TOXICITY TESTING OF SIDA CORDIFOLIA

(FLANNEL WEED) IN GOATS IN ZAMBIA

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the Degree of Master of Veterinary Medicine in Veterinary Plant Toxicology.

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DECLARATION

The contents of this dissertation are the work of Josias Jembe and has not been

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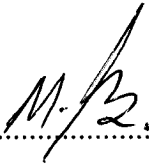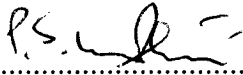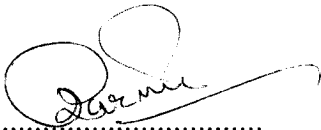Josias Jembe   •   Date

DEDICATION

To my children and wife, Agness Anyelisye, for the patience and tireless support.

APPROVAL

THIS DISSERTATION BY JOSIAS JEMBE IS APPROVED AS FULFILLING THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY MEDICINE BY UNIVERSITY OF ZAMBIA.

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ABSTRACT

Sida cordifolia, family Malvaceae, was suspected to have caused toxicity in small ruminants (sheep and goats) at one of the farms around Lusaka. To verify the suspicion the present study was planned and undertaken. The study included (a) experimental feeding of female goats with *Sida cordifolia* (b) retrospective study of liver and kidney specimens from the affected farm as well as neighboring farms with suspected plant poisoning and (c) a questionnaire survey on the same farms to gather information on farm management, animal husbandry, vegetation and disease outbreaks in case there were other agents responsible for the out break at the affected farm.

Three toxicity tests were conducted using 14 healthy female goats aged between 8 to 18 month and weighing 12 to 18kg body weight. The goats, all from one herd, were let to acclimatize for two weeks in a room with a concrete floor. During this period, the animals were clinically examined and dewormed. The plant used in the toxicity test was picked at flowering stage; and only tender aerial leaves together with flowers and seeds were dried in the shade, milled and stored at -10°C. The first experiment involved five goats. Two of the goats were drenched at a dosage of 5g (dry matter; D.M.)/kg body weight and another two received a dosage of 10g (D.M.)/kg body weight. One goat was used control. The goats were dosed for 5 consecutive days and observed for 10 days. The second toxicity test comprised six goats but only two completed the course having been drenched with 10g (D.M.)/kg of the milled plant material for 10 consecutive days and observed for 30 days. The third and final test involved three goats but only one completed the experiment on a dosage of 5g (D.M.)/kg for 20 consecutive days and observed for 20 days. Rectal temperature, respiratory rate, heart rate, pulse rate, and rumen motility were measured during the experiments. Signs of jaundice and photosensitivity were monitored. Serum metabolite indices known to be sensitive indicators of liver damage, gamma-glutamyltranspeptidase (GGT) and aspartate

aminotransferase (AST) were measured; and to assess kidney, damage, urea and creatinine were measured. At the end of each experiment the goats were sacrificed by exsanguination and postmortem examination done. From three different areas of liver and kidney, tissues were cut, preserved in 10% buffered formalin and processed for light microscopy.

In all the three toxicity tests the parameters measured did not show any statistically significant change (P > 0.05) before and after dosing. Jaundice and photosensitivity were not observed. On gross pathological examination the liver and kidneys had no lesions suggestive of any toxicity. Histological examination of the liver and kidneys did not reveal any lesions.

A retrospective study was carried out on the liver and kidney specimens brought to the pathology laboratory (University of Zambia, School of Veterinary Medicine) from the dead sheep and goats of the affected farm. Histopathologically the liver and kidney lesions showed hepatic necrosis with bile duct proliferation, periportal necrosis, mild hemorrhages and Kupffer cell infiltration. There were no pathological lesions in the kidneys. The liver and kidney specimens collected from neighboring farms were examined histopathologically. The liver showed fibrosis as the prominent lesions but hardly any significant pathological lesions in the kidneys. These lesions were not reproduced in the experimental goats.

Findings from the questionnaire survey on neighboring and the affected farm showed that agents other than *Sida cordifolia* were responsible for lesions described at the affected farm with the most probable suspec causal agent being *Corynebacterium.*

The failure by *Sida cordifolia* to produce any toxicity signs in the experimental goats or to reproduce lesions in the liver as described from the affected farm may lead to the conclusion that *Sida cordifolia* is probably not toxic to goats under normal grazing conditions in Zambia.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

3. CHAPTER THREE: MATERIAL AND METHODS

4. CHAPTER FOUR: RESULTS   48

5. CHAPTER FIVE: DISCUSSION   76

6. CHAPTER SIX: REFERENCES   93

7. CHAPTER SEVEN: ANNEX

LIST OF TABLES

LIST OF PLATES

Title   **Page**

LIST OF FIGURES

MAPS

CHAPTER ONE

1. INTRODUCTION

On every continent the area of rangeland or grassland and pastures far exceeds that planted to crops. Livestock convert the forage on these rangeland into protein, energy and numerous raw materials for a variety of industries, all of which play an important role in local, national and international economies. Poisonous plants and fungi grow in most plant communities and put grazing livestock at some degree of risk. The high cost of livestock poisoning by plants and fungi has caused it to be ranked as one of the principal causes of economic loss to the livestock industry internationally (James, 1994).

Africa is well known for the diversity and beauty of the flora and the continent has a possibly unequal variety of poisonous plants and fungi. The importance of poisonous plants and fungi to the livestock industry in Africa cannot be overestimated (Kellerman *et al.*, 1988). About 600 indigenous poisonous plants are known to occur in Southern Africa alone (Kellerman *et al.*, 1988). In this part of the world, where livestock are traditionally kept under extensive conditions on pastures that are frequently denuded by droughts, overstocking and uncontrolled fires, the animals are often forced to eat poisonous plants which they normally would avoid (Naude *et al.*, 1996). Many of the poisonous plants of Africa are unknown in other parts of the world and very little of the knowledge of poisonous plants gained elsewhere would be applicable in Africa. Too often plant poisoning cases are a problem to some experienced veterinarians from abroad who find themselves in diagnostic or research

laboratories in Africa. In countries with limited resources like Zambia, there is hardly any study performed on possible plant poisoning in animals. The situation is compounded by the fact that all Government -owned diagnostic centers including the University of Zambia, lack the necessary equipment and expertise to adequately handle any cases of plant poisoning. As such, many cases of suspected plant poisonings go without being confirmed and recorded by field veterinarians. Thus the local knowledge on poisonous plants is limited and the impact of poisonous plants cannot be defined nor could a comprehensive diagnosis be rendered. It is with these views in mind that an attempt has to be made to study plant poisoning in Zambia.

Sida cordifolia L is one of the Zambian plants that is suspected to be poisonous to livestock in Zambia. On a 5000 hectare farm, 50 km East of Lusaka the plant was found in monoculture in a large valley in which Kikuyu grass pasture had been heavily overgrazed by small ruminants. The small ruminants group turned to browsing the small leaves of *S. cordifolia* extensively which could be seen during observation by the herdsmen and by the bite marks. The plant was suspected to have caused liver damage characterized by bile duct proliferation and fibrosis in 4 sheep and a goat that had died on the farm. This study was thus initiated to prove if the suspected plant was the cause of small ruminants deaths.

CHAPTER TWO

2. 0. LITERATURE REVIEW

2.1. POISONOUS PLANTS AND MYCOTOXINS IN GENERAL

2.1.1 *Toxins*

In toxicology, poisonous plants are classified under natural occurring toxicological hazards (Garner, 1961). About 1,500 poisonous plant species have been listed world-wide (Radostis, 1994), but few cause problems because animals will hardly eat them due to either repulsive smells, irritability (due to presence of spines, thorns, leaf hairs) or just plain unpalatability as a result of high lignin or silica in the plant tissues or due to bitter taste because of the presence of toxins like alkaloids, glucosinolates and saponins (Garner, 1961: Cheeke, 1995: Cheeke, 1998). All these are characteristics of the plant's defensive mechanism. However, the most important of all is the chemical defense by means of plant toxins. These toxins can be those produced by the plant itself (intrinsic toxins) e.g. cyanide from Sorghum or those produced by microbes that live wholly in or on the plant tissues (extrinsic toxins) usually fungi. The extrinsic toxins produced by fungi are known as mycotoxins. At present the most important mycotoxins to the livestock and poultry industries are aflatoxins produced by *Aspergillus flavus* less so by *A. parasiticus*, ergot by *Claviceps purpurea*, ochratoxin by *Aspergillus* and *Penicillium* spp, zearalenone produced by seven species *Fusarium*. Another important mycotoxin is trichothecenes from various species of *Fusarium, Trichoderma* and *Stachybotrys* (Pier *et al.*, 1977 : Rodricks *et al.*, 1977).

