. It is utilized in studies of various liver diseases in ruminants (Boyd, 1962 ; Shaw,

1974, Ford and Gopinath, 1976), such as fascioliasis and in evaluation of fasciocidal drugs in sheep and cattle (Fowler, 1971, Harvey and Hoe, 1971, Alemu *et al.*, 1977). S.D.H also occurs in the kidneys of these species but at considerably lower levels than those present in the liver. The enzyme is present in other organs and tissues at very low concentrations (Ford *et al.*, 1967) and thus for all practical purposes it can be considered to be a liver specific enzyme. A kit for measurement of S.D.H is commercially available and measurement of the enzyme activity depend on the change in optical density due to oxidation of reduced nicotinamide adenine dinucleotide (N.A.D.H). Sorbital dehydrogenase determination, in conjunction with aspartate aminotranferase determination are of considerable importance in the diagnosis and prognosis of liver diseases in ruminants and in the differential diagnosis of liver and muscle diseases. This was the reason the enzymes were included in the present studies in order to investigate whether pyrethrins cause any pathological effects in these organs in sheep.

Glutamic dehydrogenase (G.L.D.H) is the enzyme of choice for detection of bovine liver diseases. It can also be used in the sheep. Boyd (1962) showed that G.L.D.H is highly concentrated primarily in ovine and bovine species and recommended its use in measuring hepatic necrosis in the species. It has become the enzyme of choice in measuring hepatic necrosis in ruminants. Glutamate dehydrogenase has been useful in studying experimental carbon tetrachloride poisoning in cattle and sheep (Boyd, 1962, Fowler, 1971), measuring necrosis after hexachlorophene administration in

sheep (Harvey and Hoe, 1971), calving (Treacher and Collis, 1977) and studying secondary effects on the liver after bile duct ligation in sheep (Ford and Gopinath, 1976).

The concentration of plasma proteins depend upon a multitude of factors, including the extent, duration, severity and primary nature of the damage, current rate of synthesis, catabolism, hepatic release and distribution. In addition, each of these factors can be affected by circulatory, inflammatory, reparative, degenerative, metabolic and regenerative processes occurring in specific liver disease. It is therefore impossible to define a typical plasma protein pattern in certain types of liver disease, but changes in certain types of patterns are characteristic of specific hepatopathies (Cornelius, 1980).

In the present studies the main postmortem changes were, extensive pulmonary congestion and oedema and ecchymotic haemorrhages in respiratory and cardiovascular systems. Other researchers have shown that pyrethrins cause very few pathological changes. Bond and DeFeo, (1969) fed pyrethrins at 380 mg/kg/day to rats for 90 days and observed only one death out of twenty animals. Pathology in these animals was minimal, but they reported that two of the rats showed some increase in eosinophilia of cytoplasm of the liver, indicating the beginning of cells necrosis. Two other animals showed slight kidney involvement.

Gombe and Oduor-Okelo (1983) found that the most outstanding postmortem changes in dogs receiving pyrethrins at 100 mg/kg body weight were cachexia and jaundice. The viscera were completely devoid of fat, the liver was yellow and

cirrhotic and the spleens were smaller. No other abnormality of the thoracic or abdominal viscera was evident. The meninges were similarly clear of any congestion or inflammation. No gastroenteritis was evident.

Externally pyrethrins caused slight to moderate ocular irritation in sheep in the present studies. The main inflammatory changes on the eyes were hyperaemia of conjunctiva, chemosis and lacrimation. On the skin, pyrethrins caused moderate irritation, erythema and oedema. Dermal toxicity of pyrethrins is negligible, because they seem to be poorly absorbed through the intact skin. The reported values of dermal LD_{50} of the pyrethrins in several species range from 1,300 mg/kg to 5,000 mg/kg.

Many humans have experienced dermatitis following exposure to pyrethrins. This dematitis is an allergic manifestation and disappears rapidly on removal of the compounds. In humans, numerous investigations of allergic responses caused by pyrethrins have been reported. One of the early records of allergic reaction is a report on pyrethrin dermatitis among factory workers by McCord *et al* (1921). Their investigation reported that 30% of the workers engaged in grinding, filling, weighing and sealing pyrethrum powder were found to have erythema venestrum, skin roughening and erythema with pruritus which cleared up on removal of exposure. Kensten and Laszelo (1931) reported two cases of dermatitis following exposure of their patients to commercial preparation of pyrethrins. Martin and Hester (1941) examined the allergen in pyrethrum flowers causing

dermatitis. They found a volatile oil to be highly active in causing dermatitis.

