EXPERIMENTAL *SOLANUM INCANUM* L POISONING IN SHEEP AND GOATS

BY

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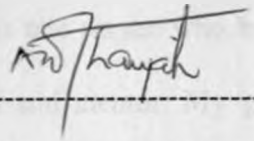
DECLARATION

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



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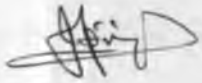

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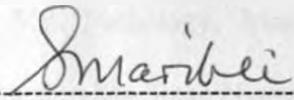

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May the blessings of the Lord be upon you

DEDICATION

This thesis is dedicated to my family and to the almighty God for His invaluable help and guidance throughout this work.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
b. wt	body weight
dl	decilitre
ED_{50}	median effective dose
g/dl	grams per decilitre
GGT	gamma glutamyltransferase
Hb	haemoglobin
H & E	haematoxylin and eosin
LD_{50}	Median lethal dose
MCV	mean corpuscular volume
mg/dl	milligrams per decilitre
mls	milliliters
PCV	packed cell volume
RBC	red blood cell count
rpm	revolutions per minute
TP	total protein
u/l	units per litre
µl	microlitre
WBC	white blood cell count

EDTA Ethylenediamine tetra-acetic acid

ABSTRACT

Solanum incanum L. is a shrub found in many areas of Kenya. The toxicity of ripe *Solanum incanum* L fruits has been carried out in sheep and goats with no effect. However, unripe fruits are more abundant and goats and sheep may be more easily attracted to eat unripe fruits. The toxicity of such unripe fruits of *Solanum incanum* L has not been tested in sheep and goats. This study was, thus, undertaken to find out the toxic effects of unripe fruits of *Solanum incanum* L in goats and sheep.

Powdered unripe fruits of *S. incanum* L were used to determine the LD_{50} and the toxic effects. Toxicity was determined by drenching the animals with the powder suspended in water in three doses; Group1 (0.25 LD_{50}), Group 2 (0.5 LD_{50}) and group 3 (0.75 LD_{50}) and observing for clinical signs and haematological, biochemical, necropsy and histopathological findings.

The LD_{50} was 4.8g and 3.0g/kg body weight in sheep and goats, respectively. In group 3: one goat had diarrhoea from day 3 until death at day 14. One other goat died on day 15 of the experiment after showing signs of coughing, anorexia, depression with the head held low, staggering gait and continuous bleating while one goat survived to the end of the experiment without signs of toxicity. One group 2 goat had bloat, shivering, progressive weakness, depression, staggering gait, lateral recumbency, leg paddling movements and continuous bleating before death on day 24 of the experiment while three others did not show clinical signs. All group 1 goats did not show clinical signs.

All the group 3 sheep (0.75 LD_{50}) showed signs of bloat, depression, coughing and anorexia. One group 3, three group 2 and one group 1 sheep, respectively, also showed colic, staggering gait, lateral recumbency, leg paddling movements, coma and death. The group 3 sheep died on days 2, 3, 4 and 13 of the experiment while group 2 died on days 4, 14, and 42, respectively. One group 1 sheep died on day 29 while the rest survived to the end. These clinical manifestations were significantly different between goats and sheep. Haematological and biochemical findings were also significantly different in terms of total protein, PCV and AP.

On gross pathology, group 3 goats (0.75 LD_{50}) showed hydroperitoneum and hydropericardium, which were absent in sheep. In group 2 (0.5 LD_{50}), goats had fibrinous pericarditis and hydroperitoneum while sheep in group 2 (0.5 LD_{50}) showed pneumonia, lung emphysema and haemorrhagic enteritis. In group 1 (0.25 LD_{50}), sheep showed emaciation, hydroperitoneum, lung emphysema and pneumonia, while the goats in group 1(0.25 LD_{50}) had no lesions.

On histopathology, the lungs had congestion and interstitial pneumonia in both species but sheep in addition showed emphysema, oedema and proliferation of alveolar epithelium. The brain in goats had microthrombi, marked wallerian degeneration of neurons and necrosis of Purkinje cells in the high and medium dose groups (group 2 and 3) while in group 1 there was congestion. The sheep brain showed widespread haemorrhage, slight necrosis of Purkinje cells and chromatolysis of neurons in groups 2 and 3 while the group 1 had marked haemorrhage and necrosis and loss of Purkinje cells,

which were absent in goats. The liver showed centrilobular necrosis but in addition sheep had proliferation of bile ducts.

In conclusion the study shows that the plant is more toxic to sheep than to goats and that there were marked differences in the clinicopathological manifestations of the toxic effects in the two animal species. Since there was less toxic effect in goats, it may seem that goats have a way of reducing the toxic effects of unripe fruits of *Solanum incanum* L.

Chapter 1

1.0 INTRODUCTION

There are about 1,500 species in the genus Solanum and among these several have been found to be toxic to animals. These include *S. aculeastrum, S. sodomoeum, S. nigrum, S. panduriforme, S. pseudocapsicum, S. kwabense, S. malacoxylon, S. fastigiatum var fastigiatum, S. bonariensis, S. dimidiatum, S. glaucophyllum, S. dulcamara,* and *Solanum tuberosum.*

Solanum incanum L. is one such Solanum species found abundantly in many areas of Kenya where goats are sheep are kept in large numbers. Limited studies in animals have been carried out with this plant. Steyn (1936) fed the ripe fruit to the goat, sheep and rabbit without effect but found that the unripe fruit was toxic to the rabbit. A sheep was fatally poisoned within three days of being dosed with c. 19 g/kg of *S. incanum* fruit (Shone *et. al.*, 1965). According to Steyn (1936), the main clinical signs of *S. incanum* poisoning in animals are salivation, diarrhoea, colic, bloat, stomatitis, tachycardia, polypnea, cramps, paralysis and occasionally, a vesicular exanthema. The principal necropsy features in animals are catarrhal enteritis (Steyn, 1936; Shone *et. al.*, 1965), hyperaemia and oedema of the lungs, ascites and hydrothorax (Shone *et. al*, 1965).

Unripe fruits and leaves of *S. incanum* L were shown to be toxic when fed to rats (Thaiyah, 1992). Clinically, affected rats showed diarrhoea and a starry coat and a generalized congestion of all the organs on post mortem examination. On histopathology, there was perivascular oedema and vacuolation of the white matter of the brain, linear

haemorrhages on glandular stomach and haemorrhages and necrosis of the kidney. The rats also showed lung pneumonia and degeneration of the germinal layer of the testis with aspermia. In the intestines, there was coagulative necrosis in the high dose groups and adenocarcinomas in the low dose groups. The liver showed hepatocyte hyperplasia in the low dose groups.

No studies have been done so far on the toxicity of unripe fruits of *Solanum incanum* L in goats while in sheep only one limited study has been done. Thus, this study seeks to determine the toxic effects of *S. incanum* L in goats and sheep.

1.1 HYPOTHESIS

This study was undertaken to evaluate the major hypothesis that "There is no difference in the toxic effects of unripe fruits of *Solanum incanum* L in sheep and goats".

The minor hypotheses were as follows:

1. That there is no difference in the clinical findings in sheep and goats fed *S. incanum* L
2. That there is no difference in the haematological and biochemical findings in sheep and goats fed *S. incanum* L
3. That there is no difference in the gross pathological lesions in sheep and goats fed *S. incanum* L
4. That there is no difference in the histological changes in sheep and goats fed *S. incanum* L.

In order to test the above hypotheses, the following experiments were performed:

a. Determination of the oral LD_{50} of *S. incanum* L in goats and sheep using dried fruit powder.

b. Determination of the effects of graded oral doses of *S. incanum* L in sheep and goats as assessed by the clinical, haematological, biochemical, pathologic and histologic observations.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 General remarks.

Plant poisoning is recognized as a major problem affecting livestock in all parts of the world. Any plant community includes poisonous plants. Overstocking, drought and other environmental variations in range areas where livestock is extensively managed may force animals to consume poisonous plants since they may be the only green plants present in abundance (James *et. al.*, 1992a). However, basing on the great diversity of plant communities including poisonous plants, it is possible that a proportionate loss in livestock production occurs in pastoral areas from poisonous plants especially during harsh weather conditions. In a single estimate in 1992, deaths and reproductive losses from plant poisoning cost 17 western USA states 340,000 US dollars (James *et. al.*, 1992a). The losses are either direct or indirect. Direct losses are manifested as deaths, weight loss and loss of production and reproduction, abortions, lengthened calving intervals and birth of weak or malformed foetuses. Indirect losses include cost of change in management, fencing, supplementary feeding and medical costs to treat poisoned animals.

Naturally animals and poisonous plants must coexist in range areas. In this regard, livestock have an inherent capacity to avoid poisonous plants such that when pastures are abundant, animals ingest non- toxic plants selectively, discriminating against the poisonous ones. Livestock newly introduced to an area may succumb to poisoning due to indiscriminate ingestion of herbage in the area. It has been shown that conditioned

aversion to poisonous plants can be induced by feeding the animals with the plant and a known toxin such as lithium chloride to induce nausea and emesis. The animal will then associate the illness to the poisonous plant and instinctively avoid it (Ralphs, 1992; Toit *et. al.*, 1992).

2.2 Measures of toxicity in animals

Each cell of an organ has a specific function and contains enzymes unique to that function. When the integrity of a cell is disrupted, enzymes escape into the surrounding fluid compartment and into serum or cerebrospinal fluid, where their activity can be measured as a useful index of that cells integrity (Kaneko, 1989). These enzymes include alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), sorbitol dehydrogenase (SDH) and gamma glutamyl transferase (GGT).

AP is found in bone and the liver and is increased in serum when there is disease affecting these organs. In normal liver, bile duct epithelium has the most AP activity and when obstruction occurs, there is an increase in hepatic AP in serum (Hoffmann *et. al.*, 1977a). The transamination of L-aspartate and α-oxaloglutarate to oxaloacetate and glutamate is catalysed by AST. This enzyme is useful when used with other enzymes as an index of hepatic or muscular cell damage (Kaneko, 1989). GGT cleaves terminal glutamyl groups and transfers them to peptides and other suitable acceptors. Most cells have GGT activity but serum GGT is mostly derived from the Liver (Braun et. al., 1978). Creatine kinase phospholirates creatinine to form creatine phosphate, which is the storage form of energy required by muscle for contraction. Only heart and skeletal muscles

contain sufficient amounts of creatine to alter serum activity in organ specific disorders (Kaneko, 1989). These enzymes will be used to measure damage to specific organs by the unripe fruits.

2.3 Toxicants in plants

There is a great diversity of components in plants that cause toxicity in animals. These vary in their biochemical, physiological and pharmacological functions. These toxic components have been comprehensively reviewed (Cheek, 1989) and the human health implications from these toxicants examined (Cheek, 1990). These components include alkaloids, cyanogenetic glycosides, terpenes, coumarins, flavonoids, estrogens, tannins and sterols among others.

2.4 Toxicological work on some Kenyan plants

Toxicological work on poisonous and medicinal plants in Kenya is little probably because of the long-term nature of such studies. Some research in this area has been undertaken in the Departments of Veterinary Pathology, Microbiology and Parasitology, Clinical Studies and Public Health, Pharmacology and Toxicology, University of Nairobi.

Both sheep and calves fed the leaves and seeds of *Burttia prunoides* developed similar lesions which included: froth in the mouth and trachea; Lung congestion and oedema; ecchymotic haemorrhages on epicardium and endocardium; hydroperitoneum and congestion in the brain and gastrointestinal mucosa (Mugera, 1970). Histologically, there was congestion, haemorrhages, necrosis and oedema of perivascular spaces in the brain; hyaline degeneration, haemorrhages and fatty infiltration on heart muscle; oedema and haemorrhages on lung tissue and haemorrhages and fatty infiltration in the liver and

kidneys. Mugera (1970) also fed *Elaeodendron buchananii* to calves, sheep and rats. All calves and sheep developed diarrhoea and pathologically, there was generalized heamorrhage in various organs in acute cases and emphysema and oedema in the lungs. The rats were resistant and only had fatty infiltration in hepatic cells after one year. The clinical signs of *Acokanthera longiflora* and *Acokanthera schimperi* poisononig in sheep and calves were muscular spasms, rapid breathing, salivation and diarrhoea (Mugera, 1970). On pathology, there was hydrothorax and hydropericardium, lung oedema, froth in the trachea and ecchymotic haemorrhages on epicardium and endocardium. *Dialotropis Africana* induced diarrhoea in sheep and calves with haemorrhagic gastroenteritis (Mugera, 1970).

Stephanorossia palustris induced a haemorrhagic diarrhoea and rapid breathing in sheep and calves and on pathology, the animals had a haemorrhagic gastroenteritis and ecchymotic haemorrhages in the lungs, epicardium and endocardium (Mugera, 1970). In bovine, *Ornithogalum longibracteatum* induces diarrhoea and tympany and on pathology, hydropericardium, hydrothorax, haemorrhagic gastroenteritis and abomasal ulceration (Mugera, 1970). *Haemanthus multiflorus* leaves and bulbs induced profuse salivation and bloat in calves with no visible post mortem lesions. However, in sheep it induced salivation, bloat and diarrhoea and on post mortem there was haemorrhagic enteritis, haemorrhages on epicardium and endorcardium and degenerative changes in liver and kidney, varying from fatty infiltration to necrosis (Mugera, 1970). *Bersamia abyssinica Fres.* induces bloody diarrhoea in bulls with a haemorrhagic, ulcerative gastroenteritis and haemorrhages and necrosis of cardiac muscle (Mugera, 1970). Leaves of *Maesa*

lanceolate induced hydrothorax, hydroperitonium and hydropericardium, diffuse fatty infiltration in hepatic lobules and coagulative necrosis of proximal convoluted tubules with hyaline cast formation in calves (Mugera, 1970). The leaves of *Crotalaria mauensis Bak.* produced extensive petechiae to ecchymotic haemorrhages on serous and mucous surfaces and parenchymatous organs and ulcerative abomasitis and duodenitis in calves (Mugera, 1970). With leaves of *Cassia floribunda Cav.* in sheep, there was constipation which progressed to diarrhoea and on post mortem, there was a serous exudates in the thoracic, pleural, pericardial and abdominal cavities. In addition, there was fatty infiltration in the liver, coagulative necrosis and interstitial haemorrhages in the kidney and necrotic gastroenteritis (Mugera, 1970).

Cassia didymobotrya Fres. induced signs of diarrhoea in sheep and on post mortem, generalized congestion, petechial haemorrhages on epicardium and endocardium, lung oedema and haemorrhagic gastroenteritis (Mugera, 1970). In sheep and bulls, *Diplocyclos palmatus (L) Jeffrey* induced diarrhoea clinically and on post mortem, hydrothorax and ascites, lung oedema, ulcerative gastroenteritis and degenerative changes in liver and kidney varying from fatty infiltration to necrosis (Mugera, 1970). *Dichlocephala chrysanthemifolia* induces bloody diarrhea in sheep and on post mortem, haemorrhagic gastroenteritis and congestion of the kidneys. Microscopically, there was fatty infitration in the liver and kidneys and a necrotizing haemorrhagic gastroenteritis (Mugera, 1970). The leaves and young shoots of *Senecio moorei* induced bloody diarrhoea in bulls and on post mortem there were ecchymotic haemorrhages in the epicardium, endocardium, pleura and diaphragm. The hepatic changes varied from a

haemorrhagic hepatitis to necrosis and hyperplasia while the kidney showed fatty infiltration (Mugera, 1970). No toxicity was demonstrated with *Ehretia cymosa* Thonn, *Lippia unkambensis* Valke, *Leonotis nepetifolia* L and *Cassia singueana* (Mugera, 1970). In cattle, *Senecio moorei* produced liver fibrosis and bile duct proliferation; in swine it produced epithelialization of lung alveoli and enlargement of nuclei in hepatocytes and proximal convoluted tubules of the kidney while in rats there were hepatocellular carcinomas, bile duct adenomas and fibroma (Kamau, 1973). *Adenia volkensii* fed to sheep, rabbits and rats produced congestion to haemorrhage in various internal organs (Kamau, 1973).