2.1.2 *Classification of Plant Toxins*

Plant toxins have probably been classified for easy identification. Due to their variation in chemical structure, range, site of action and occurrence in feed, most researchers have grouped these toxins as such. For instance Garner (1961) and Cheeke (1998) arranged some toxins according to chemical structure as listed below.

a) Glycoside: a substance containing a sugar, frequently glucose. Examples are cyanogenic glycosides from Sorghum (Sudan grass) or saponin as in *Tribulus terrestris* (Devils thorn).

b) Alkaloid: a compound containing nitrogen, usually in heterocyclic ring, bitter and very toxic. A few examples are strychnine from *Strychnos henningsii,* pyrrolizidine alkaloid from *Senecio* spp and tropane alkaloid in *Datura* species.

c) Protein: examples include ricin from *Ricinus communis* (castor bean), an enzyme, thiaminase from bracken fern and trypsin inhibitor found in Soyabean.

d) Amino acid e.g. mimosine found in tropical leguminous plant *Leucaena leucocephala.*

e) Metal-binding substances like oxalate which is highly concentrated in plants like *Halogeton glomeratus* (halogeton), *Sarcobatus vermiculutus* (greasewood), *Anagallis arvensis* and in grasses like *Panicum* and fungi such as *Aspergillus niger* and *A. flavus* (Jubb *et al.*, 1993).

f) Carbohydrates: e.g. xylose which causes eye cataracts in pigs and poultry.

g) Lipid: e.g. erucic acid in rape seed and sterculic and malvalic acids from cottonseed which cause pink albumins in stored eggs.

h) Glycoproteins: A good example are lectins (Hemagglutinins) found in soybeans and other common beans. The lectins can cause damage to intestinal mucosa and interfere with nutrient absorption (Cheeke, 1998).

i) Glycolipids: e.g. crynetoxin from ryegrass which can affect the CNS causing inco-ordination.

j) Phenolic compounds (e.g. gossypol in cotton and tannin in oak tree).

k) Terpenoids such as resin found in pine which can cause abortion in cattle (Cheeke, 1998).

Classification based on site of action depends on which part of the body or organ the toxin will have its effect. As Cheeke (1995) put it, every animal organ, tissue and system is a target for a particular plant toxin. For example, the target of pyrrolizidine alkaloids from *Crotalaria* (Sunn hemp) or *Senecio* is the liver and therefore these pyrrolizidine alkaloids are classified as hepatotoxins. Oxalates affects the renal tubules and can be called nephrotoxins while tropane alkaloid in *Datura stramonium* (Thorn apple) acts on the CNS (Kellerman *et al.*, 1988) and is known as a neurotoxin. The only problem with this type of classification is that some plant toxins may affect more than one organ making their allocation to a single group difficult (Humphreys, 1988). An example is tannin which can cause liver necrosis, nephrosis and hemorrhages on epicardia, serosal hemorrhages (Silanikove *et al.*, 1996).

Toxins have also been classified according to their presence in feed. For example trypsin inhibitors are usually found in protein supplement (soybeans, fava and field beans), cyanogenic and solanum alkaloids are common toxins found in tubers (cassava and potatoes respectively) while oxalates and saponins are common in tropical grasses. Mycotoxins and phytates are found in all grains (Cheeke, 1998). This type of classification can facilitate identification of a toxin from the suspect feed.

2.1.3. Mode of action

Briefly, the mode of action of a toxin often includes binding to macromolecules, inhibition of energy metabolism, structural antagonism, binding to cell receptors, enzymatic activity, disruption of cell membranes and oxidant and free radical damage (Cheeke, 1998).

2. 1. 4. Occurrence of poisonous plants

Poisonous plants usually exist in the natural vegetation that has not been disturbed by cultivation (Radeleff, 1970). Some are indigenous to a particular field and tend to increase with heavy grazing and others are invaders after the land has been over grazed (The Veterinary Merck Manual, 1991). Usually poisonous plants have a certain niche in most plant communities (The Veterinary Merck Manual, 1991). Hence plant poisoning will be common where animals are on pasture all year round especially under extensive grazing systems. Animals can also eat poisonous plants by accident (contaminated hay or water) and this type of exposure is usually acute rather than chronic (Timbrell, 1995). Animals that have been introduced to a new area may eat poisonous plants because of unfamiliarity with the local toxic plants (The Veterinary Merck Manual, 1991 ; Radostits *et al*, 1994). For those practicing intensive animal husbandry, plant poisoning may occur when animals are fed plant waste from the food industries (Radostits *et al.*, 1994) or when fed hay that has been contaminated with mycotoxins like *Stachybostris atra* (Radeleff, 1970) or *Lucerne* fodder contaminated fodder with *Argemon*.

However, not all poisonous plants will under normal conditions kill or harm animals. For example lupine will only kill if too much of it is eaten in a short time (The Veterinary Merck Manual, 1991). Infarct, some poisonous plants are so palatable that they attract animals to eat them e.g. the British plant *Taxus boccata* (Yew) is very attractive to horses (Garner, 1961) just as most of the oxalate containing plants are to other animals (Clarke and Clarke, 1975). Other plants will only cause toxicity if damaged e.g. Kikuyu grass (*Pennisetum clandetinum*) after being damaged by worms (Bryson, 1982); some become harmful if wilted (*Lolium spp*) or frosted and dried like *Halogeton*. Some plants are toxic when they are young (*Cyanodon* spp.) or when infested with nematode larvae (*Anguina agrostis*) as in Chewing fescue (*Festuca rubra var commutata)* (Clarke and Clarke, 1975). Generally the majority of poisonous plants have toxin amounts well below the toxic limit and can thus be grazed for a long time with little or no effect (Radeleff, 1970) until favorable factors and conditions appear (Radostits *et al.*, 1994).

2.1.5. Factors affecting plant poisoning

Knowledge of factors that can influence plant toxicity is very important to any investigator in plant poisoning. According to Radostis (Radostits *et al.*, 1994), the major risk factors that usually have a bearing on plant poisoning investigation include animal factors (species, health status, sex, size and nutriton status), environmental factors (weather and soils) and plant factors (growth stage).

Animal Factors

Animal factors are actually biological factors such as species, animal health status, age, sex, size and nutritional condition.

Species difference: There is often species difference in the way animals respond to a plant toxin. Some animal species will eat a toxic plant with impunity while others would die if they tried the same plant. The difference has been attributed to so many factors of which the common ones are differences in tissue enzymes activities and the gastrointestinal arrangement. Inside the body, toxins are detoxified by various chemical pathways in which enzymes play an important role. Mixed function oxidases (MFO), esterases, reductases and transferases are enzymes involved in the detoxification processes. MFO are comprised of cytochrome P_{450}, NADPH, NADPH-cytochrome P_{450} reductase and phosphatidylcholine (Carlton and McGavin, 1995 : Cheeke, 1998). These are responsible for oxidative reactions changing lipid soluble toxicants to polar, water-soluble metabolites which can be expelled from the body through urine. Some animals are quick in carrying out such reactions while others are slow. For example, aflatoxins are more poisonous to ducklings and trout than sheep because in the former their microsomal enzymes (MFO) rapidly convert aflatoxin B_1 to a toxic agent, aflatoxin 2,3-epoxide, whereas in sheep the reaction is slow hence the resistance (Cheeke, 1998). Difference in the levels of enzyme activities can also make a difference in the toxicity of a plant. For example sheep has higher levels of the enzyme epoxide hydrolase which renders the pyrrolizidine alkaloid jacobine in *Senecio jacobaea* harmless and thus the sheep is able to graze the plant without problem but this is not the case for cattle which have less levels of the enzyme. Species difference in salivary tannin-binding proteins separates those animals able to consume larger amounts of tannin-rich browse e.g. goats from those lacking such enzymes like cattle and sheep (Silanikove *et al.*, 1996 : Cheeke 1998).