Pyrethrins has been used orally as an anthelmintic for many years with no apparent ill effects (U.S.D.A., 1959). In the present trials pyrethrins were also found to have some *in vitro* anthelmintic activity. Placing larvae and adult *Haemonchus contortus* in solutions containing pyrethrins resulted in death of both stages of the worm. The number that died was found to be directly proportional to the concentration of pyrethrins. No recent literature is available on the anthelmintic activity of pyrethrins but many researchers have worked on anthelmintic properties of pyrethrins. A literature review on the work done on the anthelmintic activity of pyrethrum was published by Mclellan (1964). The review indicates that the earliest reference to the anthelmintic activity of pyrethrins dates back to 1855 when pyrethrins were used as an enema and evicted large numbers of *Enterobius vermiculars* from a 20 year old man. In the same review it was reported that due to destruction of the pyrethrins by the digestive juices neither oral doses of pyrethrins nor colonic washings were really effective in eliminating *Enterobius* infestations from the duodenum and ileo-caecal region. The development of special granules is reported, designed to release pyrethrins along the entire digestive tract, with these granules complete success was achieved in all subjects treated. In further trials using the granules, humans infestated with *Trichuris*, *Ascaris* and *Ancylostoma* were successfully cured. Results of the current trials are consistent with these findings, Pyrethrins were

lethal to larvae and adult *Haemonchus contortus in vitro* but when given orally to sheep infested with worms there was no significant difference in fecal egg counts per gram of faeces between the pretreatment and post treatment periods ($p>0.05$). Based on the *in vitro* trials it was concluded that pyrethrins have potential anthelmintic activity. It was not possible to conclude from the *in vivo* studies because it was a trial using only one dosage level in four sheep. The fact that pyrethrins were effective against the worms *in vitro* and not *in vivo* was probably due to rapid destruction of pyrethrins in the gastrointestinal tract and the liver of the sheep. Development of special granules designed to release pyrethrins slowly along the entire digestive tract of sheep was beyond the scope of the present trials. The presence of large amounts of mucous in the gastrointestinal tract of sheep could have rendered the treatment ineffective since mucous absorbs and inactivates pyrethrins.

In the present trials, pyrethrins did not have significant effects on the eggs of *Haemonchus contortus in vitro*, since there was no effect on hatchability. This is in good agreement with the findings of other researchers. Pyrethrins appear ineffective against the eggs, this was reported by Mclellan (1964). He reported that in cases of oxyuriasis treatment should be repeated after a 10-day interval, an ointment of 1 part pyrethrins should be applied to the anus to calm itching and kill the worms which have descended to that region. In another study on *Ascaris lumbricoides* of the pig and *Taenia* species of the dog it was found that contact with a dilute pyrethrin solution caused the worms to be "animated by

violent movements, the head detaches from the intestinal wall, and after sometimes they become paralysed and die".

The methods available for measuring anthelmintic activity include the faecal egg count reduction test (FECR) test, in vivo assays and anthelmintic efficiency test. The FECR test provides an estimate of athelmintic efficacy by comparing egg counts of groups of animals before and after treatment. The test results only estimates anthelmintic efficacy because nematode egg output does not always correlate well with the actual worm numbers and it only measures the effects on mature worms. Another short coming of this procedure is that anthelminitic treatment may cause a temporary suppression in worm egg output without any worm loss. Failure of an anthelmintic to considerably reduce egg counts indicates resistance, but most natural infection are with a mixture of species and only one species may be resistant. Therefore in addition to faecal egg counts, infective larvae derived from pre- and post-treatment faecal cultures need to be identified. Further more, egg counts will not detect the presence of immature parasites that survive treatment and which may develop to adult parasites and contribute to post-treatment egg count.

5.2 Conclusions

The acute oral LD_{50}s for pyrethrins with piperonyl butoxide and pyrethrins alone are 1,300 mg/kg and 1,500 mg/kg in rabbits respectively. Rabbits are very resistant to pyrethrin toxicity after pretreatment with phenobarbitone sodium since the oral LD_{50} for pyrethrins alone was more than

4,500 mg/kg b.w.t. The acute oral LD_{50} for pyrethrins with piperonyl butoxide in sheep was 600 mg/kg b.wt. This indicated that pyrethrins alone and with piperonyl butoxide (synergist) are slightly toxic to both sheep and rabbits, while pyrethrins alone are practically non toxic to rabbits pretreated with phenobarbitone sodium.