Gnidia latifolia fed to calves for 2-3 months produced hydrothorax, hydropericardium, hydropleura, follicular hepatic fibrosis and necrosis, haemorrhage, congestion and fibrosis in the lymphopoietic cells in the spleen and lymph nodes. Chronically, the animals had a slight anaemia and lymphopaenia and degenerative fibrosis and glomerulonephritis with hyaline casts in kidneys. In rats, the kidney, liver and adrenals showed marked cellular degeneration with hyaline casts while other organs had generalized petechiae haemorrhages (Kiptoon, 1981).

The methanolic extract of *Spirostachys venenifera* pax produced signs of immonosuppression in rats while tumor cells implanted into similarly treated rats produced malignancy attributed to the immunosuppressive status (Njiro, 1984).
Peddie volkensii Gilg and *Scutia myrtina* produced pulmonary haemorrhage and alveolar thickening in rats (Muchiri, 1987). In addition, *P. volkensi* Gilg fed rats developed

hydropericardium, hydrothorax, froth in the trachea, degeneration of proximal tubules of the kidney, hepatic and adrenal cortical haemorrhages, splenic haemosiderosis and increased red blood cell destruction. Badru (1989) studied the toxic effects of *Ajuga remota* Benth in rats and goats.

Unripe fruits and leaves of *Solanum incanum* L fed to rats produced diarrhoea and a starry coat clinically and generalized congestion of all the organs on post mortem examination (Thaiya, 1992). Histologically, there was perivascular oedema and vacuolation of the white matter of the brain; linear haemorrhages on glandular stomach; haemorrhages and necrosis of the kidney; lung pneumonia; degeneration of the germinal layer of the testis with aspermia; coagulative necrosis in the intestines in the high dose groups and adenocarcinomas in the low dose groups; and hepatocyte hyperplasia in the low dose groups (Thaiyah, 1992).

Prunus africanus in rats produced lymphocytosis in all lymphoid organs, myocardial necrosis, mild vacuolation of hepatocytes and gross reduction in the size of the prostrate gland (Gathumbi, 1995).

2.5 EFFECTS OF POISONOUS PLANTS ON ANIMALS

2.5.1 Hepatotoxic plants

Many plant poisonings in animals cause lesions in the liver. These include plants in the genera *Senecio, Heliotropium, Amsinca, Echium, Crotalaria, Symhytum* and *Trichodesma* spp containing pyrrolizidine alkaloids (Adams, 1974; Hooper, 1978), *Cestrum* (Mugera and Nderitu, 1968b), *Lantana* (Gopinath and Ford, 1969), *Tribulus terrestris* L (Van

Tonder *et. al.*, 1972), *Brachiaria decubens* (Graydon *et. al.*, 1991), *Asaemia axillaris, Pteronia pallens, Lasiospermun bipinnatum* and *Galenia africana* in South Africa (Kellerman *et. al.*, 1988).

2.5.2 Cardio toxic plants

Direct cardio toxins include cardiac glycosides (Schulz *et. al.*, 1975). A comprehensive review of plants that contain cardiac glycosides and other toxic effects in animals has been presented (Kellerman *et. al.*, 1988).

2.5.3 Nephrotoxic plants

Plants that result in nephrotoxicity include *Quercus robur* L (Neser *et. al.*, 1982), *Anagallis arvensis* L (Schneider, 1978) and Oxalate containing plants (*Spinacia olearacea L, Beta vulgaris L, Rumex acetosa L, Opuntia* spp and *Halogeton glomeratus*) (James, 1978). Others include *Tribulus terrestris* (Coetzer, *et. al.*, 1963) and *Vicia* spp (Anderson and Divers, 1983).

2.5.4 Plant poisoning that causes disturbance in reproduction

Some plant toxins cause anomalies in reproduction manifested by infertility, reproductive disturbances and fetotoxicity. A review of specific plant poisoning that affects the various stages of the reproductive cycle has been presented (James *et. al.*, 1992b). A review of plants that affect the embryonic, fetal and neonatal development, the animals affected and the most susceptible stage of gestation are also available (Panter *et. al.*, 1992).

2.5.5 Plant induced pulmonary disturbances

The most commonly reported plant induced respiratory distress in animals is acute bovine respiratory emphysema (fog fever) associated with the ingestion of lush pastures

containing high amounts of amino acid L-tryptophan (Carlson and Dickinson, 1978; Carlson and Breeze, 1984). A similar condition has been reported in cattle ingesting *Perilla frutescens*, mouldy sweet potatoes and *Ipomoea batatas* (Wilson *et. al.*, 1978). Other plants inducing pulmonary problems include *Crotalaria dura* (Kellerman *et. al.*, 1988), *Lasiospermum bipinnatum* (Williams, 1990) and *Hertia pallens* (DC) (Prozesky *et. al.*, 1986).

2.5.6 Plants affecting the haematopoietic system

Some plant poisonings that affect the haematopoietic system are known. These include *Allium cepa* (Hutchinson, 1977; Kirk and Bulgin, 1979; Verhoeff *et. al.*, 1985; Lincoln *et. al.*, 1992), *Brassica* species (Smith, 1980), *Acacia nilotica* and cereal grasses (Carrigan and Gardiner, 1982), *Pterdium aquilinum* (Evans, 1964) and *Ricinus communis* (Geary, 1950).

2.5.7 Plants that cause sudden death

Plants that contain cyanogenetic glycosides are a common cause of sudden death in livestock (Burrow and Tyrl, 1989). As reported by Conn (1978), 1000 species of plants worldwide, representing 250 genera and 80 families contain cyanogenetic glycosides. The various plants and the cyanogenetic glycosides they contain are presented by Conn (1978).

2.5.8 Plants that affect the gastrointestinal tract

Plants affecting the gastrointestinal tract include *Solanum* species (Morris and Lee, 1984), *Gnidia* species (Terblanche *et. al.*, 1966; Kiptoon *et. al.*, 1982), *Spirostachys venenifera* (Njiro, 1984), *Geigeria* species (Kellerman *et. al.*, 1988) and *Pennisetum cladestinum* (Bryson, 1982; Newsholme *et. al.*, 1983).

2.5.9 Neurotoxic plants

A wide variety of plants cause central nervous system disturbances and these include: - *Datura stramonium* L (Agnew, 1974), *Dipcadi glaucum* Bak (Kellerman *et. al.*, 1988), *Matricaria nellifolia* DC (Newsholme *et. al.*, 1984), *Cynanchum africana* (Kellerman *et. al.*, 1988), *Euphorbia mauritanica* L (Terblanche *et. al.*, 1966), *Oxytropis* and *astragalus* species (Van Kampen and James, 1969), *Phalaris* species (Hartley, 1978a), *Karwinski humboldtiana* (Chalton *et. al.*, 1971), *Cycad* palm (Hooper *et. al.*, 1974) and annual ryegrass (Lorium species) (Schneider, 1981; Lanigan *et. al.*, 1976; McIntosh and Thomas, 1967). Other sources of intoxication include the ingestion of maize cobs infected with the fungus *Diplodia maydis* (Kellerman *et. al.*, 1991) and maize infected with the fungus *Fusarium moniliforme* Sheldon (Marasas *et. al.*, 1976; Kellerman *et. al.*, 1972).

2.5.10 Plants causing lesions in the musculoskeletal system

Plant poisonings that cause damage to the musculoskeletal system are few and include *Thermopsis montana* (Baker and Keeler, 1992), *Geigeria ornativa* (Van der Luht and Van Heerdan, 1993), *Crotalaria burkeana* (Kellerman *et. al.*, 1988) and chronic selenium poisoning (Olson, 1978).

2.5.11 Plants causing lesions in the cutaneous system

Plant poisons affecting the cutaneous system are many and the lesions are varied. *Chrysocoma tenuifolia* (Kellerman, *et. al.*, 1988) causes alopecia while *Vicia villosa* causes granulomatous nodular to ulcerative dermatitis (Kellerman *et. al.*, 1988). *Euphorbia* species contain highly irritating latex that causes severe burning irritation to mucous membranes and the skin (Watt and Breyer-brandwijk, 1962). Plants in the genera

Apiaceae, Ruraceae, Fabaceae, Moraceae and *Archidaceae* contain furocoumarins and when ingested cause primary photosensitization (Kellerman *et. al.*, 1988). Gangrene formation occurs in animals ingesting cereal grains infected with the fungus *Claviceps purpurea* (Yager and Scott, 1985) while fescue foot is caused by ingestion of *Festuca arundinaceae* (tall fescue) that is infected with the fungus *Acremonium coenophialum* (Hemkem *et. al.*, 1984). Fungi in the genus *Fusarium, Myrothecium* and *Stachybotrys* when ingested with contaminated hay or feed elicit necrotic dermal lesions in the skin and mouth (Hintikka, 1977).

Solanum malacoxylon (Done *et. al.*, 1976), *Cestrum diurnum* (Krook *et. al.*, 1975) and *Trisetum flavescens* (Dirksen *et. al.*, 1974) cause calcification in various tissues including the skin while *Leucaena leucocephala* causes cataract, goitre, epithelial ulceration, atrophy of gingiva, depilation, infertility and foetal death and resorption in ruminants (Holmes *et. al.*, 1981; Hegarty *et. al.*, 1976; Falvey, 1976).

2.5.12 Plant toxicants shed in milk

Many of the toxicants in plants can be shed in milk of lactating animals and by implication be passed to the newborn or to humans thereby producing toxic effect. Toxicants shed in milk include tremetol, a compound from *Eupatorium rugostum* (Couch, 1929), pyrrolizidine alkaloids (Johnson, 1976; Dickinson and King, 1978), piperidine alkaloids (Kubik *et. al.*, 1980), quinolizidine alkaloids (Cheek and Shull, 1985), indolizidine alkaloids (James *et. al.*, 1977) and Aflatoxins M_1 and M_2 (Allcroft and Carnaghan, 1963; Stolaff, 1980).

2.6 SOLANUM SPECIES

2.6.1 Solanum species other than *S. incanum* L

There are about 1,500 species in the genus Solanum and over 30 species of alkaloid containing Solanum are used as human foodstuffs. Major edible Solanum species include *S. tuberosum* (potato), *S. melongena* (eggplant), *S. aviculare* (kangaroo apple), *S. centrale* (desert raisin) and *S. aethiopicum* (African scarlet eggplant or garden egg) (Facciola, 1990). Other Solanum species have, however, been shown to be toxic to animals in South Africa and these include *S. aculeastrum, S. sodomoeum, S. nigrum, S. panduriforme* and *S. pseudocapsicum* (Steyn, 1932; Steyn, 1949).

Solanum kwabense (bitterappel, Rooibessie) is the cause of maldronsiekte (mad-drunk-disease) in cattle, characterized by epileptiform seizures and signs of cerebellar dysfunction (Menzies *et. al.*, 1979; Pienaar *et. al.*, 1976; Riet-Correa *et. al.*, 1983). This disease is characterized by atrophy of the cerebellum, which is manifested microscopically by depletion of the Purkinje cell layer. *S. dimidiatum* in West Texas (Menzies *et. al.*, 1979), *S. fastigiatum var. fastigiatum* in Brazil and *S. bonariensis* in Uruguay have been associated with clinical signs of seizures and pathology similar to that of *S. kwabense* (Riet-Correa *et. al.*, 1983).

In South America, *S. malacoxylon* is responsible for arteriosclerosis of the aorta and major arteries, and metastatic calcification in various organs, such as the heart, lungs, kidneys and tendons (Dobereiner *et. al.*, 1971). It has also been shown that the young born of does fed *S. malacoxylon* on days 6 to 30 of gestation also develop mineralization

of soft tissues and an increase in the calcium and phosphorous levels (Gorniak *et. al.*, 1999). When the leaves of *S. glaucophyllum* are fed to rabbits on days 5, 7 and 9, the rabbits develop loss in weight, elevation of tissue calcium levels and calcinotic lesions (Dallorso *et. al.*, 2001).

Studies on *S. dulcamara* were done in mice using ripe and unripe berries collected at various times in the year. The mice receiving unripened bellies from early in the year had gastrointestinal tissue changes consistent with solanine toxicity. Unripened fruit in the latter part of the year caused behavioral changes suggestive of solanine toxicity but no gastrointestinal lesions. The ripe fruit was completely non-toxic (Hornfeldt and Collins, 1990).

Studies using Solanine, from the potato (*Solanum tuberosum*) have been done in animals (Chaube and Swinyard, 1976). The LD_{50} of solanine in rats has been found to be 67mg/kg b.wt. (Chaube and Swinyard, 1976, Nishie *et. al.*, 1975), and 27 mg/kg b.wt. and 50 mg/kg b.wt in mice and rabbits, respectively. Solanine injected into rats at the rate of 10-85 mg/kg b.wt. resulted in acute death, characterized by periorbital, nasal and oral haemorrhage and bloody ascitic and pleuritic fluid (Chaube and Swinyard, 1976). Injection of the same compound to pregnant rats in eight daily injections on days 5-12 of gestation at 5-20 mg/kg resulted in foetal death and no abnormalities (Chaube and Swinyard, 1976). Beating heart cell cultures of 1-2 day old rats when treated with alpha-solanine (80ugm/ml) and tomatine (40ugm/ml) ceased beating within a few minutes. The

addition of alpha-solanine (40ugm/ml) and tomatine (20ugm/ml), however, resulted in an increased contraction frequency lasting for at least 2 hours (Bergers and Alink, 1980).

2.6.2 *Solanum incanum* L

Solanum incanum L belongs to the family Solanaceae. It is a shrubby, prickly herb 0.6 - 1.8 meters tall. The leaves are ovate to lanceolate, with entire to lobed margins and are covered with yellow or brown star shaped hairs, usually very velvety and 5.0 - 18.0 centimeters long. The corolla is purple or violet and the anthers are yellow. The unripe fruits are round, green with pale blotching, but later are bright yellow in colour on ripening and are 1.9 - 3.8 centimeters in diameter. The prickles are present or absent from stem and petioles.

2.6.2.1 Distribution

Solanum incanum L, commonly known as "sodom apple", is fairly abundant in several ecological zones. In Kenya it is found around Mt. Elgon, Cherangani, Loita hills, along the Aberdare range, Kitale, Mumias, Kisii, Narok, Baringo, Rift Valley, Magadi, Embu, Machakos, Kajiado and Nairobi including surrounding areas of Kikuyu, Kabete and Athi River (Agnew, 1974).