The arrangement of the gastrointestinal tract of animals can make a difference in toxicity. Ruminants are more resistant to some plant toxins than non-ruminants due to the presence of

abundant microbes in their rumen which are able to degrade or detoxify some of the toxins. For instance oxalate containing plants are more toxic to monogastric animals than to ruminants because the latter's ruminal metabolism is able to combine oxalate with calcium and form insoluble salts that cannot be absorbed but excreted in faeces (Radeleff, 1970; Kellerman *et al.*, 1988). The mycotoxin ochratoxin A is degraded in the rumen and thus field outbreaks of toxicosis are limited only to monogastrics such as pigs, chickens and turkeys (Kellerman *et al.*, 1988). Equally, ruminants are able to detoxify trypsin inhibitors (found in raw soyabean), lectins (in field beans), gossypol (cotton seed cake), glucosinolates (in rape seed), some phytoestrogens (genestein and biochnin) and some pyrrolizidine alkaloids thus enabling ruminants to feed on such feedstuff with deleterious factors (Cheeke, 1998). On the other hand the rumen may increase toxicity of a toxicant. For example the rumen may increase toxicity of nitrates, cyanogens, mimosine and phytoestrogens (Cheeke, 1998). The above factors may also explain why gousiekte poisoning occurs only to ruminants, *Matricaria* intoxication is only in cattle and *Microcystis aeuroginosa* and sporidesmin cause hepatogenous photosensitivity only in ruminants (Anonymous, 1996).

Animal health status: a sick animal or that in poor condition is normally more susceptible to poisoning than a healthy one. For instance an animal with hepatic or renal disease is more susceptible to poisoning just as an animal with blood parasites or that which is stressed (Humphreys, 1988). Animals trekked or moved long distances without sufficient feed may greedily eat anything, including poisonous plants when placed in the range. Thirsty animals may also eat unpalatable plants after gaining access to water (Redeleff, 1970).

Age: very young and the very old animals often succumb quickly to poisoning simply because the young have low capacity to detoxify toxins due to their low MFO activities

(Cheeke, 1998). The old are weak and have lower metabolic and excretory capacities hence lowered resistance to poisoning (Garner, 1961).

Sex: several findings suggest little difference between males and females in susceptibility to the harmful effects of plant toxins (Humphreys, 1988). However, Timbrell (1995) said there was some differences and is mostly due to metabolic and hormonal differences between the sexes. MFO activities are higher in males than in females (Carlton and McGavin, 1995). Garner (1961) reported marked difference in the susceptibility to organophosphorus insecticide (Parathion) poisoning which was more in females than males. Phytoestrogens affect mostly females because the female reproductive tract has more estrogen receptors and this may explain why photoestrogens have no effect on the fertility of rams (Kellerman *et al.*, 1988).

Size: The amount of material ingested per unit body weight determines whether or not intoxication occurs (Humphreys, 1988). The digestive tract with its content and body fat contribute to differences in response to a toxin even if the animals were of the same weight.

Nutritional status of the animal: Animals that are undernourished have lowered resistance to so many adverse conditions including poisoning. Cheeke (1998) suggested that low dietary protein, deficiency of vitamin and minerals may reduce MFO activities thus increasing chances of toxicity.

Environmental factors

Season: certain plants become toxic during a certain period of a year e.g. in South Africa the toxicity of *Lolium* spp (annual ryegrass) occurs only in winter (Kellerman *et al.*, 1988). *Albizia versicolor* (Musansengoma in Bemba) poisoning is common in dry period when large numbers of dry pods are on the ground (Soldan *et al.*, 1996) and in the United States of America, USA, photosensitivity in sheep caused by *Panicum coloratum* L (Kleingrass), occurs during the hot humid months of July to October (Muchiri *et al.*, 1975). Some plants can be toxic through out the year. For example *Homeria pallida* is toxic both in fresh and dry state (Kellerman *et al.*, 1988).

Weather: cloudy condition promote toxicity in plants containing cyanogenic glycosides (Seawright, 1982) while drought conditions cause plants to wilt a factor that promotes toxicity in some plants like *Tribulus terrestis* and *Sorghum* spp (Kellerman *et al.*, 1988). *Microcystis aeruginosa* (blue-green alga) toxicity depends on calm weather, high temperature, high pH and low hydraulic flows (Codd *et al.*, 1989 as cited by Van Halderen *et al.*, 1995). In New Zealand facial eczema (pithomycotoxicosis) occurs when the weather is humid with grass minimum temperature of 12°C (Flåøyen and Frøslie, 1997).

Soil: Soil fertility can influence the concentration of poison in plants. For example too much nitrogen in the soil (resulting from application of nitrogenous fertilizer) increases the cyanide content of *Sorghum* spp and nitrite content of *Amaranthus* spp. Soils high in selenium are equally dangerous to plants (Seawright, 1982). Too much molybdenum and sulfates in the soil can result in the accumulation of the two minerals in the plants; and such plants if grazed

may reduce dietary copper absorption in the digestive tract and thus induce copper deficiency. The deficiency occurs as a result of sulfur reacting with molybdate to form thiomolybdate which in turn combines with copper to form an insoluble copper thiomolybdate (Cu MoS_4) thereby lowering the absorption of dietary copper (McDonald *et al.*, 1987). On the other hand absence of certain minerals in the soil e.g. calcium may cause certain plants (halogeton and greasewood) to become toxic (Radeleff, 1970). It has also been reported that low molybdenum in soil does promote chronic copper poisoning especially in sheep (McDonald *et al.*, 1987).

Plant factor

Growth stage of a plant may be very important. Some plants are more toxic when they are just shooting up. Such plants include *Pteridium aquilinum* and *Melica decumbens*. Other plants are more toxic when flowering for example *Crotalaria* and *Senecio* spp; and some when they are in a mature form for example *Datura,* and *Sesbania* seeds and *Albizia* pods (Radostits *et al.*, 1994). Acceptability and palatability of the plant also matter; e.g. only young *Senecio* is acceptable to sheep. However, certain plants vary in toxicity that they can sometimes be grazed or fed to animals with impunity e.g. *Sarcostemma viminale* and as such its difficult to ascertain factors that govern their toxicity (Kellerman *et al.*, 1988).

2.1.6. Economic importance

The economic importance of poisonous plants and mycotoxicoses to the livestock industry is difficult to estimate (Senti, 1977). Most authorities try to gauge losses by estimating the cost of mortalities and the cost in maintaining the grazing land. For example, in Southern Africa where more than 600 indigenous poisonous plants exist, annual stock losses, in certain years

are thought to be 10-25% attributed to plant poisoning (Naude, et, al., 1996). In South Africa alone, the annual cost of the plant poisoning and mycotoxicoses to the livestock industry is conservatively estimated at R 104,506,077 (approximately 300 million US dollars) (Kellerman and Naude, 1996). Such cost could be the same in most sub-Sahara African countries. In Western USA, James *et al.*, (1992 as cited by Kellerman *et al.*, 1996) estimated an annual loss of 300 million dollars as a result of poisonous plants. In New Zealand, Mortimer and Ronaldson (1983 as cited by Flåøyen and Frøslie, 1997) mention losses amounting to 100 million USA dollars as a result of facial eczema (photosensitivity). Probably this is the same in Australia which is regarded as the land qf poisonous plants (Cheeke, 1998).

Other losses that are more difficult to measure include loss of weight gain and milk yield, reproductive failure such as abortion, decreased libido, protracted gestation, still birth, birth defects and interference with estrus (Nielsen and James 1972 as cited by Kellerman *et al* 1996). In addition, mycotoxins tend to suppress immunity of animals thereby increasing chances of infection (Pier *et al.*, 1977). Indirect losses such as fencing, supplement feeding, veterinary expenses, temporary or permanent non-utilization of toxic pasture and the diminished value of the infested land also make their toll on the livestock industry (Kellerman *et al.*, 1996).

2.1.7. Diagnosis of plant poisoning

Many researchers have described on how to diagnosis plant poisonings. In this text a brief overview is given. As stated by Seawright (1982), diagnosis of plant poisoning is not a straight forward exercise because it is not easy to determine whether a particular plant is poisonous or whether a sick animal is suffering from plant intoxication. One has also

differentiate plant poisoning from other toxicants such as herbicides, fertilizers, disinfectants, drugs, defoliants, desiccants and other chemicals. As if this is not enough a task for differential diagnosis, one must also distinguish plant poisoning from other causes of disease such as viruses, bacteria, fungi, nutritional deficiencies, endoparasites and ectoparasites.