The main clinical signs of acute pyrethrins toxicity in sheep and rabbits were hyperexcitation, tremors convulsions, paralysis and death. Pentobarbitone sodium and diazepam controlled signs of pyrethrins toxicity in rabbits. Externally pyrethrins caused mild and short-lived irritation of the eyes and skin. The main inflammatory changes on the eyes of sheep were hyperaemia of conjunctiva, chemosis and lacrimation while on the skin irritation, erythema and oedema occurred.

The main postmortem changes in acute pyrethrins toxicity in rabbits and sheep were pulmonary congestion and oedema and ecchymotic haemorrhages in respiratory and cardiovascular systems indicating that death occurred probably due failure of both respiratory and cardiovascular systems.

Pyrethrins administered orally as a single dose at 210 mg/kg and 420 mg/kg b.wt caused very slight effects on haematological and biochemical parameters in sheep indicating that pyrethrins do not cause significant pathological effects on the haemopoetic system, the liver or the kidney in sheep.

The *in vitro* studies indicated that pyrethrins have a potential anthelmintic activity due to their lethal effects on

both larvae and adult *Haemonchus contortus*. However, the *in vivo* studies were not conclusive since only one dosage level was investigated in four sheep. When administered orally as a single dose at 168 mg/kg b.wt to a group of four sheep infested with helminths, pyrethrins did not cause significant drop on the faecal egg counts per gram of faeces.

In conclusion, the present study indicate that pyrethrins stimulate the central nervous system in both rabbits and sheep. They are mild irritants of the skin and eyes and have potential anthelmintic activity. The acute LD_{50} for pyrethrins alone is 1,500 mg/kg. The toxicity is enhanced by piperonyl butoxide and reduced by pretreatment with phenobarbitone sodium. Clinical signs of pyrethrin toxicity can becontrolled by administration of pentobarbitone sodium or diazepam.

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APPENDICES

Appendix 1: Serum levels of aspartate aminotransferase (A.S.A.T), Glutamate dehydrogenase (G.L.D.H),sorbital dehydrogenase (S.D.H), creatinine and total proteins before and after oral administration of pyrethrins at 210 mg/kg (Sheep No. 03)

	Time (days)										
Parameter	0	1	2	5	6	7	8	9	12	13	14
A. S. A. T (S-F Units)	99	56	49	84	97	94	100	95	80	105	85
Creatinine (mg/100ml)	1.87	1.25	2.12	2.15	1.96	1.7	1.5	1.78	1.65	1.1	1.75
G. L. D. H (U/l)	3.0	5.5	1.0	8.8	8.8	8.0	7.6	-	-	-	-
S. D. H (sigma units)	472	503	324	337	539	711	497	594	-	-	-
Total proteins (g/100 ml)	7.0	9.0	8.0	8.6	8.0	8.0	8.4	8.8	8.0	7.8	8.0

(-) sample not taken

Appendix 2: Serum levels of A.S.A.T, G.L.D.H, S.D.H, creatinine and total proteins before and after oral administration of pyrethrins at 210 mg/kg. (Sheep No. 06)

	Time (days)										
Parameter	0	1	2	3	6	7	8	9	12	13	14
A. S. A. T (S-F Units)	73	121	76	104	110	167	95	44	93	20	42
Creatinine (mg/100ml)	1.9	2.18	2.65	1.86	2.3	1.63	1.36	1.23	1.15	1.07	1.65
G. L. D. H (U/l)	7.9	4.7	2.4	1.8	6.2	8.2	6.4	-	-	-	-
S. D. H (sigma units)	297	259	296	423	244	430	-	-	-	-	250
Total proteins (g/100 ml)	7.0	6.8	7.2	7.0	7.0	6.6	6.2	7.0	6.6	7.0	6.8

(-) sample not taken

Appendix 3: Serum levels of A.S.A.T, G.L.D.H, S.D.H, creatinine and total proteins before and after oral administration of pyrethrins at 420 mg/kg. (Sheep No. 07)

Parameter	Time (days) 0	1	2	3	4	7	8	9	10	11	14
A. S. A. T (S-F Units)	87	75	124	79	25	121	32	55	16	107	187
Creatinine (mg/100ml)	2.1	2.08	2.33	1.8	1.43	1.5	1.5	1.13	1.65	1.55	1.78
G. L. D. H (U/l)	5.4	1.6	4.5	6.5	1.6	1.6	5.8	-	-	-	-
S. D. H (sigma units)	804	571	629	417	232	461	-	-	-	1110	-
Total proteins (g/100 ml)	7.4	7.8	7.6	7.0	6.8	6.2	6.6	6.8	7.2	7.0	7.0