This shrub is referred to by various local names. The Abaluhya refer to the plant as *indalandalwa* or *maduranzura* and by the Marakwet as *lobotwa*. The Luo name for the plant is *ochok* or *machoge* while the Taita refer to the plant as *mrong*. The Digo name for the plant is *mtunguzo* while that for the Kamba is *mukondu* or *mutongu*. The Kikuyu and

Meru call the plant *mutongu* while the Masai name for the plant is *endallelei* (Kokwaro, 1976)

2.6.2.2 Use of *Solanum incanum* L in traditional medicine

This shrub is used widely in traditional medicine both in Eastern and Southern Africa. In Tanzania, the Pedi take a decoction of the plant, made with *Fagara capensis* for chest troubles; and some parts of the plant, roasted, for pleurisy and pneumonia (Watt *et. al.*, 1962), while the Sotho use the plant as a remedy for toothache and sore throat (Phillips, 1917). The root is a remedy for abdominal pains, liver troubles and carbuncle while the fruit is used as a snakebite remedy Watt *et. al.* (1962).

In West Indies, the root and leaf are used as a snakebite remedy. In Tropical and Southern Africa, the root and leaf are used as a remedy for cough, colic, sore throat, gonorrhoea and syphilis (Watt *et. al.*, 1962). The Zulu use the juice as a ringworm remedy. The roots are also used for the treatment of sexually transmitted diseases in the Guruve District, Zimbabwe (Kambizi and Afolayan, 2001).

According to Kokwaro (1976), a decoction of the roots is used for abdominal pains, dyspepsia, fever, stomachache and indigestion. Roots can also be used for toothache by scrubbing the affected tooth with pieces of the root. Young leaves are chewed and rubbed hard onto a recent snakebite, while an infusion of leaves is applied to the ear as a remedy for earache. For fresh cuts and wounds, the fruit is broken and the contents applied. The fruits although known to be poisonous are given to children as an emetic (Kokwaro, 1976).

The fruit juice is applied into the sheep's nostrils as a cure for sheep cough (Kokwaro, 1976). An infusion of the fruit is said to be effective in removing external benign tumours including an epithelioma on the back of a racehorse by local application; carcinoma on the hock of a mule by injection of an aqueous solution and half a dozen melanomata in a horse treated the same way as the mule (Watt *et. al.*, 1962).

2.6.2.3 Solanum incanum L toxicity in man

After oral ingestion, *Solanum incanum* L causes toxicity in man that is characterized by headache, severe colic, vomiting, diarrhoea, apathy, cyanosis, accelerated weak pulse, fever, profuse perspiration, dizziness, mydriasis, lassitude, disturbances in speech and sight, hallucinations and unconsciousness. Death is due to heart failure or respiratory paralysis (Verdcourt and Trump, 1969). The part of the plant causing these signs is not described in the literature.

2.6.2.4 Active principle

The active principle in Solanum species is a glycoalkaloid. Solanine is the most common glycoalkaloid in Solanum species and is several times more toxic than its corresponding aglycone solanidine (Cheek and Shull, 1985; Morris and Lee, 1984). Glycoalkaloids affect mainly the nervous system and the gastrointestinal tract (GIT). The nervous signs are attributed to inhibition of cholinesterase, and the GIT effects to the saponin-like properties of Glycoalkaloids (Cheek and Shull, 1985; Morris and Lee, 1984). Other steroidal alkaloids include incanumine, Solamargine, solasodine, ursolic acid, and ursolic acid derivatives (Chun-Nan *et. al.*, 1990, Fukuhara and Kubo, 1991))

2.6.2.5 Toxicity studies in animals

Studies on the toxicity of *Solanum incanum* L in animals were carried out by Steyn (1936) who fed the ripe fruit to the goat, sheep and rabbit without effect but found that the unripe fruit was toxic to the rabbit. A sheep was fatally poisoned within three days of being dosed with 19 g/kg of *S. incanum* fruit (Shone *et. al.*, 1965). Steyn (1936) lists the main clinical signs of *S. incanum* poisoning in animals as salivation, diarrhoea, colic, bloat, stomatitis, tachycardia, polypnea, cramps and paralysis. Occasionally, a vesicular exanthema may be present (Steyn, 1936). The principal necropsy features are catarrhal enteritis (Steyn, 1936; Shone *et. al.*, 1965), hyperaemia and oedema of the lungs, ascites and hydrothorax (Shone *et. al*, 1965).

Thaiyah, (1992) fed the unripe fruits and leaves to rats and found these materials to be toxic. Clinically, the rats showed diarrhoea and a starry coat and had a generalized congestion of all the organs on post mortem examination. On histopathology, there was perivascular oedema and vacuolation of the white matter of the brain, linear haemorrhages on glandular stomach and haemorrhages and necrosis of the kidney. The rats also showed lung pneumonia and degeneration of the germinal layer of the testis with aspermia. Other lesions include coagulative necrosis in the intestines in the high dose groups, adenocarcinomas in the low dose groups and hepatocyte hyperplasia in the low dose groups.

From this review, no studies have been undertaken with unripe fruits of *Solanum incanum* L in goats while in sheep only limited studies have been done. Thus, this study seeks to determine the toxic effects of unripe fruits of *S. incanum* L in goats and sheep.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Plant material

Unripe fruits of *S. incanum* L were collected from around Kabete and chopped into pieces. The material was then dried in an oven at 50°C for three days. Thereafter, the dried material was ground into a fine powder in an electric mill and the powder stored at room temperature in appropriately labeled sealed plastic bags until use.

3.2 Experimental animals

All experimental animals (sheep and goats) were obtained from the semi-arid area of Machakos District in Eastern Province and were housed in stalls in the large animal compound, Department of Clinical Studies, Kabete. On arrival, a faecal sample was taken from each animal for determination of the worm loads and the animals were then drenched with Albendazole (Valbazen^{R}) at 3mg/kg body weight.

All animals were allowed 28 days to acclimatize to the environment and were fed on a ration of good quality hay, water and a commercial salt lick, (Maclick super®, Cooper (K) Ltd) *ad. libitum*. A high concentrate diet [Dairy meal, Unga (K) Ltd] was also fed twice a day.

All experimental animals were weighed on arrival and weekly thereafter. The clinical picture was observed before and during the experiment.

3.3 Experiment 1: Determination of acute LD_{50} of *Solanum incanum* L unripe fruits in sheep and goats after oral administration

3.3.1 Description of method used in LD_{50} determination

The moving average method of determination of median effective dose (ED_{50}) described by Weil (1952) was applied. The LD_{50} was the ED_{50} in these studies. This method reduces the cost and time of calculation of ED_{50} and its confidence interval to a minimum without sacrificing accuracy. A group of tables had been calculated according to the formulae presented in the original article. These published tables allow for the use of 2,3,4,5,6, or 10 animals per dose level, with 4 or more dosage levels tested (using K=3), provided the logarithms of successive dosage levels differ by a constant factor denoted by R and d = logarithm of R. The LD_{50} refers to the least dosage expected to kill 50 percent of animals that received it. The moving average method of determination of median effective dose (ED_{50}) requires simpler calculations than other methods of determination of LD_{50} like probit analysis and other methods of curve fitting such as logistic functions using maximum likelihood and weighted least squares.

Moving averages are widely used in mathematics and statistics in time series analysis and its advantages are: (i) its free from assumption as to the precise type of fundamental curve involved but it is capable of taking into account more of the data than other methods that use only data from both sides of the 50% level of effectiveness, (ii) only simple computations are involved and (iii) it replaces the fitting of complex mathematical curves.

For one to use this method effectively, several criteria must be met which include:

(a) Dosing a constant number of animals at each level (n= number dosed per level)

(b) Spacing the dosage levels so that they are in geometric progression

(c) Dose animals on at least K+1 level of dosage.

When these requirements are met, a set of mortality data (r-values in the tables) is obtained from the animals that match one of those in the published tables for the given value of n and K (appendix 2). The general formula for the calculation of m, the estimated LD_{50} is:

$$\text{Log } m \equiv \log D_a + D.\ (f+1) \text{ for } K=3$$

With Log D_a = log of the lowest of the four dosages used.

In estimating the 95% confidence interval, we take that bounded by antilog [Log m $\pm$ 2.σ log m].

3.3.2 Determination of acute oral LD_{50} of *Solanum incanum* L in goats and sheep

3.3.2.1 Dose determination experiments

Dose determination experiments were first done using two goats per dosage to determine the dosage range and observing for mortalities within 24hours. First a high dose of 15g/kg was tested resulting in both goats dying within 24 hours. Other dosages that were tried in a descending order were 10, 8, 5, 3, and 1.5g/kg. The 1.5g/kg dosage did not result in mortality.

Dose determination experiments were similarly done in sheep to establish the dosage range using dosages of 15, 10, 5 and 3g/kg. The 3g/kg dosage did not cause mortality.

3.3.2.2 Determination of the LD_{50} of *S. incanum* L in goats

Fifteen adult East African goats of both sexes were randomly divided into five groups of three goats each. Each of the five treatment groups was randomly assigned to a dosage level. The dosage levels were in geometric progression; the geometric factor (R) was 1.5, with dosage levels of 0, 1.5, 2.25, 3.38 and 5.06g/kg unripe fruit powder. The fruit powder was administered as a drench by stomach tube and a syringe and the goats were then observed for 24hrs and a set of mortality data (r-value) recorded for calculation of oral median lethal dose.

3.3.2.3 Determination of the LD_{50} of *S. incanum* L in sheep

Fifteen adult local sheep of both sexes were then similarly divided and received dosages of 0, 3.0, 4.5, 6.8 and 10.1g/kg unripe fruit powder as a drench by stomach tube and were observed for mortalities within 24 hours.

3.3.2.4 Clinical examination of animals in the LD_{50} experiment

Immediately after oral administration of the fruit powder, the sheep and goats were critically examined for behavioral changes, survival times and clinical signs of toxicity.

3.3.2.5 Post mortem examination of animals in the LD_{50} experiment

Post mortem examination was performed on all the sheep and goats that died within 24 hours after dosing with the unripe fruit powder. All animals with prolonged signs of toxicity and showing no signs of recovery at 48 hours were also sacrificed and post mortem examination performed. Gross pathological changes were recorded in all the cases. The LD_{50} was then computed according to the method of Weil (1952).

3.4 Experiment 2: Determination of the prolonged toxic effects of unripe fruits of *S. incanum* L. in goats

Sixteen adult East African goats were obtained and housed in stalls in the large animal compound, Department of Clinical Studies. They were housed and fed as described above. Body weights and blood with and without anticoagulant was taken on arrival and weekly thereafter during the acclimatization period to obtain baseline data on packed cell volume, haemoglobin concentration, total white cell count, total protein, bilirubin content, creatinine and the enzymes gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), and alkaline phosphatase (AP).

The goats were randomly divided into four groups of four animals each. Groups 1, 2 and 3 received oral doses of 0.25 LD_{50}, 0.5 LD_{50} and 0.75 LD_{50} Solanum powder suspended in water, respectively, each day for three months. Group 4 was set aside as the control.

3.4.1 Clinical examination of animals

The animals were observed individually every day for signs of poisoning by a complete examination of all systems and any clinical signs were noted. Body weights of all the animals were taken weekly.

3.4.2 Blood sampling

All animals were bled at the beginning of each week by venupunture from the jugular vein after disinfecting the injection sites with surgical spirit. About 10mls of blood was withdrawn from each animal using a sterile disposable syringe and 18G disposable needle. 2mls of blood was put in glass bijou bottles containing EDTA anticoagulant while

8mls was put in a glass universal bottle without anticoagulant. This procedure was repeated weekly until the end of the experiment.

3.4.3 Handling of blood samples

Blood in EDTA was immediately placed on an electric rotator to allow for thorough mixing with anticoagulant and was analyzed in a coulter counter within 3 hours of collection. The blood without anticoagulant was placed on the bench in the lab for 20-30 minutes to allow for clotting to occur. It was then centrifuged at 1,000rpm for 20minutes and the serum drawn out with a pipette into plastic containers for serum enzyme analysis. The serum was stored at ¯20°C and was analyzed within 48hours.

3.4.4 Post mortem examination of dead animals

All dying animals together with those sacrificed at the end of the experiment were subjected to a thorough necropsy and all gross lesions noted. Samples for histopathology were taken from the gross lesions and also from the brain, liver, kidney, heart, skeletal muscle, lungs, testicles, ovaries, rumen, abomasum, duodenum and large and small intestines. The tissues were fixed in neutral buffered Formalin, cut at 5µm, stained with Haematoxylin and Eosin (H&E) and observed under the light microscope.

3.5 Experiment 3: Determination of the prolonged toxic effects of unripe fruits of *S. incanum* L. in sheep

The experimental procedure as described above in goats was repeated using sixteen adult local sheep. Clinical examination, blood sampling and post mortem of dead animals was carried out as in goats

3.6 Procedures for the tests

3.6.1 Haematological examination

Blood for haematology was examined using standard protocols (Jain, 1986). Red blood cell count (RBC), haemoglobin concentration (HB), mean corpuscular volume (MCV) and white blood cell count (WBC) were determined electronically in a coulter counter (coulter electronics). Differential leucocyte counts for neutrophils (N), Lymphocytes (L), eosinophils (E) and macrophages (M) were obtained from Giemsa stained blood films. Packed cell volume (PCV) was measured using the microhaematocrit method while total proteins were estimated using a refractometer.

3.6.2 Serum enzyme and protein examination

The plasma parameters were measured using Boehringer Mannheim Gmbh kits (Germany). The activity of serum aspartate aminotransferase (AST) was determined calorimetrically at 546nm using the principle of Reitman and Frankel (1957). The activity of alkaline phosphatase (AP) in serum was determined calorimetrically at 405nm, according to the principle described by Kaneko (1989). Creatinine was assayed using the Jaffe method with deproteinization (Seelig and Wust, 1969). Gamma glutamyl transferase (GGT) was assayed using the optimized kinetic method recommended by the German society for clinical chemistry (Persijn and Van der Slik, 1976). Bilirubin was assayed using the method of Jendrassik (1938) at 560-600nm. Total protein was assayed calorimetrically at 546nm using the Biuret method (Weichselbaum, 1946).

3.7 Statistical analysis

Analysis of variance (ANOVA) was done using Genstat® statistical program to determine statistical significance for differences in clinical presentation and haematology, biochemistry and pathological observations. LD_{50} was computed by the method of Weil (1952).

CHAPTER 4

4.0 RESULTS

4.1 Results of the dose determination experiment

The results of the above experiment are summarized in table 1 below. From the results only the 1.5g/kg and 3.0g/kg dosages did not cause mortalities in goats and sheep respectively and were selected as the baseline for the LD_{50} determination.

Table 1. Mortalities recorded in sheep and goats in the dose determination experiment

	Goats		Sheep		
Dosage g/kg	No. per group	No. died	Dosage g/kg	No. per group	No. died
10.0	2	2	10.0	2	2
8.0	2	2	7.0	2	2
5.0	2	2	5.0	2	2
3.0	2	1	3.0	2	0
1.5	2	0			

4.2 Result of the LD_{50} determination

4.2.1 LD_{50} determination in goats

The set of mortality data obtained in this experiment is shown in table 2. Using the formula as given by Weil (1952) namely: Log m ≡ log D_a + d.(f+1) for K=3 and antilog [log m ± 2.σlog m] the LD_{50} for *S. incanum* L in goats was calculated to be 3.0g/kg body weight with a 95% confidence limit of 2.3 to 4.1g/kg body weight (appendix 1).