As a general guideline, diagnosis of plant poisoining is arrived after linking up history of the affected animal, clinical signs, necropsy findings histopathology and chemical investigations on the tissues and organs and dosing trials (in certain cases) (Seawright, 1982; The Veterinary Merck manual, 1991). Alternatively a toxin may be identified first (by spectrometry) followed by listing of plants containing the toxin and then examining the environment for the presence of the offending plant on the list (Raddostis *et al.*, 1994). Whichever method is used, it is important to identify the suspected poisonous plant to which the animals were exposed. If there is no specific plant being suspected, then one has to follow the animals as they graze and collect parts of the plant the animals are seen to have grazed and try to identify which one is poisonous. The plant specimen for identification must have roots, stems, leaves, flowers and fruits. For preservation, the plant should be dried or taken to the botanist as quickly as possible.

2.2 PLANT POISONING OF LIVESTOCK IN ZAMBIA

2.2.1 Background information

2.2.1.1 Geography of Zambia

Zambia is a landlocked south-central African country lying between latitudes 8°S and 18°S and longitudes 22°E and 34°E. It borders Angola to the west; Botswana and Namibia to the south; Congo Democratic Republic to the north; Malawi to the east; Mozambique to the south-east and Tanzania to the north-east. It covers a total surface area of 750,000 square kilometers which is mostly high plateau (rising to the highest point of 1,841 meters above sea level) consisting of bush and savannah out of which 75% is grazing area (Hussain, 1986).
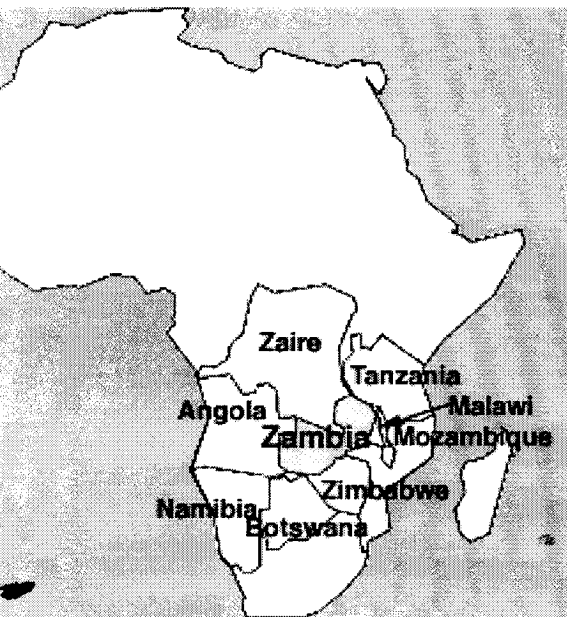
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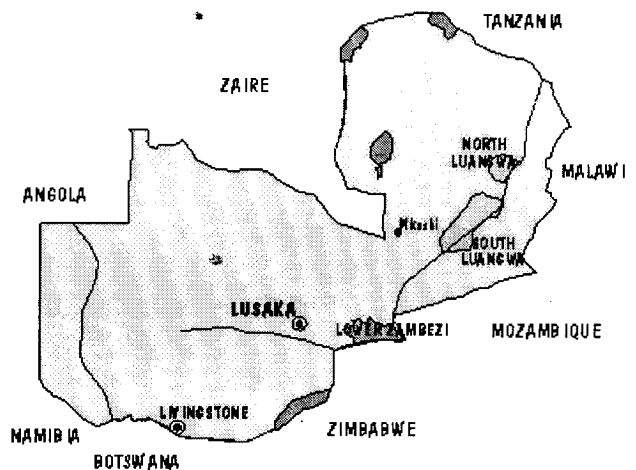
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A. Map of Africa showing geographical location of Zambia.

B. Map of Zambia.

The country has abundant fresh water bodies in form of rivers and lakes. Major rivers include Kafue, Luangwa, Luapula and Zambezi all of which are fed by numerous perrenial rivers and streams. Lakes are Bangweulu, Itezhi tezhi (man-made), Kariba (man-made and shared with Zimbabwe), Mweru (shared with Democratic Republic of Congo) and Tanganyika (shared with Tanzania). Currently, Zambia's population stands at approximately 9 million inhabitants (according to 1990 census projections). Maize (*Zea mays*) is the major cash crop and staple food.

2.2.1.2 Climate

Zambia lies in the subtropical zone; and has three seasons. These are hot wet season (November to April) cold dry season (May to August) and hot and dry season (September to October). The country has a maximum temperature of 32° Celsius in October and minimum temperature of 9° Celsius in June. The mean annual rainfall is 1300 mm in the north and 800 mm in the south. Relative humidity is 79% during the month of November to March and 55% during the cold season (April to August) and 45% during dry hot season (September to October). The hottest areas are in Western Province and coldest are in Northern Province (Mbala and Mporokoso). The prevailing winds are easterly. Even though Zambia lies within the subtropics the climate is tempered by altitude and can be compared favorably with climates of more temperate regions (Fanshawe, 1969).

2.2.1.3. Vegetation and forest

Studies on Zambian vegetation and forests have been done by many scholars of which a few examples are Fanshawe (1969), Storrs (1982), Evison and Kathuria (1982), Carr (1984) and the latest is that of Chipungu and Kunda, (1994). What is described here is a summary of

their findings. Most parts of Zambia consist of a plateau, 1070 to 1220m elevation (Evison and Kathuria, 1982) with the capital city (Lusaka) having an altitude of 1300-1500m. The highest parts are in the north east with the plateau gradually sloping to the south east. Generally Zambian vegetation ranges from woodland formations, bushes, thickets, forests and grasslands (Kulich, 1996). The greater portion of the country (71%) is covered by woodlands of which the most extensive is miombo woodland. The miombo woodland is itself dominated by various species of *Brachystegia, Isoberlinia* and *Julbernardia* (Fanshawe, 1969). Literally miombo woodlands consist of a two storeyed woodland with an open or slightly closed canopy of semi-evergreen trees. It is basically a low tree-high grass association with majority of trees being leguminous *(Acacia)* and this type of vegetation forms the greater part of maize and cattle country (Storrs, 1982). The second most extensive woodland is the Kalahari (Western, North-Western and Southern Provinces) followed by Mopane and Munga (Chipungu and Kunda, 1994).

Natural forests are mainly restricted to northern high rainfall areas with average rainfall of 1000 mm; and in the these forests, the important and dominant tree species is *Pterocarpus angolensis* (Mukwa). Industrial plantations (*Pine* and *Eucalyptus spp*) are mainly in the Copperbelt Province with 52,000 hectares and the rest of the country having 7,000 hectares (Anonymous, 1985). Of late there has been some indiscriminate deforestation in areas surrounding towns. Up to 1985, the major cause of deforestation had been demand for wood to supply the growing urban population; but now, according to Chipungu (Chipungu and Kunda 1994), 80% of deforestation is due to large scale agriculture. Other causes of deforestation are shifting cultivation (Chitemene) by peasant farmers, settlements, overgrazing, burning and over cutting (Anonymous, 1985).

The dominant grass, especially in the undisturbed and unshaded areas, is *Hyparrhemia* spp (Kulich, 1996). Other natural grasses belong to the genera *Digitaria, Setaria, Panicum, Urochloa, Andropogon, Sporobolus,* and *Eragrostis* (Kulich, 1996). In the dambos (swampy areas) and flood plains common grasses are of genera *Panicum, Acroceras, Vossia, Echinochloa, Leersia* and *Loudetia.* Where soil fertility is good, Elephant grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*) and *Brachiaria* spp are found. The Zambian vegetation is of a fire climax type. Burning of grass starts as soon as it will burn beginning as early as April until September when virtually all dry areas, including harvested fields are affected. Kulich (1996) approximated annual burnt land as 80% of the country. Only moist riverine forest, dambos and overgrazed areas survive burning. Authorities have tried to encourage early burning (April) so that by dry season palatable forage is regenerated. Unfortunately the advice is rarely heeded as more late burning (September) has occurred which has in turn encouraged excessive bush encroachment which is one of the major problems of cattle management in Zambia (Kulich, 1996).