(-) sample not taken

Appendix 4: Serum levels of A.S.A.T, G.L.D.H, S.D.H, creatinine and total proteins before and after oral administration of pyrethrins at 420 mg/kg. (Sheep No. 11)

Parameter	Time (days) 0	1	2	3	4	7	8	9	10	11	14
A. S. A. T (S-F Units)	93	123	144	38	25	140	156	86	71	134	136
Creatinine (mg/100ml)	1.52	2.13	1.9	1.6	1.43	1.98	1.48	1.38	1.55	1.28	1.33
G. L. D. H (U/l)	3.0	7.9	8.4	8.2	1.6	8.0	4.8	-	-	-	-
S. D. H (sigma units)	573	404	285	315	232	-	247	215	-	507	331
Total proteins (g/100 ml)	7.3	7.2	6.4	7.0	6.8	6.2	7.7	7.2	7.0	7.2	6.8

(-) sample not taken

Appendix 5: Serum levels of A.S.A.T, G.L.D.H, S.D.H, creatinine and total proteins in the control (Sheep No.04)

Parameter	Time (days) 0	1	2	3	4	7	8	9	10	11	14
A. S. A. T (S-F Units)	102	81	115	90	110	126	92	92	100	95	90
Creatinine (mg/100ml)	1.1	1.3	1.48	3.18	-	1.33	2.0	2.0	0.8	1.5	1.16
G. L. D. H (U/l)	7.1	7.9	1.6	2.6	1.8	8.9	5.6	-	-	-	-
S. D. H (sigma units)	304	272	167	-	398	196	-	-	-	-	215
Total proteins (g/100 ml)	6.8	6.4	7.2	7.5	7.4	7.0	7.6	7.6	7.1	7.8	6.8

(-) sample not taken

Appendix 6: Serum levels of A.S.A.T, G.L.D.H, S.D.H, creatinine and total proteins in the control (Sheep No. 10)

Parameter	Time (days) 0	1	2	3	5	6	7	8	9	12	13	14
A. S. A. T (S-F Units)	62	30	115	88	78	142	102	49	49	17	37	90
Creatinine (mg/100ml)	1.73	1.85	1.48	1.55	1.4	1.83	1.2	-	0.28	0.93	1.1	0.95
G. L. D. H (U/l)	2.6	1.6	1.6	10	8.3	5.8	3.0	-	-	-	-	-
S. D. H (sigma units)	321	209	167	191	711	144	200	-	-	-	-	293
Total proteins (g/100 ml)	7.0	7.9	7.2	7.4	6.8	7.0	7.0	8.1	7.2	6.4	6.2	6.4

(-) sample not taken

Appendix 7: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts values before and for 14 days after oral administration of a single dose of pyrethrins at 210 mg/kg.(Sheep No.03)

Parameter	Time (days) 0	1	2	5	6	7	8	9	12	13	14
M.C.T (%)	30	29	28	26	25	24	25	24	24	26	25
Hb. (g/100 ml)	12.8	12.9	12.0	11.2	11.2	11.0	11.0	10.7	10.3	11.0	10.8
RBC count (10^6)	12.2	10.45	8.3	7.85	7.45	7.85	6.95	8.4	12.25	7.4	7.45
M.C.H.C (%)	42.7	44.5	42.9	43.1	44.8	45.8	44.0	44.6	42.9	42.3	43.2
M.C.V (cu. microns)	25	28	34	33	34	31	36	29	20	35	34
WBC x 10^3	15.0	14.8	15.5	13.2	12.7	11.2	15.8	10.7	12.4	10.9	10.8
Neutrophils %	20	48	32	25	30	25	29	29	22	37	30
Lymphocytes %	76	50	65	73	69	74	70	68	77	62	67
Eosinophils %	4	2	3	2	1	1	1	3	1	1	3

Appendix 8: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts values before and for 14 days after oral administration of a single dose of pyrethrins at 210 mg/kg.(Sheep No.06)