Table 2: Mortality data per dose group in the LD_{50} experiment in goats

GROUP	I	II	III	IV	V
DOSAGE (g/kg)	0	1.50	2.25	3.38	5.06
SURVIVED	3	3	3	0	0
DIED	0	0	0	3	2

4.2.1.1 Clinical manifestation for goats in the LD_{50} experiment

Group I was set aside as the control. Groups II and III showed only bloating on drenching. Groups IV and V had an immediate bloat followed by vomiting and muscle shivering within 10minutes. The goats then went down onto sternal recumbency accompanied by salivation and a dazed appearance. They then progressed to lateral recumbency with continuous bleating before death.

4.2.1.2. Post mortem examination of goats in the LD_{50} experiment

On postmortem, all the treated groups showed stripped haemorrhages from distal abomasum to proximal duodenum, lung emphysema and haemorrhagic enteritis extending from the colon to the caecum.

4.2.2 Results of LD_{50} determination in sheep

The set of mortality data in this experiment is shown in table 3. Using the formula as given by Weil (1952) namely: Log m ≡ log D_a + d.(f+1) for K=3 and antilog [log m ± 2.σ

log m] the LD_{50} for *S. incanum* L in sheep was calculated to be 4.8g/kg body weight with a 95% confidence limit of 3.0 to 7.7g/kg body weight (appendix 1).

Table 3: Mortality data per dosage group in sheep in the LD_{50} experiment.

GROUP	I	II	III	IV	V
DOSAGE (g/kg)	0	3.00	4.50	6.75	10.13
SURVIVED	3	3	2	0	0
DIED	0	0	1	3	3

4.2.2.1. Clinical manifestation for sheep in the LD_{50} experiment

Group I was set aside as the control. In all the treatment groups (group II to V) there was an immediate bloat and defaecation. Three group II and two group III sheep did not show further signs. One sheep in group III and all sheep in groups IV and V then developed increased salivation with frothing at the mouth, depression, muscle shivering and recumbency followed by death.

4.2.2.2. Postmortem examination of sheep in the LD_{50} experiment

On post mortem, all the treated animals showed froth in the trachea with mucosal haemorrhages; lung emphysema; congestion in the brain, kidneys, liver and intestines; haemorrhages and ulceration on the abomasums extending to the pyrolus; haemorrhagic enteritis from the duodenum to the ileum.

4.3 Results of prolonged toxicity of *S. incanum* L in sheep and goats

4.3.1 Results of clinical manifestation

4.3.1.1 Clinical manifestation of toxicity in goats

4.3.1.1.1 Clinical manifestation in Group 3 goats

Group 3 goats exposed to 0.75 LD_{50} (2.25g/kg) mainly showed bloat, shivering, coughing and colic. One goat had diarrhoea from day 3 until death at day 14. One other goat died on day 15 of the experiment after showing signs of coughing, anorexia, depression with the head held low, staggering gait and continuous bleating. One goat survived to the end of the experiment without signs of toxicity.

4.3.1.1.2 Clinical manifestation in group 2 goats

One goat in group 2 exposed to 0.5 LD_{50} (1.5g/kg) showed bloat, shivering, progressive weakness, depression, staggering gait, lateral recumbency, leg paddling movements and continuous bleating before death on day 24 of the experiment. The three other goats did not show signs and were euthanized after three months from the start of the experiment.

4.3.1.1.3. Clinical manifestation in group 1 goats

No clinical signs were recorded in group 1 goats exposed to 0.25 LD_{50} (0.75g/kg)

4.3.1.2 Clinical manifestation of toxicity in sheep

4.3.1.2.1 Clinical manifestation in group 3 sheep

All the sheep in group 3 exposed to 0.75 LD_{50} (3.6g/kg) showed signs of bloat, depression, coughing and anorexia, while one sheep also showed further signs of frothing at the mouth, colic, diarrhoea, staggering gait, lateral recumbency, leg paddling movements, coma and death. The group 3 sheep died on days 2, 3, 4 and 13 of the experiment.

4.3.1.2.2. Clinical manifestation in group 2 sheep

Three sheep in group 2 exposed to 0.5 LD_{50} (2.4g/kg) had signs of bloat, increased salivation, staggering gait, lateral recumbency and leg paddling movements before death while one sheep also showed vomiting, diarrhoea, weakness, coughing and depression before death. Two sheep died on days 4 and 14 while the other two died on day 42 of the experiment

4.3.1.2.3 Clinical manifestation in group 1 sheep

All group 1 sheep exposed to 0.25 LD_{50} (1.2g/kg) had signs of bloat. In addition, one sheep also showed weakness, staggering gait, lateral recumbency and leg paddling movements before euthanasia at the terminal stage on day 29. Three sheep also showed coughing. Three sheep survived and were euthanized at the end of the experiment.

The clinical signs seen per group are summarized in table 4.

Table 4. Main clinical signs recorded per species per dosage group

	Goat groups				Sheep groups			
Clinical sign	*1	2	3	4	1	2	3	4
Bloat	**0/4	0/4	4/4	0/4	4/4	4/4	4/4	0/4
Shivering	0/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4
Vomiting	0/4	0/4	1/4	0/4	0/4	1/4	0/4	0/4
Salivation	0/4	0/4	0/4	0/4	0/4	4/4	1/4	0/4
Diarrhoea	0/4	0/4	1/4	0/4	0/4	1/4	1/4	0/4
Weakness	0/4	1/4	1/4	0/4	1/4	1/4	0/4	0/4
Colic	0/4	0/4	4/4	0/4	0/4	0/4	1/4	0/4
Depression	0/4	1/4	1/4	0/4	0/4	1/4	4/4	0/4
Coughing	0/4	0/4	1/4	0/4	3/4	2/4	3/4	0/4
Staggering gait	0/4	1/4	1/4	0/4	1/4	3/4	1/4	0/4
Anorexia	0/4	0/4	1/4	0/4	0/4	2/4	4/4	0/4
Lateral recumbency	0/4	1/4	0/4	0/4	1/4	3/4	1/4	0/4
Leg paddling	0/4	1/4	0/4	0/4	1/4	3/4	1/4	0/4
Continuous bleating	0/4	1/4	2/4	0/4	0/4	0/4	0/4	0/4
***Death	0/4	1/4	3/4	0/4	1/4	4/4	4/4	0/4

Key: * = Group1, 2 and 3 represent 0.25, 0.5, 0.75LD_{50} respectively. Group 4 represents the control.
** = 0-4 represents the number of animals that manifested a particular clinical signs.
*** = Eight goats and three sheep survived to the end of the experiment.

4.4 Results of change in weight in sheep and goats

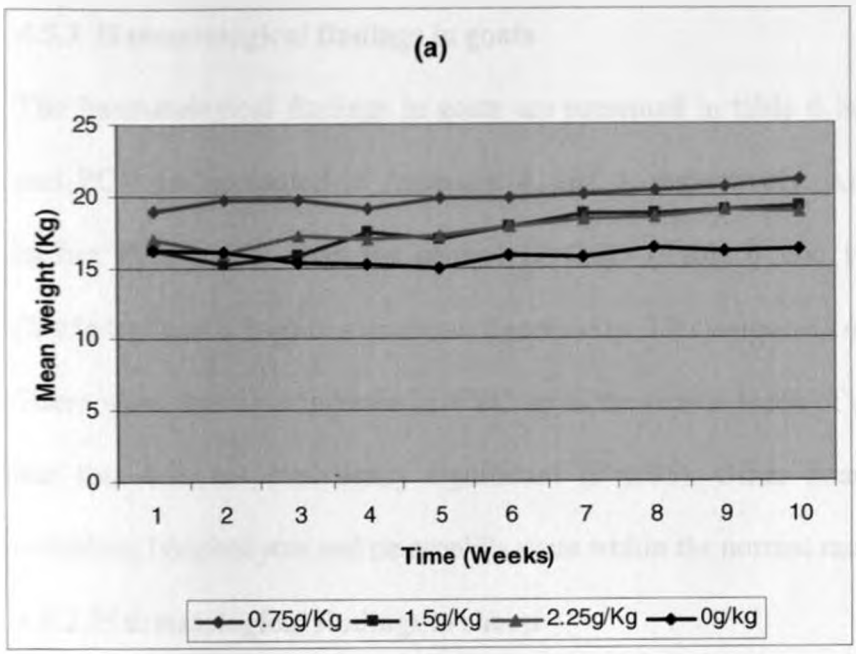
The results of the change in body weight are presented in Fig. 1 and Appendix 3. In goats (Fig. 1a), there was a significant difference in weight ($P<0.01$) between the control and all treatment groups and also within treatments ($P<0.01$).

In sheep (Fig. 1b) there was a significant difference between the control and groups 2 (2.4g/kg) and 3 (3.6g/kg) ($P< 0.01$). There was also a significant difference within treatments ($P<0.01$). However, there was no difference in weight between sheep and goats ($P>0.05$). Table 4 shows the ANOVA table for weighs in sheep and goats.

Table 5. ANOVA table for weights in sheep and goats

		Animal groups			
	d.f.	1	2	3	4
Goats	3	20.1 ± 0.6^{a}	17.4 ± 0.6^{ab}	17.4 ± 1.0^{ab}	15.8 ± 0.6^{ab}
Sheep	3	18.8 ± 0.6^{a}	15.9 ± 0.8^{ab}	15.0 ± 1.6^{ab}	17.8 ± 0.6^{b}

*Means with a common superscript are statistically significant.



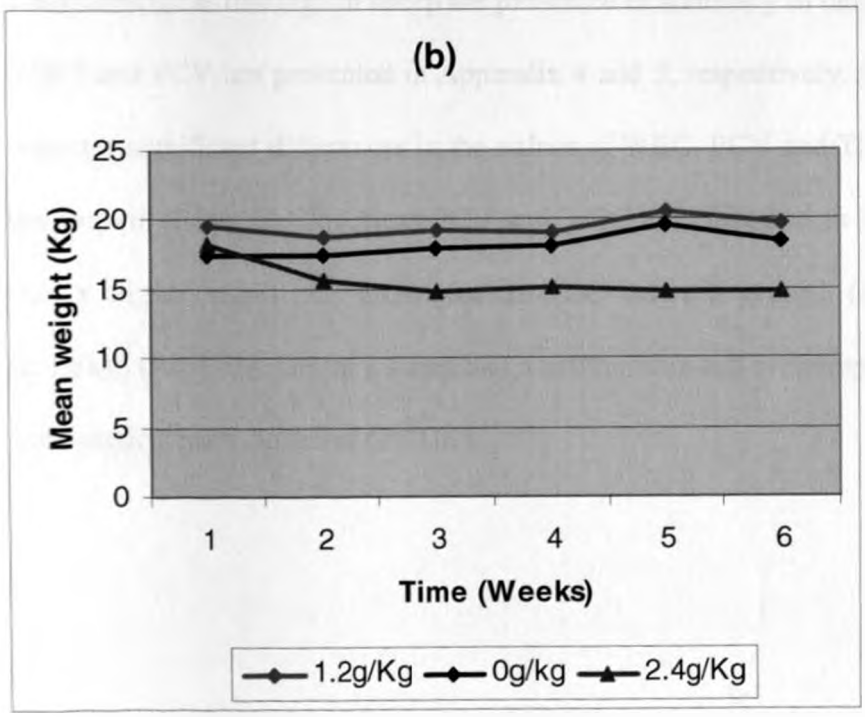


Figure 1. Mean weight (Kg) against time (weeks) in (a) goats and (b) sheep.

All treatment groups in goats had a slight increase in weight compared to the control, group 1 ($0.25LD_{50}$) continuing to gain weight. Group 2 ($0.5\ LD_{50}$) sheep had a decrease in weight to the second week remaining low thereafter.

4.5 RESULTS OF THE HAEMATOLOGICAL INVESTIGATION

4.5.1 Haematological findings in goats

The haematological findings in goats are presented in table 6 below while mean WBC and PCV are presented in Appendix 4, and 5, respectively. All treatment groups had higher PCV values than the control ($P<0.01$) (Table 6 and Fig. 2a) while group 3 (2.25g/kg) had a highly significant decrease in TP compared to the control ($P< 0.01$). There was a transient increase in WBC up to the second week of the experiment (Fig. 3a) but this was not statistically significant ($P>0.05$). Other haematological parameters including lymphocytes and neutrophils were within the normal range ($P>0.05$).

4.5.2. Haematological findings in sheep

Haematological findings in sheep are presented in summary in table 6 below while mean WBC and PCV are presented in Appendix 4 and 5, respectively. From the results, there were no significant differences in the values of WBC, PCV and TP ($P>0.05$) between all the groups (Table 6). The mean PCV and WBC are presented in Fig. 2b and 3b. There was a highly significant difference in RBC between group1 (1.2g/kg) and group 2 (2.4g/kg) ($P< 0.01$). Group 1 sheep had a neutrophilia and lymphopaenia and these values were significantly different ($P<0.01$).

Table 6. Mean values of PCV, WBC, RBC and total protein in sheep and goats per dose group

	Goats				Sheep			
Treatment	WBC /µl	RBC ($x10^6$/µl)	PCV (%)	Total protein (g/dl)	WBC /µl	RBC ($x10^6$/µl)	PCV (%)	Total protein (g/dl)
Group 1	9,517	4.9 ± 0.8	29.4	7.3 ± 0.2	9,931	9.0 ± 0.8	31.2 ± 2.0	6.6 ± 0.3
Group 2	10,235	4.7 ± 1.0	30.2	7.2 ± 0.2	10,388	11.7 ± 1.2	33.4 ± 2.6	6.2 ± 0.4
Group 3	12,177	4.9 ± 1.6	33.0	6.9 ± 0.2	10,840	9.6 ± 2.2	33.1 ± 2.4	7.1 ± 0.3
Group 4 (control)	11,008	3.8 ± 0.8	26.3	7.4 ± 0.2	9,600	10.3 ± 1.0	32.1 ± 2.4	6.8 ± 0.4
Normal values*	4,000-13,000	8.0-18.0	22-38	6.0-7.5	4,000-12,000	9-15	27-45	6.0-7.5

Source: Jain, N.C (1993). In: Essentials of Veterinary Haematology. Lea & Febiger, Philadelphia, Pg 7.