2.2.1.4. Livestock situation in Zambia

Livestock production accounts for 35% of total agricultural output (Colby 1995). There are approx. 2.6 million cattle, 684,000 goats, 75,000 sheep and 313,000 pigs (Mangani and Imakando, personal communication, 1996). According to Anonymous (1994), 82% of cattle, 96% of goats and 65% of sheep are kept by traditional sector and almost all of these animals are of local breeds with minimum management inputs. A steady decline (12%) in cattle was recorded from 1990 to 1993 and was attributed to various tick borne diseases mentioned below. On the other hand there was an increase in the population of small ruminants (Witola, 1998).

2.2.1.4.1. Livestock management

Traditional

The majority of farm animals (ruminants) in Zambia are under traditional management system with hardly any supplementary feeding or veterinary care. Breeding is uncontrolled and grazing is communal. According to Colby (1995) cattle the calving rate is less than 50%, adult mortality is 10% and off-take rates 5-6%. Husbandry (depending on tribes) is practiced in a limited way. For instance, people of Southern, Central, Western and parts of Eastern Provinces rely on oxen to plough their fields. In these areas only working animals are given special care (they are routinely sprayed or dipped, dewormed, vaccinated, given mineral supplements; and in localities where trypanosomosis is a threat, trypanocides are administered (Personal experience). Only if there is a real threat of tick borne disease do farmers dip all the cattle. Castration (to make more oxen and not to control inbreeding) is done on 1-2 years old male calves. Some tribes (Tonga and Lozi) have a tendency of allowing cattle to wander around for months in the plains without anyone herding them. This occurs in the dry season when there is shortage of pasture in the higher lands. But now this trend is being discouraged because of cattle rustling. Generally cattle are herded during the day and housed in kraals (wooden fences) during the night. Lack of discrete unit of grazing land has led to rangeland degradation. Due to over grazing there has been soil erosion, deforestation and bush encroachment especially in the provinces where cattle are concentrated (Southern 42%, Western 19%, Central 18% and Eastern 18% (Chipungu and Kunda, 1994). As for small ruminants, management depends on the season, availability of land and manpower. Three simple management systems are practiced by traditional farmers. These are tethering, semi-extensive and extensive management systems. In tethering, animals are tied to trees with long ropes to allow movement for grazing and browsing. This is commonly practiced by

households with limited manpower and during the cultivating season (November to April) so as to avoid the animals straying in the crop field. In semi-extensive, the most favored management system, animals are shepherded during the day and housed during the night. The houses are made of either wooden fences or mud. In extensive management, animals are left to roam around without housing them; and they spend nights under trees. This management system is commonly practiced after harvest. Though almost all traditional animals rely on natural vegetation and harvest remains (maize stovers, sorghum and millet) or browsable shrubs and trees including a range of pods, fruits, weeds of fallow lands and fallen leaves (Manson and Maule, 1960) efforts are being made to produce pasture specifically for traditional farmers. The pasture will consist of Siratro, Lablab, Stylo, Leucaena, Sunn-hemp, Elephant grass, Common Buffalo grass, Gamba grass and green panic (Kulich, 1996). Though the indigenous animals are able to withstand severe nutritional deficiencies of dry season (albeit at the expense of production and weight gain) they do succumb to severe drought conditions more especially the young.

Commercial

Commercial farmers practice intensive system of management where animals are housed at night and released to paddocks by day time. Supplementation and veterinary care are observed. With superior management, calving rates range between 65%-70%, there is also a low calf mortality rate (1-2%) and off take rate of 17-18% annually (Colby, 1995). According to Kulich (1996) commercial farmers are advised to cultivate Lucerne, Siratro, Stylo, Glycine, Sunn-hemp, Leucaena, Rhodes grass, Common Buffalo grass, Green panic and Star grass.

2.2.2. Common poisonous plants of livestock in Zambia

There is hardly any published case of plant poisoning in Zambia. There is however, knowledge of the existence of poisonous plants including a few mycotoxins and agricultural pollutants that have affected livestock. This author has seen suspected cases of *Albizia versicola*, *Lantana camara* and *Microcystis* (algae) poisoning in ruminants during field practice but could not confirm. Equally traditional farmers would report mysterious paralyses and deaths during dry season (personal experience) and again no confirmatory diagnosis could be done. In his book "More about Trees", Storrs (1982) mentioned the existence of more than 100 known toxic plants in Zambia (most of which were toxic to humans and fish). The Department of Forestry (Ndola) also has a check list of poisonous plants of Zambia (including fungi) by D.B Fanshawe and C.D. Hough. Ferreira (Ferreira1977) mentions herbs (*Ranunculus* and *Guidia* spp.) found in the dambos to be poisonous. Tim Ayliffe (Ayliffe, 1994, Personal Communication) has prepared a check list of well known poisonous plants, exclusively for livestock, which are frequently encountered in Zambia and confirms the suggestions by Kellerman (Kellerman *et al.*, 1988) that Southern Africa poisonous plants are spread in all the states belonging to Southern African Development Community (SADC). Thus, it will be repetitive to describe each plant as this has already been done by Kellerman (Kellerman *et al.*, 1988). The list (Table 1) shows well known plants of Zambia that are poisonous to livestock. The list also shows distribution of these poisonous plants in Zambia, toxins involved and the animal species affected.

Table 1. Common poisonous plants of livestock in Zambia

Family	Genus/specie	Toxin	Livestock affected	Distribution
Amaranthaceae	*Amaranthus retroflexus*	Oxalates/nitrates	Pigs, cattle, sheep	Wide spread
Apocynaceae	*Nerium oleander*	Oleandroside/nerioside	Cattle, sheep, horses	Wide spread
Cactaceae	*Opuntia spp*	Oxalates	?	?
Combretaceae	*Combretum spp*	Saponin	pigs	Wide spread
Combretaceae	*Terminalla spp*	Tannin	Cattle	Wide spread
Compositae	*Xanthium strumanium*	Carboxyatractyloside	pigs	Lusaka/CB
Compositae	*Senecio latifolius*	Retrosine	cattle, horses	Southern province
Compositae	*Giegeria africana*	Sesquiterpenoidlactones	cattle sheep	Southern province
Crassulaceae	*Cotyledon spp*	Bufadienolides	livestock	Wide spread
Dennstaedtiaceae	*Pteridium acquilinum*	Thiaminase	Cattle	N/Western
Dichapetalaceae	*Dichapetalum*	Munofluoratic acid	Ruminants	S/P Kalomo.
Euphorbiacea	*Rinus communis*	Ricin	All species	Wide spread
Euphorbiacea	*Phyllanthus engleri*	Glycoside	Cattle, Goats	Low rainfall areas
Fabaceae	*Acacia nilotica*	Saponina/tanins	Cattle, goats	Widespread
Leguminosae	Cassia spp	Anthraquinone	Cattle	Wide spread
Leguminosae	*Crotalaria junceae*	Monocrotaline	Cattle, sheep, horse	CB
Leguminosae	*Albizia versicolor*	Pyridoxine	Cattle	Wide spread
Liliaceae	*Urginea spp*	Bufadienolide	Cattle	S/P Mazabuka
Loganiaceae	*Strychnos henningsii*	alkaloid strychnine	Cattle, dog	High rainfall areas
Meliaceae	*Melia azedarach*	Tetranortriterpenes	Pigs, sheep, poultry	Widespread
Papaveraceae	*Argemone mexicana*	Berberine/protopine	Cattle	?
Phytolaccaceae	*Phytolacca dodecandra*	Oxalate/Saponins	Cattle Sheep	?
Poaceae	*Sorghum spp*	Cyanogenic glycoside	Cattle	Wide spread
Rubiaceae	*Pachystgma pygmaeum*	Cardiac glycoside	Ruminants	Copperbelt
Rubiaceae	*Pavetta schumanniana*	Cardiac glycoside	Ruminants	Wide spread
Ranunculaceae	*Ranunculus spp*	Ranunculin	Livestock	Wide spread
Solanaceae	*Datura stramonium*	Tropane alkaloid	Cattle, Horses	Wide spread
Solanaceae	*Cestrum aurantiacum*	Carboxyatractyloside	Cattle Goats sheep	Wide spread
Verbenaceae	*Lantana camara*	Triterpenoids.	Cattle	Wide spread
Zygophylaceae	*Tribulus terrestris*	Saponin	Sheep	Low rainfall areas