Parameter	Time (days) 0	1	2	3	6	7	8	9	10	13	14
MCT %	26	27	31	29	29	28	28	29	27	25	29
Hb.	14.3	13.0	13.0	12.4	13.0	12.2	12.2	13.0	12.1	11.2	12.3
RBC counts x 10^6	12.85	10.2	11.8	9.4	9.8	9.3	8.9	9.85	10.45	10.6	8.95
MCHC %	55.0	48.1	41.9	42.8	44.8	43.6	43.6	44.8	44.8	44.8	42.4
MCV (cu. microns)	20	26	26	31	30	30	31	29	27	24	32
WBC x 10^3	11.1	14.0	12.8	11.8	12.5	10.2	11.7	20.1	10.2	11.0	10.8
Neutrophils %	34	28	31	22	33	25	69	14	14	21	27
Lymphocytes %	64	68	67	75	66	71	25	82	83	73	70
Eosinophils %	2	5	2	3	0	4	6	4	3	6	3

Appendix 9: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts values before and for 14 days after oral administration of a single dose of pyrethrins at 420 mg/kg.(Sheep No.07)

Parameter	Time (days) 0	1	2	3	7	8	9	10	11	14
M.C.T (%)	31	29	30	25	26	28	26	27	27	27
Hb. (g/100 ml)	12.0	12.3	13.5	12.4	10.9	11.8	11.1	11.6	11.9	11.3
RBC count (10^6)	8.0	7.8	8.45	7.05	8.75	7.55	7.45	7.5	7.6	7.45
M.C.H.C (%)	38.7	42.4	45.0	49.6	41.9	42.1	42.7	43.0	44.1	41.9
M.C.V (cu. microns)	39	37	36	35	29	37	35	36	36	36
WBC x 10^3	11.1	10.1	9.3	12.7	7.3	7.1	7.2	7.8	6.7	8.0
Neutrophils %	46	50	47	42	40	32	37	27	33	25
Lymphocytes %	51	50	51	56	56	68	61	69	63	75
Eosinophils %	3	0	2	2	4	0	2	4	4	0

Appendix 10: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts values before and for 14 days after oral administration of a single dose of pyrethrins at 420 mg/kg.(Sheep No.11)

Parameter	Time (days) 0	1	2	3	4	7	8	9	10	11	14
M.C.T (%)	33	35	33	31	30	30	32	30	27	29	30
Hb. (g/100 ml)	13.6	14.6	13.7	13.2	12.8	12.1	13.8	12.7	11.8	12.8	12.1
RBC count (10^6)	9.5	9.35	8.5	8.0	8.0	8.55	8.8	7.95	7.75	8.35	7.5
M.C.H.C (%)	41.2	41.7	41.5	42.6	42.7	40.3	43.1	42.3	43.7	44.1	40.3
M.C.V (cu. microns)	35	37	39	39	38	35	36	38	35	35	40
WBC x 10^3	11.5	10.4	9.3	12.0	9.3	9.3	8.5	10.7	8.4	13.0	12.3
Neutrophils %	47	50	35	29	41	28	26	29	33	31	34
Lymphocytes %	51	50	64	70	58	70	73	68	65	68	55
Eosinophils %	2	0	1	1	1	2	1	3	2	1	11

Appendix 11: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts levels in the control (Sheep No.04)

Parameter	Time (days) 0	1	2	3	4	7	8	9	10	11	14
M.C.T (%)	25	34	29	33	31	30	26	28	35	28	27
Hb. (g/100 ml)	11.2	14.6	12.8	13.0	11.9	12.5	13.3	11.2	12.6	10.3	11.9
RBC count (10^6)	8.35	10.15	10.6	9.3	8.1	12.3	11.75	10.35	11.35	8.8	11.2
M.C.H.C (%)	44.8	42.9	44.1	39.3	38.3	41.6	51.2	40.0	36.0	36.8	44.1
M.C.V (cu. microns)	30	33	27	35	38	24	22	27	31	32	24
WBC x 10^3	14.2	10.4	10.2	11.7	11.0	14.4	13.1	9.3	10.9	10.9	14.1
Neutrophils %	28	48	39	47	44	27	31	37	42	50	28
Lymphocytes %	67	51	57	51	53	69	67	62	58	50	68
Eosinophils %	5	1	4	2	3	4	2	1	0	0	4

Appendix 12: MCT, Hb., RBC, MCV, MCHC, WBC and differential leucocyte counts levels in the control (Sheep No.10)