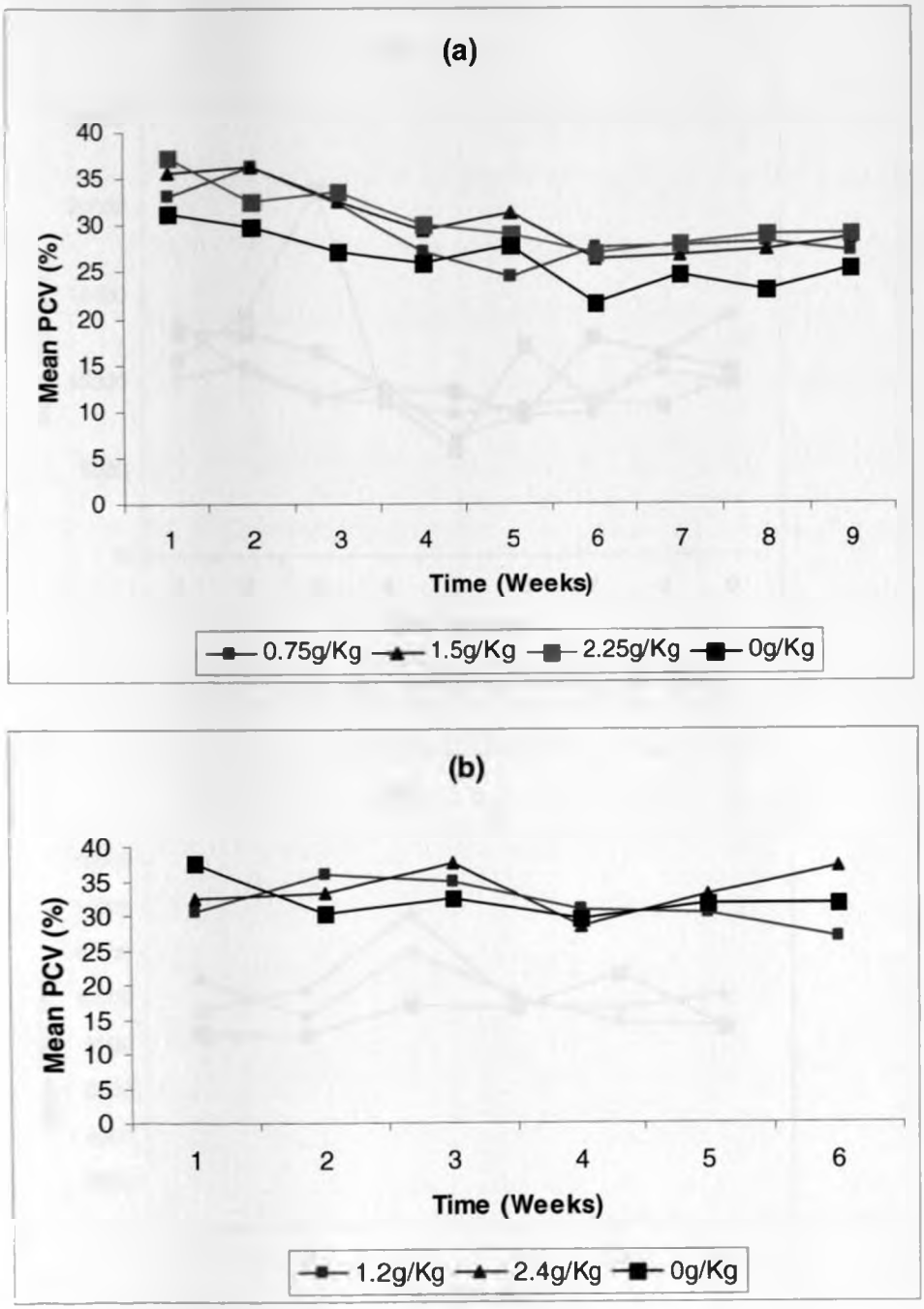
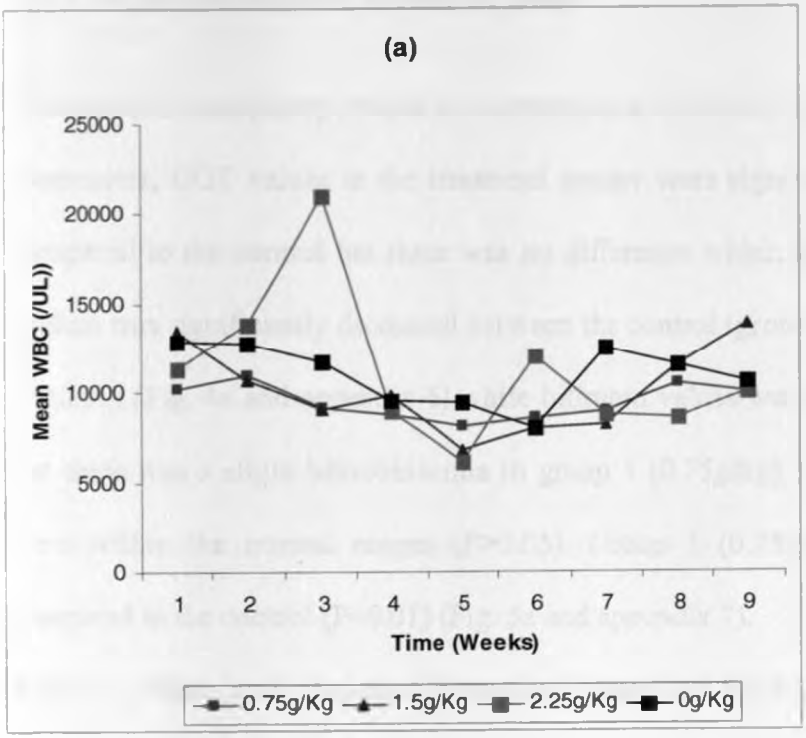


Figure 2. Mean packed cell volume (%) against time (weeks) for (a) goats and (b) sheep

All treatment groups in goats had a higher PCV than the control ($P<0.01$) while PCV in sheep was within the normal range ($P>0.05$). Sheep had higher PCV values than goats and this difference was significant ($p<0.01$).



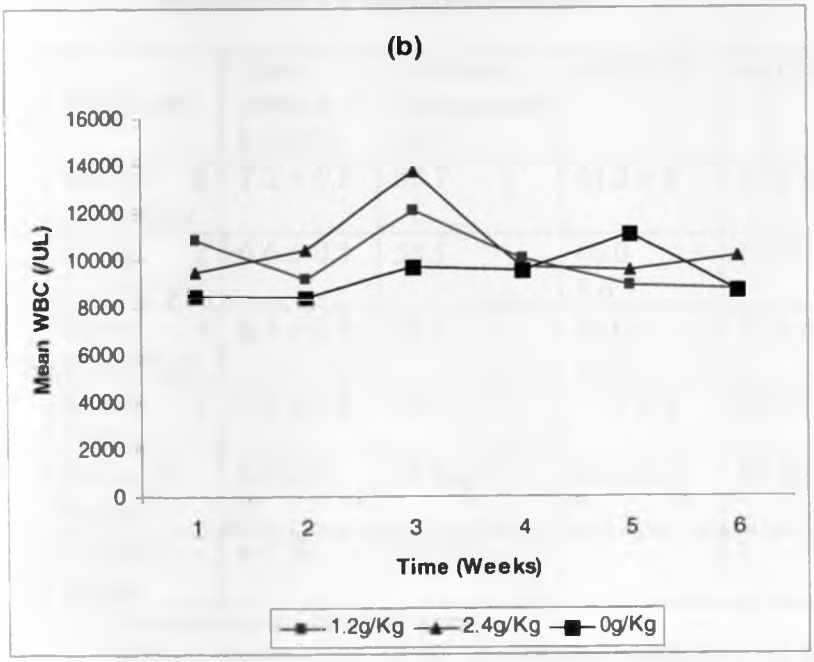


Figure 3. Mean white blood cell counts (/ul) against time (weeks) in (a) goats and (b) sheep.

There was a leucocytosis in 2nd and 3rd weeks of the experiment in goats and sheep respectively, the values normalizing thereafter (P>0.05). There was no significant difference in the WBC counts between the two species (P>0.05).

4.6 RESULTS OF ANALYSIS ON SERUM BIOCHEMICAL PARAMETERS

4.6.1 Serum biochemical findings in goats

The serum biochemistry results are summarized in table 7 below. On the biochemical parameters, GGT values in the treatment groups were significantly decreased ($P<0.01$) compared to the control but there was no difference within the treatment groups. Total protein was significantly decreased between the control (group 4) and group 3 (2.25g/kg) ($P<0.01$) (Fig. 4a and appendix 6) while bilirubin values were not significantly different but there was a slight bilirubinaemia in group 1 (0.75g/kg). AST and creatinine values were within the normal ranges ($P>0.05$). Group 1 (0.75g/kg) had high AP values compared to the control ($P<0.01$) (Fig. 5a and appendix 7).

Table 7. Mean and standard deviation values for total protein, AP, AST, GGT, Bilirubin and Creatinine in goats

Treatment	Total protein (mg/dl)	Alkaline phosphatase (u/l)	AST(U/l)	GGT (u/l)	Bilirubin (mg/dl)	Creatinine (mg/dl)
Group 1 (0.75g/kg)	7.2 ± 0.3	85.7	61.2 ± 8	19.5 ± 1	0.5 ± 0.2	0.9 ± 0.1
Group 2 (1.5g/kg)	6.6 ± 0.3	58.5	60.0 ± 8.6	18.2 ± 1	0.3 ± 0.2	0.9 ± 0.1
Group 3 (2.25g/kg)	6.4 ± 0.4	55.0	63.6 ± 13.4	17.3 ± 1.6	0.4 ± 0.4	0.8 ± 0.1
Group 4 (0g/kg)	7.1 ± 0.3	57.4	74.9 ± 8	22.9 ± 1	0.3 ± 0.2	0.9 ± 0.1
[1]Pre-expt values	6.9±0.9	77.1±27	68.2±8.3	19.4±3.7	0.3±0.2	0.7±0.1
[2]Normal values	4-7.0	93-387			0-0.1	1.0-1.8

[1]Pre-experimental values for goats.

[2]Source: Kaneko, J. J. (1980). In: Clinical biochemistry of domestic animals. 3rd edition. Academic Press Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney and Tokyo. Pg 792-3.

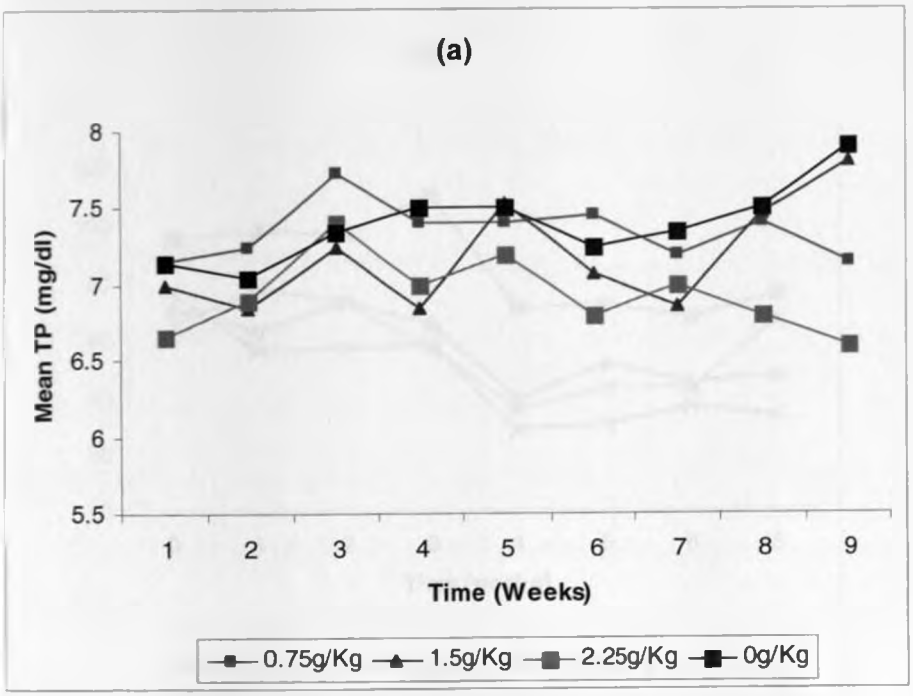
4.6.2 Serum biochemical findings in sheep

The biochemical parameters are summarized in table 8 below. Total protein values were within the normal range ($p>0.05$) (Fig.4b and appendix 6). Alkaline phosphatase values were significantly decreased in groups 1 (1.2g/kg) and group 3 (3.6g/kg) compared to the control ($p<0.05$) (Fig. 5b and appendix 7) and the difference was significant even within treatments ($P<0.05$). Groups 2 (2.4g/kg) and 3 (3.6g/kg) had a slight increase in bilirubin but this increase was not significant ($P>0.05$). Creatinine values were within the normal range.

Table 8. Mean and standard deviation values for Total Protein, Alkaline phosphatase, Bilirubin, Creatinine and Aspartate aminotransferase in sheep

Treatment	Total protein (mg/dl)	AP (u/l)	Bilirubin (mg/dl)	Creatinine (mg/dl)	AST (u/l)
Group1 (1.2g/kg)	6.6 ± 0.3	53.5	0.4 ± 0.3	0.9 ± 0.1	60.6 ± 8
Group2 (2.4g/kg)	6.2 ± 0.4	78.6	0.7 ± 0.4	1.0 ± 0.1	65.4 ± 10
Group3 (3.6g/kg)	7.1 ± 0.7	37	1.26 ± 0.6	1.1 ± 0.2	51.2 ± 17
Group 4 (0g/kg)	6.8 ± 0.4	92.2	0.4 ± 0.3	1.1 ± 0.1	65.6 ± 9
Normal values*	6.0-7.9	68-387	0.1-0.42	1.0-1.8	48-128

Source: *Kaneko, J. J. (1980). In: Clinical biochemistry of domestic animals. 3rd edition. Academic Press Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney and Tokyo. Pg 792-3.



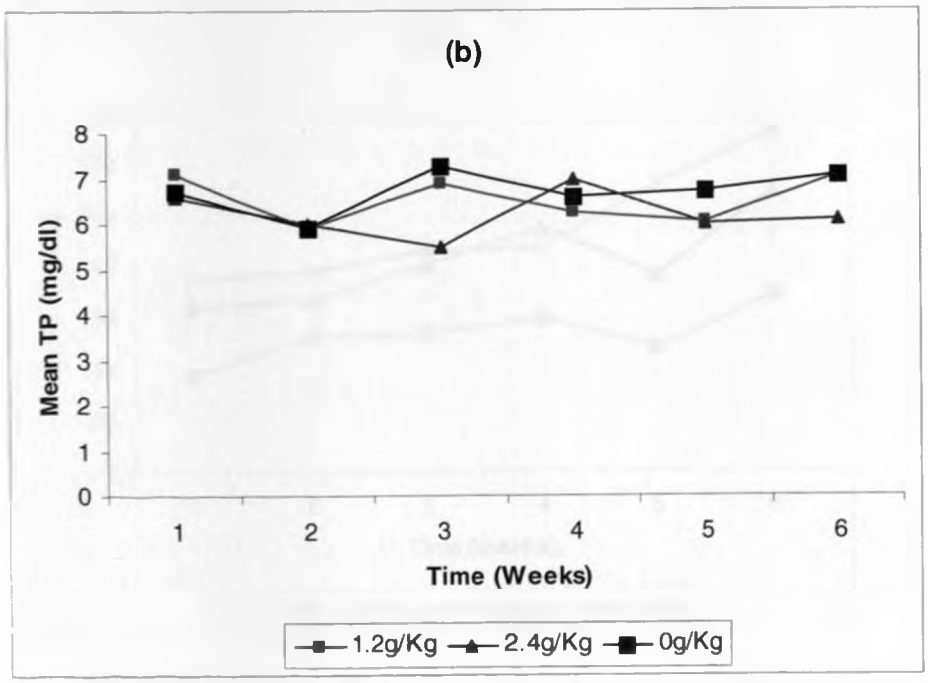
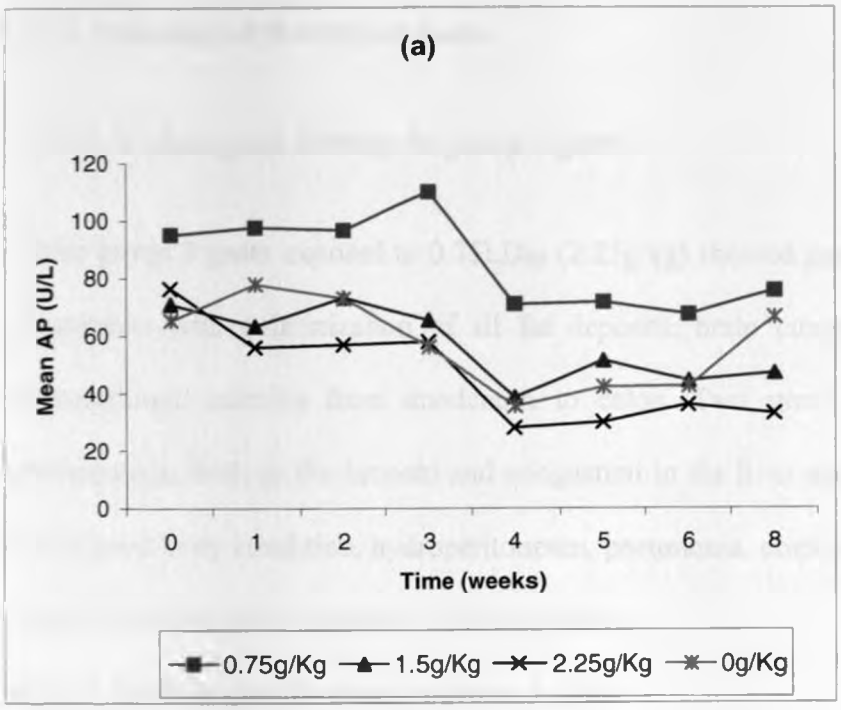


Figure 4. Mean total protein (mg/dl) against time (weeks) in (a) goats and (b) sheep
Group 3 (2.25g/kg) goats showed hypoproteinaemia which was significant ($P<0.01$) while the other groups had normal values. In sheep TP was within the normal range ($P>0.05$) in all groups. Overall, there was a significant difference in TP between goats and sheep ($P<0.01$).