2.2.3 Common mycotoxins of livestock in Zambia

Fusarium graminearum, *Diplodia maydis* and *Aspergillus flavus* are fungi that are known to cause problem to livestock in Zambia. Zambia's staple food is predominantly maize on which the first two fungi harbor causing cob rot. *F. graminearum* cob rot appears as a distinct pink rot which starts at the tip and eventually affecting the whole cob. A survey conducted by Bupe (1988) found maize grown by Lusaka farmers to be contaminated with zearalenone and deoxynivalenol (*F. graminearum* mycotoxins) in the range 0.08 - 0.60 mg/kg and 0.5 - 16 mg/kg respectively. In Zambia, such mouldy maize is usually fed to animals as feed supplement thereby increasing chances of the mycotoxins (especially zearalenone and zearalenol) getting into cattle milk thus creating a potential health hazard to humans. It is also very likely tat the hyperestrogenism-associated fertility problems commonly seen in pigs in Zambia are caused by *F. graminearum* from mouldy maize. This fungus can also be found on hay and sorghum (Senti, 1977). Though the fungi cause problems mainly in swine, cattle, chickens and turkeys may also be affected (Senti, 1977).

Diplodia maydis, the toxin of which has not yet been identified, produces whitish mycelia between grains on the maize cob. The brown rot starts at the base of the cob and progresses towards the tip. The fungi affects mostly cattle causing nervous signs such as ataxia, paresis and paralysis (Kellerman *et al.*, 1988). Occasionally sheep is affected causing birth of non viable lambs or stillbirth (Anonymous, 1996). According to available literature, there has been no documented positive case of diplodiosis in Zambia, but suspected cases have been reported after maize harvest.

Aspergillus flavus produces a mycotoxin, aflatoxin, which causes liver disease in animals and man. Aflatoxin has been well studied in Zambia albeit in relation to its effect on human health (Samuel, 1988 : Bupe, 1988). As regards livestock, aflatoxin is a problem in dairy herds because of feeding on contaminated maize meal by-products from millers (Bupe, 1988). This posses a serious public health hazard as aflatoxins can be transmitted to humans vthrough cattle milk. The fungus is common on ground nuts but can also be found on other cereals such as maize, beans, rice, sorghum and sunflower seeds.

2.3. SIDA CORDIFOLIA

2.3.1. Description of *S. cordifolia* (adapted from Exell and Wild, 1960).

Sida cordifolia L, family Malvaceae, is a herbaceous perennial shrublet with a stout woody taproot covered with velvety gray hairs throughout. The leaves are broadly oval, heart shaped at base and often yellowish, especially the venation. The margin is coarsely toothed and apex usually obtuse. Flowers are arranged in a dense, many-flowered clusters on terminal and lateral branches bearing small leaves with weak filiform and stipules about 5mm long. The plant may be confused with plants such as *Watheria indica* L (Family, Sterculiaceae), *Sida rhumbifolia, Sida alba* and *Sida cappinofolia.*

Plate 1. Showing *Sida cordifolia* in its natural habitat

Plate 2. Branches and leaves of *Sida cordifolia*

2.3.2. Habitat

Sida cordifolia is a perennial weed in waste places and old cultivated fields, particularly in sandy soils. It appears in the summer-autumn season and is possible invader of pastures. It is known to be resistant to several herbicides including pre-emergence ones such as Metribuzin (Dalldorf, 1976).

2.3.3. Distribution

S. cordifolia is a world-wide distributed plant especially in the tropics Ghosal (Ghosal *et al.*, 1975) reported its distribution not only in the tropic areas of India but also in the subtropical plains. Carvalho and Pitelli (Carvalho and Pitelli, 1992) mention its existent in the Brazilian plains. In Australia this plant is known as Flannel sida. Watt and Brandwijk (Watt and Brandwijk, 1962) have describe the plant as being wide spread in Southern Africa. In Zimbabwe the Ndebele people call it "Inama". In Zambia, Exell and Wild (Exell and Wild, 1960) found the plant concentrated in the North-Western province where it is known as Kavuvu (in Lunda and Luvale) and Kabuhu (in Luchazi language). Other Zambian provinces where the plant is found are Northern (Mbala District), Copperbelt, Central (Chisamba

District), Southern (Livingstone District) and Lusaka Province (Palabana area, from where test plants were picked).

2.3.4. Extracts of *S. codifolia* and their use

The first information on the extracts of *Sida cordifolia* was by Chopra (Chopra, 1933) who had isolated asparagin, an amino acid. In 1962, Watt and Breyer -Brandwijk indicated the presence of extracts such as alkaloids, fixed oils, phytosterols, resin acids, mucins and potassium nitrates from *Sida cordifolia* of which the alkaloids constituted up to 0.085% of all the extracts (calculated on dry material basis). In 1975, Ghosal extracted eight true alkaloids from the roots of this plant (Ghosal *et al.*, 1975). These were three β-phenethylamines (ephedrine, β-phenethylamines and ψ-ephedrine), two carboxylated tryptamines (*S* -(+)-N_b-methyltryptophan methyl ester and hypaphorine) and three quinazoline alkaloids, (vasicinone, vasicine and vasicinol). Small amounts of choline and betaine were also isolated. According to Ghosal *et al*, (1975) the seed and other aerial parts of the plant contained much of ephedrine while the rest of the extracts were found in the roots.

β-phenethylamine, ψ-ephedrine.

S-(+)-N_b-methyltryptophan methyl ester,

Hypaphorine

Vasicinone

Vasicine, R=H

Vasicinol, R= OH
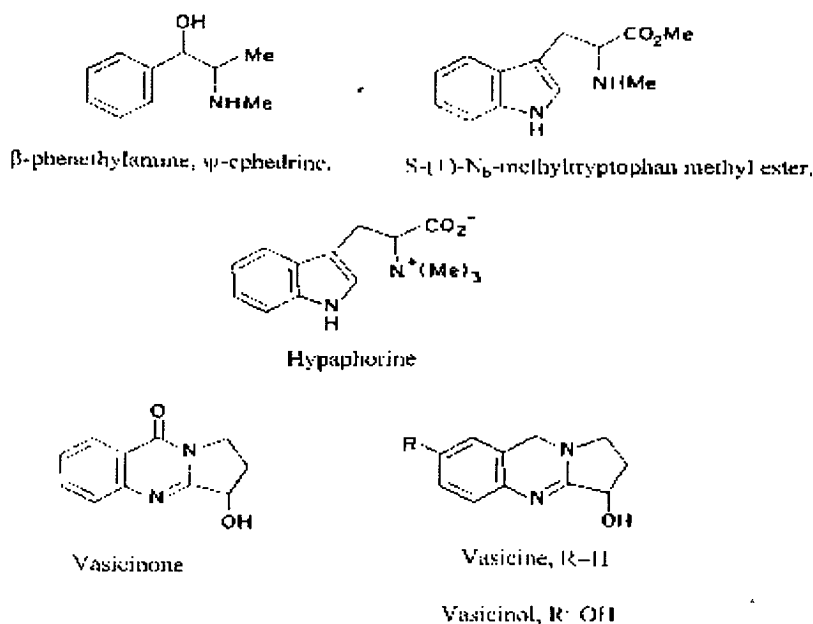
Fig. 1. *Sida cordifolia* alkaloid molecules

The presence of these extracts in *Sida cordifolia* seems to promote its usage in human medicine. The dominant alkaloid in this plant is ephedrine (Ghosal *et al.*, 1975) and latest information indicates ephedrine content ranges from 0.8% to 1.2% of the total alkaloids in the plant (Anonymous, 1996. Internet). The use of ephedrine in the treatment of human ailments is well documented. Bowman and Rand, (1980) wrote that ephedrine could be used in the treatment of myasthenia gravis, allergic rhinitis, heart block and bedwetting in children (as it reduces the depth of sleep due to its weak stimulating effect on central nervous system). Other usages of ephedrine mentioned were treatment of chronic broncho-pulmonary conditions characterized by bronchospasms, and coughs. The ephedrine in *Sida cordifolia* is reported to be the same as that found in other plants of the *Ephedra* species such as Ma-Huang (a Chinese plant used in treating hay fever) (Brown and Malone, 1978) and *Ephedra nevadensis* (a plant used by Indian Americans in the treatment of venereal infections). Brown

and Malone, (1978) mentioned the use of *Ephedra* species by Asian communities in the treatment of rheumatism, syphilis, cardiac insufficiency, malaria, fever, influenza, post-partum difficulties, typhoid fever, joint pain and induction of sweating. Thus with ephedrine being the dominant alkaloid in *S. cordifolia,* it is no wonder the plant is widely used in various herbal medicines.