Parameter	Time (days) 0	1	2	3	5	6	7	8	9	12	13	14
M.C.T (%)	30	28	27	28	25	27	25	25	26	23	23	23
Hb. (g/100 ml)	13.0	13.1	12.4	12.1	10.5	11.0	10.5	10.7	14.2	9.6	10.4	10.2
RBC count (10^6)	10.9	11.35	9.25	8.8	7.3	7.9	7.6	6.9	8.15	8.35	7.0	9.65
M.C.H.C (%)	43.3	46.8	45.9	43.2	42.0	40.7	42.0	42.8	54.6	41.7	45.2	44.3
M.C.V (cu. microns)	27	25	29	32	34	34	33	36	32	26	33	24
WBC x 10^3	10.4	5.3	5.3	0.8	11.5	16.3	20.6	29.2	26.0	29.9	32.9	34.2
Neutrophils %	40	46	38	55	66	71	81	80	75	86	82	87
Lymphocytes %	57	41	59	43	30	29	19	20	25	14	18	13
Eosinophils %	3	13	3	1	0	0	0	0	0	0	0	0

Appendix 13: Scores obtained on evaluation of dermal irritation in sheep after 24 and 72 hours

Sheep No.	Intact skin				Abraded skin			
	Erythema		Edema formation		Erythema		Edema formation	
	24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs	24 Hrs	72 Hrs
03	0	0	2	0	1	1	1	1
04	0	0	0	0	1	0	1	1
06	0	0	0	1	0	0	0	0
07	0	0	0	0	2	1	1	0
11	1	0	1	1	1	1	1	1
13	1	0	1	1	2	2	1	1
14	0	0	1	0	1	1	2	1
15	0	0	1	1	0	0	2	1

0, no erythema; 1, very slight erythema; 2, well defined erythema

Appendix 14 Scores obtained in evaluation of ocular irritation in sheep

Sheep No.	Cornea										Iris					Conjunctivae														
	Opacity					Area invoved					Changes					Redness					Chemosis					Discharge				
	24 Hr	48 Hr	72 Hr	4 D	7 D	24 Hr	48 Hr	72 Hr	4 D	7 D	24 Hr	48 Hr	72 Hr	4 D	7 D	24 Hr	48 Hr	72 Hr	4 D	7 D	24 Hr	48 Hr	72 Hr	4 D	7 D	24 Hr	48 Hr	72 Hr	4 D	7 D
03	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	2	0	0	0	0	1	1	0	0	0	1	1	0	0
04	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	2	0	0	0	0	0	1	0	0	0
06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
07	1	1	0	0	0	2	2	0	0	0	0	1	0	0	0	2	1	1	1	0	1	1	0	0	0	0	1	0	1	0
11	0	1	0	0	0	0	2	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	0	0	0	0	0	1	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Hr, hours D, days

Appendix 15: Number of eggs per gram of faeces before and after administration of pyrethrins to sheep as a single oral dose of 168 mg/kg b.w.t

Sheep No.	Pretreatment period (Days)					Post treatment period (Days)												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	22	29	36
13	1700	2800	3000	300	800	8900	800	2000	2000	-	-	-	2600	700	1200	7800	800	400
14	3300	4200	1900	3400	7800	3500	1800	1600	1200	11400	3600	6300	3300	3000	7300	5400	1800	800
15	2100	900	4100	1500	3900	3200	6200	1800	100	3700	2500	4300	2000	1600	11500	5900	2600	2500
16	1700	5300	3200	2200	2900	6300	12700	4400	4000	3000	5900	10400	5100	5400	17100	11100	8000	2800

(-) Sample not taken

Appendix 16: Normal haematological and biochemical values in sheep

Parameter	Normal values
Haematology	
Microhaematocrit (PCV)	38 (25-50) %
Haemoglobin	12.0 (8.0-16.0) g/dl
Red blood cell counts (RBC)	12.0 (8.0-16.0) x 10^6/l
MCHC	33.5 (31.0-38.0) %
MCV	33.0 (23.0-48.0) fl
Total white blood cell counts (WBC)	8,000 (4,000-12,000) x 10^9/l
Neutrophils	30 (10-50) %
Lymphocytes	62 (50-75) %
Eosinophils	5 (0-10) %
Biochemistry	
Aspartate aminotransferase (ASAT)	100 (0-150) sigma-Frankel units
Creatinine	0.9-2.0 mg/dl
Sorbital dehydrogenase (SDH)	210-1,236 sigma units (3.5-20.6 U/l)
Total proteins	5.9-7.9 g/dl
Glutamate dehydrogenase (GLDH)	upto 12 U/l