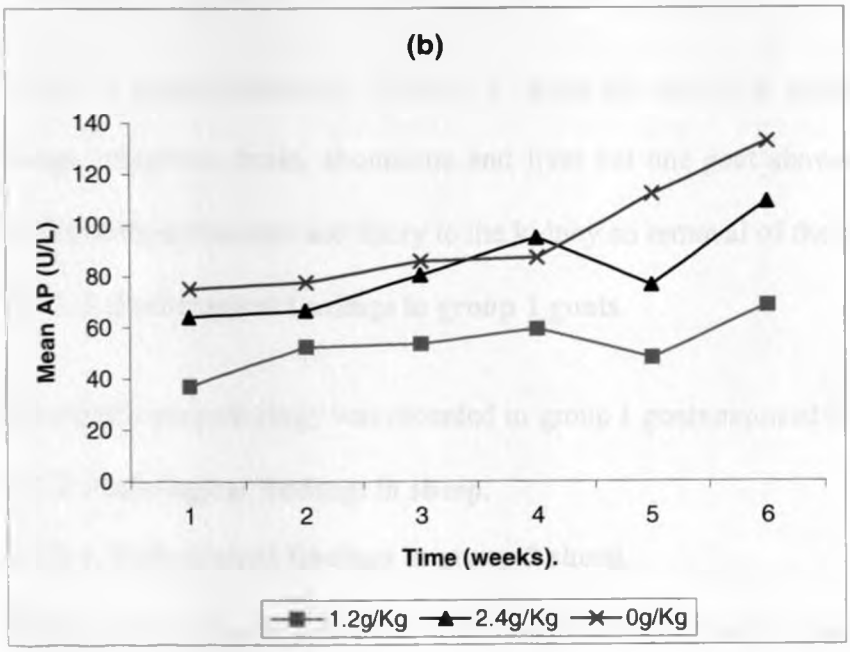


Figure 5. Mean Alkaline phosphatase (u/l) against time (Weeks) in (a) goats and (b) sheep.

AP was significantly increased in group 1 goats (0.75g/kg) compared to the control ($P<0.01$) but the increase in groups 2 and 3 was not significant. There was a significant decrease in AP in sheep in groups 1 (1.2g/kg) and 3 (3.6g/kg) compared to the control ($P<0.05$). However, there was no difference in AP between sheep and goats ($P>0.05$).

*Group 3 sheep data could not be plotted due to few measurements.

4.7 RESULTS OF PATHOLOGICAL EXAMINATION

4.7.1 Pathological findings in goats.

4.7.1.1. Pathological findings in group 3 goats.

Three group 3 goats exposed to $0.75LD_{50}$ (2.25g/kg) showed generalized congestion and emaciation with gelatinization of all fat deposits, brain congestion and oedema and haemorrhagic enteritis from duodenum to colon. Two goats had hydropericardium, perihepatitis, froth in the bronchi and congestion in the liver and kidney while one goat had a good body condition, hydroperitoneum, pneumonia, emphysema and haemorrhagic ulcers on abomasums extending to distal pyrolus.

4.7.1.2. Pathological findings in group 2 goats.

Group 2 goats exposed to $0.5LD_{50}$ (1.5g/kg) did not show significant pathology in the lungs, intestines, brain, abomasum and liver but one goat showed fibrinous pericarditis with hydroperitoneum and injury to the kidney on removal of the capsule.

4.7.1.3. Pathological findings in group 1 goats.

No significant pathology was recorded in group 1 goats exposed to $0.25LD_{50}$ (0.75g/kg).

4.7.2 Pathological findings in sheep.

4.7.2.1. Pathological findings in group 3 sheep.

Three group 3 sheep exposed to $0.75LD_{50}$ (3.6g/kg) showed a good body condition while one was emaciated. All sheep in this group had pneumonia, froth in the bronchi, emphysema and congestion in the liver and kidneys. Three sheep had perihepatitis,

haemorrhagic ulcers on distal abomasum to proximal duodenum while two sheep had haemorrhagic enteritis from duodenum to colon.

4.7.2.2. Pathological findings in group 2 sheep.

All group 2 sheep exposed to $0.5LD_{50}$ (2.4g/kg) had a good body condition, congestion in the brain, pneumonia, froth in the bronchi, lung emphysema and haemorrhagic ulcers on distal abomasum to proximal duodenum. Three sheep had congestion of the kidneys and liver and haemorrhagic enteritis from duodenum to colon.

4.7.2.3. Pathological findings in group 1 sheep.

Three group 1 sheep exposed to $0.25LD_{50}$ (1.2g/kg) had a good body condition while one had generalized emaciation with gelatinization of all fat deposits. Three sheep had pneumonia, froth in the bronchi, lung emphysema and congestion in the brain. Two sheep had hepatitis and perihepatitis while one had hydroperitoneum and roughening of the surface of the abomasums.

The results of the gross pathological findings in sheep and goats are summarized in table 9 below.

Table 9. Gross pathological findings in sheep and goats

	Goat groups				Sheep groups			
Gross Pathological finding	*1	2	3	4	1	2	3	4
Carcass in good condition	**4/4	0/4	1/4	0/4	3/4	4/4	3/4	0/4
Emaciation with fat gelatinization	0/4	0/4	3/4	0/4	1/4	0/4	1/4	0/4
Increased peritoneal fluid	0/4	0/4	1/4	0/4	1/4	0/4	0/4	0/4
Increased pericardial fluid	0/4	1/4	2/4	0/4	0/4	0/4	0/4	0/4
Brain congestion	0/4	0/4	3/4	0/4	3/4	4/4	0/4	0/4
Brain oedema	0/4	0/4	3/4	0/4	0/4	0/4	0/4	0/4
Perihepatitis	0/4	0/4	2/4	0/4	2/4	0/4	3/4	0/4
Hepatitis	0/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4
Heamorrhagic enteritis from duodenum to colon	0/4	0/4	3/4	0/4	0/4	3/4	2/4	0/4
Froth in bronchi	0/4	0/4	2/4	0/4	3/4	4/4	4/4	0/4
Lung emphysema	0/4	0/4	1/4	0/4	3/4	4/4	4/4	0/4
Pneumonia	0/4	0/4	1/4	0/4	3/4	4/4	4/4	0/4
Haemorrhagic ulcers on distal abomasum to proximal duodenum	0/4	0/4	1/4	0/4	0/4	4/4	3/4	0/4
Congestion on kidney, liver,	0/4	0/4	2/4	0/4	0/4	3/4	4/4	0/4

Key: * = Group 1, 2 and 3 represent 0.25, 0.5 and 0.75 LD_{50} respectively while Group 4 represents the control.

** 0-4 = number of animals showing a particular pathological finding.

4.8 RESULTS OF HISTOLOGICAL FINDINGS IN SHEEP AND GOATS

4.8.1 Histological findings in goats

4.8.1.1. Histological findings in group 3 in goats

All goats in group 3 exposed to $0.75LD_{50}$ (2.25g/kg) had wallerian degeneration of neurons, necrosis of Purkinje cells and formation of micro-thrombi in the brain (Fig. 6a); congestion, emphysema, interstitial pneumonia and proliferation of alveolar epithelium in the lungs; congestion and centrolobular necrosis in the liver (Fig. 7a) and congestion, intertubular haemorrhage and tubular necrosis with hyaline cast formation in the kidneys. Three goats showed coagulative necrosis of the epithelium infiltrated with plasma cells and lymphocytes with areas of ulceration from duodenum to colon and skeletal muscle necrosis. One goat did not show ulceration of the epithelia. Two goats showed ulceration and haemorrhage in the abomasums while two had epithelial erosions on abomasums.

4.8.1.2. Histological findings in group 2 goats

One group2 goat exposed to $0.5LD_{50}$ (1.5g/kg) had congestion, mild haemorrhage, perivascular vacuolation, central chromatolysis of neurons and necrosis of Purkinje cells in the brain (Fig. 6c) and chromatolysis of neurons and vacuolation in the spinal cord while the three others had mild chromatolysis of neurons and necrosis of Purkinje cells; three goats had mild lesions in other organs which included proliferation of alveolar epithelium, interstitial pneumonia, congestion and oedema; mild coagulative necrosis from duodenum to colon with hyperplasia of glands of the colon; congestion, centrilobular necrosis and intersinusoidal haemorrhage in the liver (Fig. 7c) and congestion, glomerular necrosis and tubular necrosis with hyaline cast formation in the kidneys.

4.8.1.3. Histological findings in group 1 goats

The lesions in group 1 goats exposed to $0.25LD_{50}$ (0.75g/kg) were lung congestion and mild interstitial pneumonia, mild erosion of the intestinal epithelium, mild hepatic centrilobular necrosis (Fig 7e), mild renal tubular necrosis and cerebral congestion (Fig. 6e).

The normal liver and brain is shown in fig. 8b and d.

4.8.2 Histological findings in sheep

4.8.2.1. Histological findings in group 3 sheep

All group 3 sheep exposed to $0.75LD_{50}$ (3.6g/kg) had widespread haemorrhage (Fig 6b), chromatolysis of neurons, slight degree of wallerian degeneration and necrosis of Purkinje cells of the brain; lung emphysema, oedema, interstitial pneumonia and proliferation of alveolar epithelium; haemorrhagic enteritis infiltrated with lymphocytes and plasma cells and ulceration from duodenum to colon; haemorrhage and ulceration in the abomasums; hepatic bile duct proliferation and necrosis around central vein with the degenerative changes varying from cloudy swelling to pyknosis of nuclei (Fig. 7b) and congestion, intertubular haemorrhage and coagulative necrosis of tubules with hyaline cast formation in the kidneys. Two males showed degeneration of basement membrane of testis with aspermia. Two female sheep had necrotic changes in the uterus and ovaries.

4.8.2.2. Histological findings in group 2 sheep

All group 2 sheep exposed to $0.5LD_{50}$ (2.4g/kg) had widespread haemorrhage, perivascular oedema and vacuolation, chromatolysis of neurons and necrosis of Purkinje cells in the cerebellum and wallerian degeneration of neurons(Fig. 6d); lung emphysema,

oedema, interstitial pneumonia and proliferation of alveolar epithelium; coagulative necrosis with areas of ulceration from duodenum to colon and hyperplasia of colonic glands; fatty degeneration of sinusoids, intersinusoidal haemorrhage, bile duct proliferation in the liver (Fig. 7d) and massive intertubular haemorrhage, congestion, glomerular necrosis and widespread tubular necrosis with hyaline cast formation in the kidneys.

4.8.2.3. Histological findings in group 1 sheep

All group1 sheep exposed to $0.5LD_{50}$ (1.2g/kg) had widespread haemorrhage, perivascular vacuolation, chromatolysis of neurons (Fig. 6f), and necrosis of the Purkinje cells of the cerebellum; lung emphysema, proliferation of alveolar and bronchiolar epithelium and interstitial pneumonia, centrilobular necrosis and bile duct proliferation in the liver (Fig. 7f), epithelial erosion on duodenum and ileum, hyperplasia of glands in the colon with epithelial erosion and widespread tubular necrosis with hyaline cast formation and glomerular necrosis in the kidneys.

The normal brain and liver in sheep are shown in fig. 8a and c.

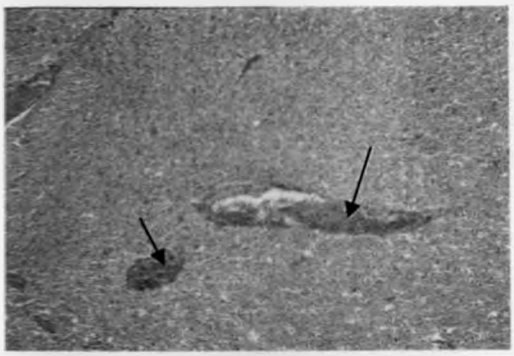


Fig. 6a. Brain tissue from a goat fed 0.75LD_{50} (2.25g/kg) unripe fruit powder that died on day 6 showing microthrombi (arrows) in the gray matter (H&E x 200)

Fig. 6b. Brain from a sheep fed 0.75LD_{50} (3.6g/kg) unripe fruit powder that died on day 2 showing haemorrhage from a blood vessel (H&E x 200).