Another extract from *S cordifolia*, vasicinone, is a potent bronchodilator and this may explain the usage of this plant in the treatment of Asthma by the Indian Americans (Brown and Malone, 1978).

The remaining extracts seem to control dysentery (Watt and Breyer -Brandwijk, 1962) and in the Ayurvedic system of medicine for many purposes including as a diaphoretic diuretic, as an aphrodisiac, anti-inflammatory and blood purifier (Banu *et al.*, 1989; Nadkarni, 1958:).

The plants extracts were also used as a weak insecticide; Banu *et al.*, (1989) used the plant in combination with other plants as an antifilaria drug, Filarin (Filarin tablet contains : 1g *Terminalia cebula*, 0.5g *Tinospora cordifolia*, 0.375g *Melia azadirachta*, 0.375g *Sida cordifolia* and *Tribulus terrestris*) which was used in the inhibition of pyruvate reduction in both female and male adult *Setaria digitata,* (Nematoda: Onchocercidae).

In Zambia, the medicinal use of this plant has not been documented but local people have used it to make fibers for chairs (Watt and Breyer -Brandwijk, 1962). The same usage was reported in Malawi by Vickery and Vickery, (1979) and in Zimbabwe and Mozambique by Exell and Wild (1960).

2.3.5. Toxic properties of *S. cordifolia*

According to the available literature there is no information on the toxicity of *S. cordifolia* in animals. Probably this is due to the fact that the plant consists chiefly of ephedrine a relatively weak psychostimulant when taken orally (Bowman and Rand, 1980). However, too much of ephedrine may cause tremors, restlessness and convulsions (Jones, 1965). Choline, found in the roots of the plant, if taken in excess, may cause hindquarter paralysis, dyspnea and imperceptible pulse (Bowman and Rand, 1980). From the same family of Malvacea, the only known toxic plant is *Gossypium* species (cotton plants) and another species called *Althaea officinalis* (Clarke *et al.*, 1981).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. History of outbreak

Sida cordifolia was suspected to have caused poisoning in small ruminants at Morester farm, 50 km East of Lusaka. In 1995 the farm had 350 sheep and 90 goats (the farmer bought one extra billy goat which had tick bites). By mid 1996 there were only 150 sheep and 60 goats remaining, the rest having died. The animals were dying at the rate of 4 a month; and the most affected were six week old suckling lambs (suckling from ewes that appeared ill). Many young ones died without showing clinical signs while those that did, showed ascites and loss of condition. Both adult sheep and goats showed pronounced cachexia, extended abdomen, sometimes abscesses, bloat and staring coat. In addition, sheep had wool falling off. There was, however, no photosensitization observed in both sheep and goats. Morbidity lasted a month before death. According to the farmer the disease did not cause any infertility i.e. ewes went on heat, conceived and lambed without problems. The lambs were born healthy and stayed like that up to six week of age. Postmortem examination (done by the farmer) revealed hard pale livers and abscess in the lungs. Bacteriological tests (at UNZA) isolated *Corynebacteria spp.* and histologically the livers showed mild diffuse fibrosis and bile duct proliferation as the main characteristics (Lutz W., 1996., personal communication).

The farmer was advised to vaccinate the animals with *Corynebacterium* vaccine and this was done in September 1995 and May 1996. But the problem continued. The farmer then decided to shift the animals to another farm, but those that were sick did not recover. There were however no new cases. In January 1997 when the animals were shifted back to the original farm they started showing poor body condition despite the abundance of forage (in April,

1997). According to the clinician who used to attend to the farm (Lutz W., 1996., personal communication) the goats of this mixed group turned to browsing the small leaves of *Sida cordifolia* extensively which could be seen during observation by the herdsmen and by the bite marks.

3.2. Study area

Morester farm situated 50Km east of Lusaka town covers 5000 hectares of land and is at an altitude of between 920 and 1075 meters above sea level. The farm consists of small hills with vegetation (miombo woodland) sloping away into the valley. The pasture consists of perennial grasses with Kikuyu grass (*Pennisetum clandestinum*) and *Sida cordifolia* dominating during the dry season. The area is of upper valley and has a sandy/loamy soil type (see Table 2 for mineral content of the affected paddock). Normally the rains start from October and end in April with maximum monthly rainfall of 300mm in the months of January, February and March (Meteorological Department, 1996). However, during the 1991-1992 and 1993-1996 rain seasons the area experienced drought with total monthly rainfall averaging at 150mm instead of the usual 300mm (Fig 2). The study area has maximum temperature of 30°C with August, September and October being the hottest and driest months. Average minimum temperature is 12°C with June and July being the coldest months.

Table 2.
Mineral content of soil from the paddock infested with *S. cordifolia*

K(me/100g)	Na(me/100g)	Ca(me/100g)	Mg(me/100g)	Cu(mg/kg)	Fe(mg/kg)	Mn(mg/kg)	Zn(mg/kg)
0.12	0.21	3.49	4.03	0.10	15.47	3.23	0.35

me = milliequivalent, mg = milligram
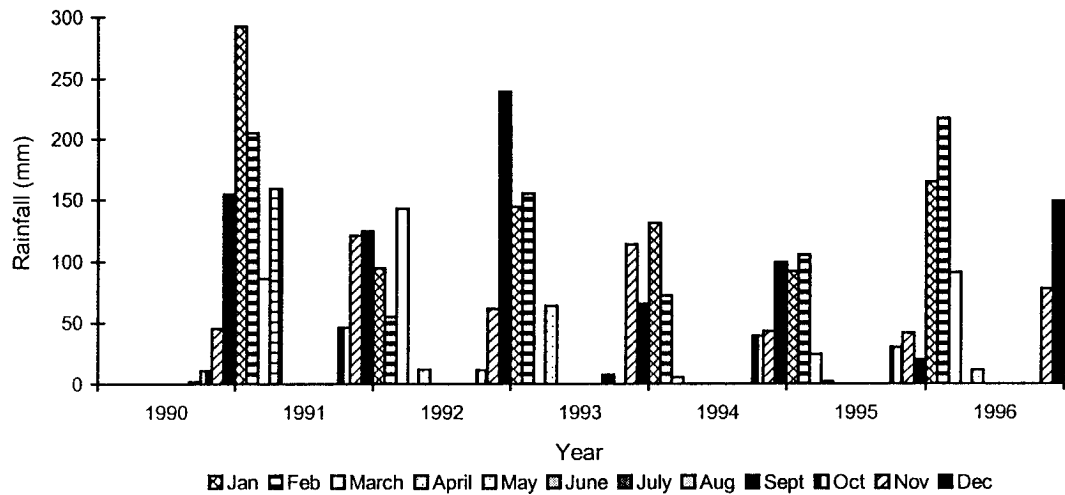
Fig. 2. Monthly rainfall 1990-1996 for Lusaka Province
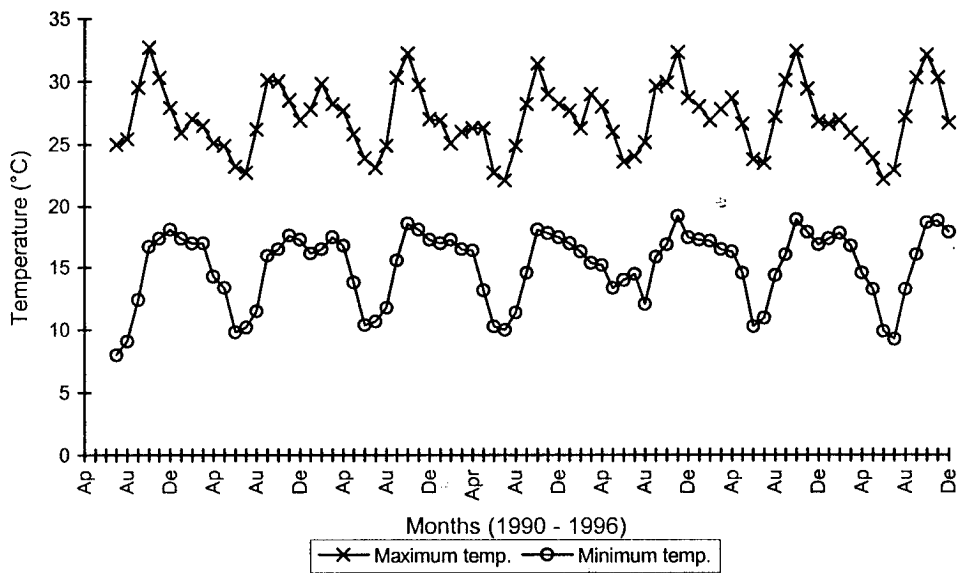
Fig. 3. Variation in atmospheric temperature for Lusaka Province