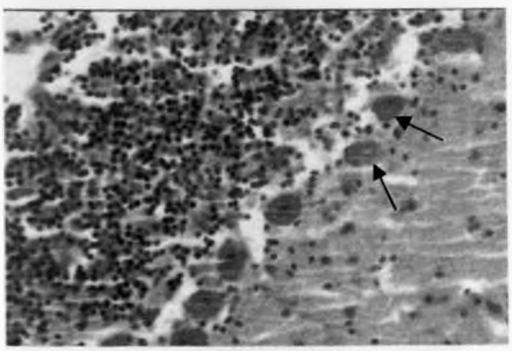


Fig. 6c. Brain tissue from a goat fed 0.5LD_{50} (1.5g/kg) unripe fruit powder that died on day 49 showing necrosis of purkinje cells of the cerebellum (H&E x 400)

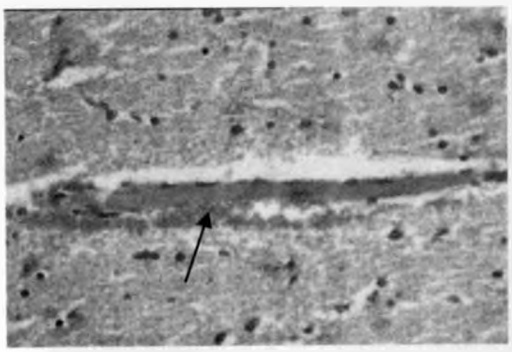


Fig. 6d. Brain from a sheep fed 0.5LD_{50} (2.4g/kg) unripe fruit powder that died on day 4 showing marked haemorrhage from a blood vessel (arrow) and wallerian degeneration of neurons (H&E x 200)

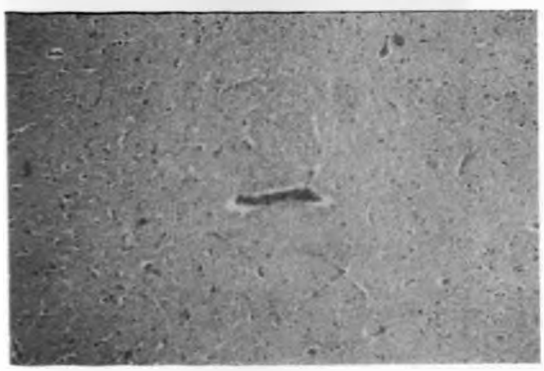


Fig. 6e. brain tissue from a goat fed 0.25LD_{50} (0.75g/kg) unripe fruit powder that was euthanized after 3 months showing congestion of blood vessels (H&E x 200)

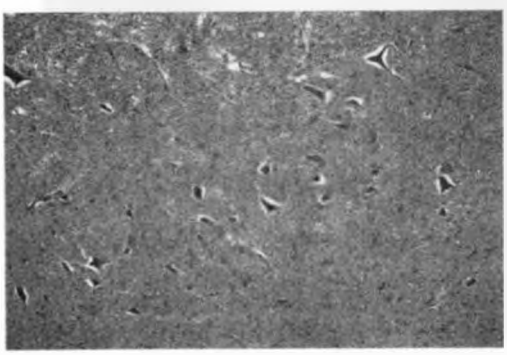


Fig. 6f. Brain tissue from a sheep fed 0.25LD_{50} (1.2g/kg) unripe fruit powder that died on day 29 showing widespread chromatolysis of neurons (H&E x 200)

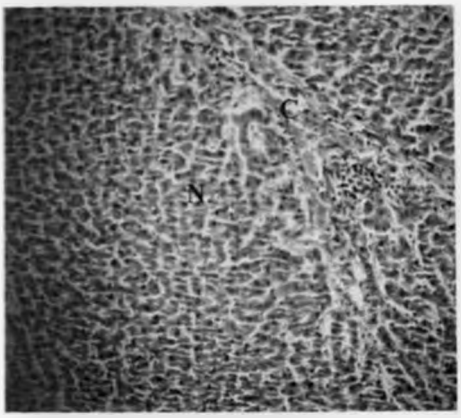


Fig.7a. Liver of goat fed 0.75LD_{50} (2.25g/kg) unripe fruit powder that died on day 6 showing congestion (C) and hepatic necrosis (N) (H&E x200)

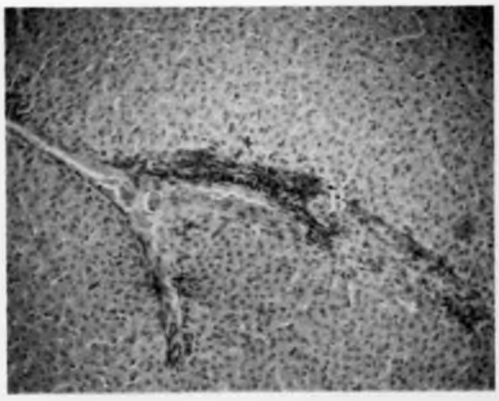


Fig. 7b. Liver of sheep fed 0.75LD_{50} (3.6g/kg) unripe fruit powder that died on day 2 showing congestion (C) and proliferation of bile ducts (B). (H&E x 200)

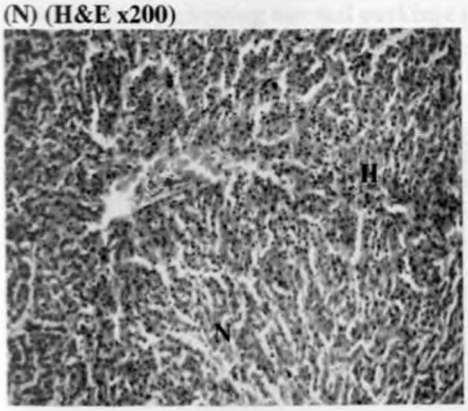


Fig .7c. Liver from a goat fed 0.5LD_{50} (1.5g/kg) unripe fruit powder that died on day 49 showing intersinusoidal haemorrhage (H) and hepatic cell necrosis (N) (H&E x200)

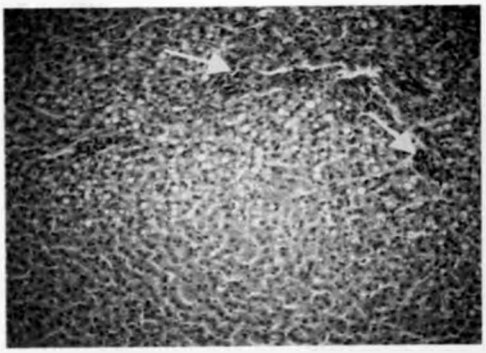


Fig. 7d. Liver of sheep fed 0.5LD_{50} (2.4g/kg) unripe fruit powder that died on day 4 showing fat necrosis of sinusoids and bile duct proliferation (arrows). (H&E x 200)

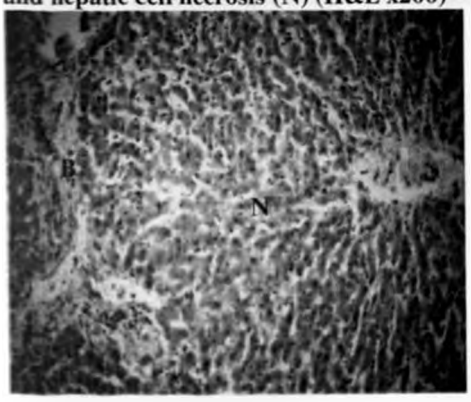


Fig 7e. Liver from a goat fed 0.25LD_{50} (0.75g/kg) unripe fruit powder and euthanized after three months showing bile duct proliferation (B) and hepatic necrosis (N) (H&Ex200)

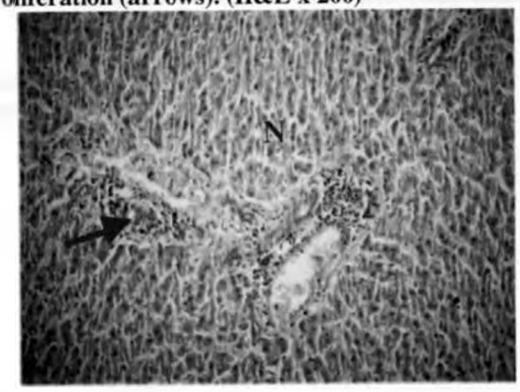


Fig. 7f. Liver of sheep fed 0.5LD_{50} (1.2g/kg)unripe fruit powder that died on day 29 showing bile duct proliferation (arrow) and hepatic necrosis (N). (H&E x 200)

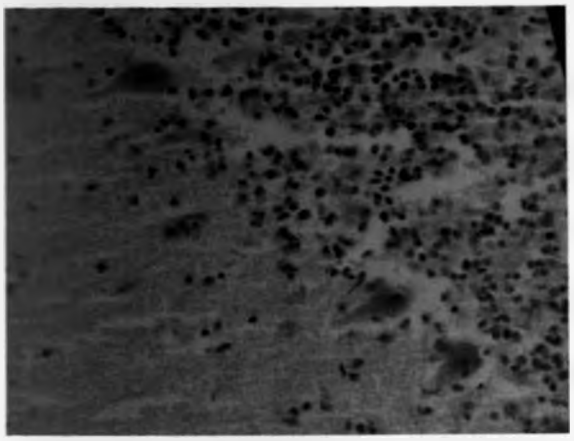


Fig. 8a. Normal tissue from the cerebellum of a control sheep showing normal purkinje cells (H&E x400).

Fig. 8b. Normal tissue from cerebellum of control goat showing normal purkinje cells (H&E x 200)

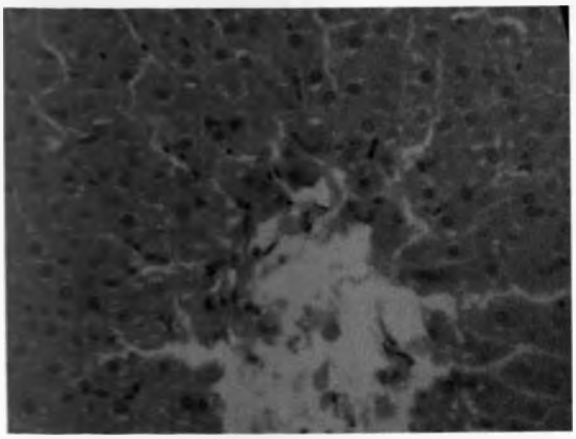


Fig 8c. Normal liver tissue from a control sheep (H&E x 400)

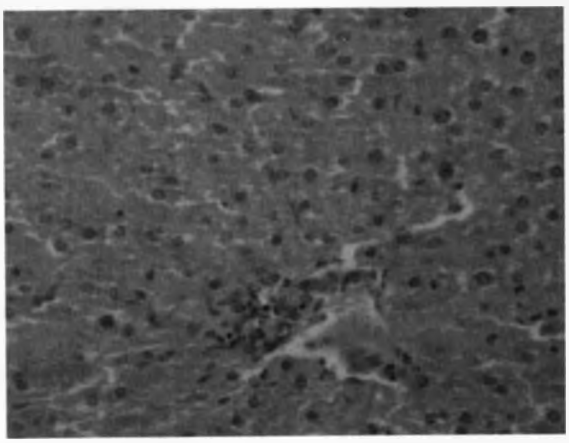


Fig. 8d. Normal liver tissue from a control goat

CHAPTER 5

5.0 DISCUSSION

The LD_{50} in sheep was 4.8g/kg (320mg/kg) with a 95% confidence limit of 3.0 – 7.7 while the LD_{50} in goats was 3.0g/kg (200mg/kg) with a 95% confidence limit of 2.25 - 4.13 g/kg. This shows that the plant is quite toxic to both species of animals as the LD_{50} falls within the range 50- 500mg/kg range (Clarke *et. al.*, 1981). However, the toxic dose in goats is lower than that in goats but it appears that when goats are exposed to *S. incanum* L for a prolonged period of time, they appear to detoxify the materials.

The oral LD_{50} for solanine in rats has been recorded as 590 mg/kg (Dalvi & Bowie, 1983) while in sheep, the LD_{50} of total glycoalkaloids is 500 mg/kg b.wt with toxic effects noted at 225 mg/kg b.wt. (Jadhav *et al.*, 1981). This LD_{50} is similar to the one determined for sheep in this study where the entire fruit was used. There is, however, no literature showing LD_{50} values for the other Solanum species and to date this is the first such determination. In this experiment there was a difference in the survival of the two species, which was statistically significant with the goats surviving longer than sheep. Animals at the lowest dosages survived for a longer period than the other dosages.

Clinically, both groups of animals had different clinical manifestations, which were more severe in sheep. This is shown by the fact that all goats in groups 1 (0.75g/kg), three in group 2 (1.5g/kg) and one in group 3 (2.25g/kg) did not show any clinical signs of intoxication whereas similar sheep groups (1, 2 and 3) had severe signs of intoxication. Prior exposure of ruminal bacteria to toxic plants increases the rate of detoxification,

indicating an adaptive response by the bacteria to these substrates (Carlson and Breeze, 1984). Rumen microbes from naïve cattle and sheep that had not been exposed to various alkaloids (sparteine, lupanine, cystine, atropine, quinidine, nicotine, harmaline, arecoline, caffeine, and senecionine) were not able to degrade these alkaloids whereas animals that were exposed degraded them (Ramon and Michael, 2005). Rumen microorganisms from a sheep preconditioned to lupin alkaloids tolerated lupanine much better than non-adapted microorganisms (Ramon and Michael, 2005).

Sometimes, degradation activities are related to adaptive changes in the rumen microorganism populations, which result in an acquired resistance to specific toxins (Duncan *et al.*, 2000, Duncan and Milne, 1992; Newbold *et al.*, 1997). Goats being browsers sometimes feed on the green berries of *S. incanum* L and this could lead to adaptation of the rumen microbes to this toxin. It is, therefore, possible that some degree of detoxification of unripe berries of *S. incanum* L occurs in goats through this adaptation.

Species differences in this detoxification process have also been demonstrated by Shull *et. al.*, (1976) who showed that incubation of *Senecio jacobaea* with rumen fluid from a steer reduced the toxicity of the plant to rats while similar rumen fluid from a sheep did not. The detoxification of pyrrolizidine alkaloids by rumen microbes is also documented (Culvenor, et. al., 1962, Lanigan, 1970) and it is possible that there is detoxification of *Solanum incanum* L in goats and not in sheep.

Steyn (1949) had listed the main clinical signs of *S. incanum* L poisoning in animals as salivation, diarrhoea, colic, bloat, stomatitis, tachycardia, polypnea, cramps, paralysis and occasionally, a vesicular exanthema. Kingsbury (1964) also reported signs of progressive weakness leading to paralysis and unconsciousness. These signs are similar to those recorded in this study but both species of animals also showed nervous signs of depression, staggering gait and lateral recumbency with leg paddling movements which had not been recorded by Steyn (1949) and Kingsbury (1964). In this experiment, sheep did not show the continuous bleating, which was recorded in goats.

Neurological diseases in ruminants caused by ingestion of different *Solanum* species have been described in South Africa, North America, South America, Australia and Western Uruguay (Raquel *et al.*, 2006; Verdes *et al.*, 2006; Medeiros, *et al.*, 2004; Porter *et al.*, 2003; Bourke, 1997; Summers *et al.*, 1995; Riet-Correa *et al.*, 1983; Menzies *et al.*, 1979; Pienaar *et al.*, 1976). *Solanum fastigiatum var. fastigiatum* (Riet-Correa *et al.*, 1983) and *Solanum bonariense* (Verdes *et al.*, 2006) cause a syndrome in cattle characterized by periodic attacks of ataxia, hypermetria, opisthotonus, nystagmus and falling to the side or backwards with animals appearing normal between episodes.

However, *Solanum viarum* (Porter *et al.*, 2003) causes a syndrome similar to the one in goats in this study, where the goats developed ataxia and head tremors, recumbency with inability to rise and death after grazing on pastures for 6-12 months. In all these studies including *S. incanum* L, the animals exhibited signs of cerebellar disease and the variations in clinical manifestation may be due to species differences within the *Solanum*

plants. It is also important to note that rats fed *Solanum incanum* L unripe fruit powder mixed with feed did not show the signs of cerebellar degeneration (Thaiyah, 1992).

On haematology there was a transient elevation in white cell counts in the two species, which was more marked in goats but the difference was not significant ($P>0.05$). Goats also showed a lower PCV than sheep ($P<0.01$). Other haematological parameters were within the normal range in both species. In *Solanum viarum* (Porter *et al.*, 2003) all the haematological parameters were within the normal range except for a moderate anaemia, which was also recorded in goats in this study.

Serum enzyme parameters were also significantly different between the two species. Bilirubinaemia was observed in group 2 and 3 sheep and in group 1 goats but the increase was not significant ($P>0.05$). The levels of creatinine and aspartate aminotransferase were normal in goats and sheep. Similar results were also obtained with *Solanum viarum* poisoning in goats (Porter *et al.*, 2003). This failure of serum enzymes from the liver to increase can be associated with the hepatic protective property of *Solanum incanum* L. (Chin, 1989). When crude extracts from the total *Solanum incanum* plant are administered to rats poisoned with carbon tetrachloride, the elevated levels of AST and ALT due to carbon tetrachloride are significantly decreased (Chin, 1989). In this study marked hypoproteinaemia was recorded in group 3 (2.25g/kg) goats ($P<0.05$) while in sheep serum total proteins were normal. Alkaline phosphatase levels were elevated in group 1 (0.75g/kg) goats but reduced in group 1(1.2g/kg) and 3 (3.6g/kg) sheep.

Pathological changes were recorded in various organs of the body. Group 3 goats (2.25g/kg) had hydroperitoneum and hydropericardium, which were also recorded in group 1 (1.2g/kg) sheep. This fluid accumulation could be as a result of hypoproteinaemia, which was recorded at these dosages. It is interesting to note that gross pathology as recorded in other organs apart from the brain in sheep and group 3 (2.25g/kg) goats was not recorded with *S. fastigiatum var fastigiatum* in cattle (Raquel *et al.*, 2006; Riet-Correa *et al.*, 1983), *S. bonariense* in cattle (Verdes *et al.*, 2006), *S. viarum* in goats (Porter *et al.*, 2003), *S. kwebense* in cattle (Pienaar *et al.*, 1976) and *S. cinereum* in goats (Bourke, 1997). From the above literature, only the study with *Solanum bonariense* (Verdes *et al.*, 2006) indicated the use of leaves for the poisoning while the rest do not state the part of the plant used.

Unripe fruits of *S. incanum* L contain steroidal glycosides the major ones being solanine, incanumine, solamargine, solasodine, ursolic acid and other ursolic acid derivatives (Chun-Nan, 1990). It is possible that all or some of these chemical compounds could cause gross pathological lesions observed in goats and sheep, which has not been reported before.

Histopathological lesions were also significant. In the lungs, both sheep and goats had severe interstitial pneumonia and emphysema and proliferation of alveolar epithelium, which was manifested clinically by coughing. The interstitial pneumonia could have been due to lowering of immunity by the toxin, which also produced proliferative changes in the alveoli. In the liver, there was centrilobular necrosis and bile duct hyperplasia, which

was more marked in sheep. These findings are consistent with those found in other plant poisonings affecting the liver which include plants in the genera *Senecio, Heliotropium, Amsinca, Echium, Crotalaria, Symhytum* and *Trichodesma* spp (Adams, 1974; Hooper, 1978), *Cestrum* (Mugera and Nderitu, 1968b), *Lantana* (Gopinath and Ford, 1969), *Tribulus terrestris* L (Van Tonder *et. al.*, 1972), *Brachiaria decubens* (Graydon *et. al.*, 1991), *Asaemia axillaris, Pteronia pallens, Lasiospermun bipinnatum* and *Galenia africana* in South Africa (Kellerman *et. al.*, 1988). This liver pathology was more severe in sheep than in goats and this is well indicated by the high bilirubin levels in group 2 (2.4g/kg) and 3 (3.6g/kg) sheep and this finding has not been reported before.

According to Jones and Hunt (1983) the tissue changes in *S. incanum* L poisoning accounting for nervous signs have not been described and histopathological studies have not been made. The current study fills this gap by giving detailed description of histopathological findings in the brain namely; wallerian degeneration of neurons in the white matter, chromatolysis of neurons and necrosis and vacuolation of the Purkinje cell layer. In addition, sheep showed widespread haemorrhage into nervous tissue and depletion of the Purkinje cells. These findings were, however, not seen in rats fed *Solanum incanum* L orally mixed with chicken mash, which only showed congestion and vacuolation in nervous tissue (Thaiyah, 1992).

According to Cheeke and Shull (1985) and Morris and Lee (1984), the nervous signs in glycoalkaloid poisoning are due to inhibition of cholinesterase. The nervous signs in this study may also be associated with the severe pathology seen in the nervous tissue and

especially the cerebellum. Other studies with *Solanum* species have shown cerebellar disorders (Raquel *et al.*, 2006; Verdes *et al.*, 2006; Medeiros, *et al.*, 2004; Porter *et al.*, 2003; Bourke, 1997; Summers *et al.*, 1995; Riet-Correa *et al.*, 1983; Menzies *et al.*, 1979; Pienaar *et al.*, 1976). It is important to note that most of the current knowledge on *Solanum* poisoning has been obtained through description of field cases except in *Solanum bonariense* where fresh leaves were fed to cattle (Verdes *et al.*, 2006). Thus for *Solanum cinereum* (Bourke, in 1997) in goats, signs were only recognized after 12 months of pasturing the animals in the field. The parts of the different *Solanum* plants causing the toxicity were not documented.

This present study thus differs from the rest in that actual unripe fruits were used within a specified period of time under controlled conditions and the results are actually attributable to the unripe fruits of *Solanum incanum* L and constitute new findings. This has not been done before. In addition, the toxin appears to have some effect on blood capillaries in sheep resulting in haemorrhage by diapedesis.

In the kidneys, there were nephritic changes manifested by widespread tubular necrosis with hyaline cast formation and degenerative changes in the glomeruli. In the gastrointestinal tract the changes varied from coagulative necrosis to deep ulceration of the mucosal lining from the abomasum to the colon with hyperplasia of colonic glands. Similar findings have been reported in sheep poisoned with *Solanum carolinense* (Upenn, 2005). In rats, however, there was also development of adenocarcinoma in the small intestines (Thaiyah, 1992), which was not recorded in this experiment.

This study has shown that *Solanum incanum* L is toxic to sheep and goats but the toxicity was more severe in sheep as shown by clinical signs, gross pathology and histopathology. Further studies would include the determination of the factors causing this reduced toxicity in goats. Effect of rumen degradation on the toxins should be evaluated and the active principles in unripe fruits responsible for producing the effects of toxicity should be identified.

CHAPTER 6

6.0 CONCLUSION

1. The LD_{50} was 3.0 and 4.8g/kg in goats and sheep, respectively.
2. There are differences in the clinical manifestation of *S. incanum* L toxicosis in both species of animals. In sheep, the main clinical signs were bloat, vomiting, hypersalivation, staggering gait, depression and lateral recumbency with leg paddling movements and death and these signs are pathognomonic in sheep. However, signs in goats were bloat, diarrhoea and shivering.
3. There are significant differences in haematological and biochemical findings between sheep and goats as exemplified by hypoproteinaemia in group 3 goats (2.25g/kg) and bilirubinaemia in sheep groups 2 (2.4g/kg) and 3 (3.6g/kg). Overall sheep had higher PCV values than goats.
4. Differences in the gross pathology between the two species of animals were noted especially in group 1 (0.75g/kg) and 2 (1.5g/kg) goats that did not have gross pathological signs.
5. There are differences in the histology as noted in the various organs and especially the brain. In the brain of goats in group1, there was only congestion while goats in group 2 had mild necrosis of Purkinje cells in the cerebellum and chromatolysis of neurons in the brain. In all sheep groups, there was severe chromatolysis of neurons and necrosis and loss of Purkinje cells in the cerebellum. Overall, in the diagnosis of this poisoning, histological examination will confirm the diagnosis using the differential lesions described above.

6. Unripe fruits of *S. incanum* L are toxic to both sheep and goats but the toxicity is more severe in sheep. This is shown clearly by the survival pattern where goats lived longer than sheep in all dose groups and by the clinical, pathological and histopathological findings.

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8.0 LIST OF APPENDICES

Appendix 1: Calculation of LD_{50} in goats and sheep (Weil, 1952)

A). Calculation of LD_{50} in goats

The LD_{50} is the median lethal dose that will kill 50% of the population and is calculated by the method of Weil (1952) as follows:

The general formula for this calculation is:

$\text{Log } M = \text{Log } D_a + d.(f + 1)$ for K = 3 (for n=3, the number of animals dosed per level)

Where:

LD_{50} = antilog M

Log D_a is the log of lowest dosage used. In this experiment the lowest dose used was 1.5g/kg and therefore, Log 1.5 = 0.17609.

K is a constant and for 3 dosage levels and above, K=3.

d is the log of the constant ratio between the dosages. In this experiment d=1.5 and log 1.5 = 0.17609.

F value is obtained from the tables already calculated (appendix 2) and for mortalities in the range 0,0,3,2 mortalities, the F value = 0.75.

Then:

$\text{Log } M = 0.17609 + 0.17609(0.75 + 1)$

$= 0.17609 + 0.30815$

$= 0.4842$

LD_{50} = antilog 0.4842

$= 3.049 \approx 3.0$g/kgm

To calculate the 95% confidence limits for the LD_{50} the following formula is used:

95% confidence interval = $\log M \pm 2 \times \sigma_{\log m} = 2d \times \sigma_f$

where, $\sigma_{\log m} \equiv d \times \sigma_f$

$\sigma_f = 0.37500$ (appendix 2)

$2d \times \sigma_f = 2(0.17609) \times 0.375$

$= 0.1321$

The confidence interval for the $LD_{50} = 0.4842 \pm 0.1321$

$= 0.6163$ to 0.3521

The LD_{50} for *S. incanum* L in goats is 3.0g/kg with a 95% confidence level of 2.25 to 4.13 g/kg.

B). Calculation of LD_{50} in sheep

The LD50 for *S. incanum* L in sheep was similarly calculated using the same formulae and procedure:

Log M = Log D_a + d. (f + 1) for K = 3 (for n=3, the number of animals dosed per level)

Where n = 3 (number of animals per dosage group).

K = constant for 3 dosage levels and above =3.

Log D_a = log of lowest dosage used. In this experiment $D_a = 3$ ∴ **Log 3** = 0.47712

d, the log of constant ratio between the dosages was 1.5 and log 1.5 = 0.17609.

The mortality figures obtained were 0,1,3,3 and the the f value = 0.16667 (appendix 2)

Log M = log D_a + d · (f + 1),

Log M = 0.47712 + 0.17609(0.16667 + 1) = 0.68256

The LD_{50} = antilog M = 4.815g/kg body weight

= 4.8g/kg

The 95% confidence interval was also calculated using the same formula, which is:

$\log M \pm 2 \times \sigma_{\log m} = 2d \times \sigma_f$

where,

$2d \times \sigma_f = 2(0.17609) \times 0.57735$

$= 0.20333$

95% confidence limit = 0.68255 ± 0.20333

$=0.88589$ to 0.47922.

The calculated LD_{50} for *S. incanum* L in sheep is 4.8g/kg with a 95% confidence interval of 3.0 – 7.7 g/kg.

Appendix 2. Extract of table from biometrics: Tables for convenient calculation of medium effective dose (LD_{50} or ED_{50}) and instructions on their use. Biometrics, 249-255.

r-values	F	σ f
0,0,2,3	0.83333	0.57735
0,0,3,3	0.50000	0.00000
0,1,1,3	0.83333	0.81650
0,1,2,3	0.50000	0.81650
0,1,3,3	0.16667	0.57735
0,2,2,3	0.16667	0.81650
1,0,2,3	0.75000	0.51539
1,0,3,3	0.25000	0.37500
1,1,1,3	0.75000	0.71807
1,1,2,3	0.25000	0.80039
2,0,2,3	0.50000	1.11803
0,0,3,2	0.75000	0.37500
0,1,2,2	0.75000	0.80039
0,1,3,2	0.25000	0.51539
0,2,2,2	0.25000	0.71807
0,1,3,1	0.5000	1.11803

Appendix 3: Mean weight (kg) over time (weeks) in goats and sheep

	Goats groups				Sheep groups			
Time (weeks)	1	2	3	4	1	2	3	4
1	19.0	16.3	17.0	16.2	19.6	18.3	15.6	17.5
2	19.8	15.3	16.0	16.1	18.8	15.7	13.0	17.4
3	19.7	15.8	17.2	15.4	19.3	14.8	-	17.9
4	19.2	17.4	17.0	15.3	19.1	15.1	-	18.1
5	19.9	16.9	17.2	15.0	20.5	14.8	-	19.5
6	19.9	17.8	17.8	15.9	19.7	14.8	-	18.4
7	20.1	18.7	18.3	15.7	18.2	-	-	18.1
8	20.4	18.7	18.5	16.4	18.3	-	-	18.2
9	20.7	19.0	19.0	16.1	17.4	-	-	16.3
10	21.1	19.2	18.8	16.3	18.2	-	-	17.8

Appendix 4: Mean white blood cells (/μl) against time (weeks) in goats and sheep

	Goat groups				Sheep groups			
Time (weeks)	1	2	3	4	1	2	3	4
1	10,240	13,500	11,400	12,875	10,875	9,500	10,575	8,500
2	10,975	10,675	13,850	12,825	9,200	10,450	12,475	8,433
3	9,133	9,050	20,950	11,775	12,100	13,700	-	9,700
4	8,825	9,875	9,000	9,525	10,050	9,750	-	9,567
5	8,125	6,767	6,100	9,475	8,900	9,600	-	11,067
6	8,825	8,033	12,000	7,975	8,800	10,150	-	8,733
7	8,950	8,167	8,800	12,525	-	-	-	-
8	10,500	11,533	8,600	11,500	-	-	-	-
9	10,000	13,800	10,000	10,600	-	-	-	-

Appendix 5: Mean packed cell volume (%) against time (weeks) in goats and sheep

	Goat groups				Sheep groups			
Time (weeks)	1	2	3	4	1	2	3	4
1	33.2	35.8	37.3	31.3	30.5	32.5	35.0	37.7
2	36.3	36.3	32.5	29.8	36.0	33.3	30.0	30.3
3	32.3	32.5	33.5	27.3	35.0	37.5	-	32.3
4	27.3	29.5	30.0	26.0	30.8	28.5	-	29.3
5	24.5	31.3	29.0	27.8	30.3	33.0	-	31.7
6	27.5	26.3	27.0	21.5	26.7	37.0	-	31.7
7	27.8	26.7	28.0	24.8	-	-	-	-
8	28.3	27.3	29.0	23.0	-	-	-	-
9	27.3	28.7	29.0	25.3	-	-	-	-

Appendix 6: Mean total protein (mg/dl) against time (weeks) in goats and sheep

	Goat groups				Sheep groups			
Time (weeks)	1	2	3	4	1	2	3	4
1	7.6	6.8	5.9	7.3	7.1	6.6	7.2	6.7
2	6.7	6.5	5.9	5.1	5.9	6.0	7.6	5.9
3	7.2	6.3	2.7	6.6	6.9	5.5	-	7.3
4	6.6	6.0	2.7	6.6	6.3	7.0	-	6.6
5	7.6	5.6	3.8	7.5	6.1	6.0	-	6.7
6	8.1	5.5	3.7	7.5	7.1	6.1	-	7.1
7	6.7	4.7	3.0	7.0	-	-	-	-
8	7.1	5.1	3.0	7.4	-	-	-	-
9	7.4	5.4	2.9	7.7	-	-	-	-

Appendix 7: Mean alkaline phosphatase (u/l) against time (weeks) in goats and sheep

	Goat groups				Sheep groups			
Time (weeks)	1	2	3	4	1	2	3	4
1	95.3	71.0	76.5	65.5	37.3	64.3	37.8	75.3
2	97.8	63.5	56.0	78.0	52.5	66.7	34.8	77.7
3	96.8	73.3	57.0	73.0	53.8	80.5	-	86.0
4	110.0	65.5	58.0	56.3	59.5	95.0	-	87.3
5	71.0	38.7	28.0	35.5	48.3	76.5	-	112.0
6	71.8	51.3	30.0	42.3	68.7	109	-	132.7
7	67.5	44.3	36.0	42.5	-	-	-	-
8	75.8	47.0	33.0	66.5	-	-	-	-