Water source for the animals is from one bore hole that feeds two troughs. The farmer also keeps 100 plus herd of cattle and grows maize. The goats (cross Boer) and sheep (doper) are

herded and housed as a mixed group since 1986. Grazing is free range but they preferred a paddock which had large quantities of *Sida cordifolia*. This paddock remained uncultivated for 3 years (i.e. 1994, 1995 and 1996 period).

Breeding of sheep is controlled; rams are put in for breeding in November while goats' breeding is not controlled. The lambs and kids are weaned at 6 months of age but they are let to graze together with adults. During the dry season the animals are supplemented with maize bran, and occasionally molasses, salt and Dicalcium phosphates but in 1995-96 period supplementation was not done. During the rain season the animals are dipped every 3rd week with Paracide (Alphamenthrin, 70% m/v) (excluding the suckling ones) and every 6th week during the dry season. The farm had a spell of drought from 1992 to 1996.

Vaccination of goats and sheep is done twice per year against the following diseases: Corynebacteria (started after experiencing death), Pasteurella and *Clostridia perfringens*. Worm control using Ranide (Rafoxanide + thiabendazole), oral albendazole (Valbazene, SmithKline Becham) and Sepnover (CAPS) is done once a month during rain season and every 3 month in the dry season. Diseases such a Riftvalley fever, coccidiosis and mange are rare. Mineral deficiency was suspected.

3.3. Retrospective study and survey

One of the approaches taken to investigate the possible toxicity of this plant was to histologically look at tissue specimen that were earlier submitted to the school of veterinary (UNZA) from the affected farm (Morester) and also look at tissues from the neighboring farms with similar problems of plant poisoning and compare them with tissues from experimental goats dosed with *S. cordifolia*. Thus tissues, (four sheep liver and four sheep kidney and one goat liver) previous collected and fixed in 10% buffered formalin by the

attending clinician were routinely processed, embedded in paraffin wax and cut to thickness of 4 μm. The sections were then stained with haematoxylin and eosin. For comparison, sections of liver and kidney of 15 other suspected plant poisoning cases (cattle 10, sheep 4, horse 1) from neighboring farms were subjected to the same treatment. A questionnaire survey (Annex 1) was conducted to gather extra information on farm management, husbandry vegetation and disease outbreaks of the affected farm and from the neighboring farms. Such type of information was necessary to see if there were any other agents that caused mortalities at Morester farm and to compare similarities and difference between the affected farm and others.

3.4. Experimental studies

These were conducted with the aim of reproducing clinical signs as witnessed at Morester farm. Three toxicity tests were conducted involving a total of 14 female goats. The first test involved five goats, the second six and the third three goats.

3.4.1 Preparation of *Sida cordifolia* for dosing trials

Leaves together with flowers of *S. cordifolia* (the plant was identified by Dr. P.S.M. Phiri, Plant Taxonomist, Biology Department, University of Zambia) were collected from Morester farm during November-December 1996, dried slowly in the laboratory at room temperature until mid February 1997 when it was milled (using a miller designed by R.K.J, Ikemoto Scientific Tech., Japan) to fine powder and stored at -10°C. The Dry Matter (DM) content was measured (using the Official Method of Analysis 11th Ed. Association Official Analytical Chemistry Washington D.C.) and found to be 90 percent.

3.4.2. Experiment no. 1

3.4.2.1. Preparation of experimental animals

Five mixed breed goats (cross breeds of various grades) from the same herd, aged between 6-18 months old and weighing 12 to 16kg body weight, healthy with no history of liver disease were acquired from a commercial farmer (Kudu farm) in Lusaka West. They were housed in a room with a concrete floor and allowed to acclimatize for two weeks during which general clinical examination and appropriate intervention were carried out. Blood and fecal samples were collected from each goat to check for any protozoa and bacterial infection and internal parasites respectively. Usually Zambian goats harbor several different species of helminths (Nalubamba, 1996). To counteract this, a combination of antihelmintics was given to all the goats. The day when the goats arrived each goat was injected with 0.5ml (intramuscular) of Levamisole (Levaject, Milborrow). Three days later 20% oxytetracycline L.A (Oxyject Dopharma) at a dose of 1ml/10kg BW intramuscular was given to counteract any bacterial infection. On the 5th day the goats were given Ivermectin 10mg (Iveen, Adwia) at a dosage of 0.2ml/kg to cure any subclinical mange. On day 7 Sulphadimidine sodium 33% (Suphazine, Milborrow) was injected subcutaneously at a dosage of 30ml/45kg to control coccidia. Oral Albendazole (Valbazene, SmithKline Becham) was given at dose of 2.5ml/10kg on day 10 (5 days before dosing).

The goats were put on 400g/goat/day of no.3 maize meal which was mixed with 100g dicalcium phosphate and 100g salt. Hay and water was given *ad libidum*. The animals were examined daily for temperature, pulse rate, respiratory rate, heart rate and rumen activity. They were also weighed at weekly interval until dosing started. Levels of aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) as well as concentrations of creatinine and urea were ascertained on day 5 and 10 days before dosing started. On the same

dates and subsequent 5 days before dosing, pulse rate, rhythm of the heart, rectal temperature, respiratory rate and rumen activity were recorded.

3.4.2.2. Dosing

The milled plant was weighed on a Libra scale and dosed according to the weight of the goat (Table 3). Each measure was diluted with about 1-2 liter of water until a running slime was made. Dosing was done every day for 5 consecutive days (days 0 to 4) using an empty 300ml Coca-Cola bottle (failed to use a stomach tube as the slime could not pass through). Goats 1 and 2 received a dosage of 10g/kg and goats 3 and 4 were given a dosage of 5g/kg. Goat 5 was control. These dosages were assigned at random by drawing lots.

Table 3.
Dosage rate of goats in experiment 1.

Goat no.	Wt of goat (Kg)	Dosage	Total amount given
1.	12	10g/kg DM	120g x 10 days = 600g
2.	15	10g/kg DM	150g x 5 days = 1,500g
3.	14	5g/kg DM	70g x 5 days = 350g
4.	16	5g/kg DM	81g x 5 days = 400g
5.	14	Control	Control

DM = Dry Matter

3.4.2.3. Clinical examination post dosing

On days 0 to 10 (consecutively) the animals were examined for rectal temperature, pulse rate, respiratory rate, heart rate and rumen activity. The measurements were done in the afternoon after dosing. On days 0 to 14 the animals were observed daily for photosensitivity and jaundice.

3.4.2.4. Blood sampling post dosing

On days 0, 3, 6, 8, 10, 12, and 14 blood was collected by jugular venepuncture into labeled plain vacutainer tubes. The blood was let to clot at room temperature for 3 hours after which serum was harvested and the clot was discarded. The serum was then transferred into clean labeled centrifuge tubes, centrifuged at 2000 rpm, for 15 minutes. The clear serum was pippeted into clean test tubes and placed into water bath at 25°C, ready for analysis.

3.4.2.5. Blood chemistry

Using Boehringer Mannheim method, the activities of the following enzymes and chemicals were determined: Gamma-glutamyltransferase (GGT, EC 2.3.2.2.), Aspartate aminotransferase (AST, EC 2.6.1.1), Urea and Creatinine. Only AST were assayed at 30°C while the rest were measured at 25°C.

3.4.2.5.1. Creatinine assay (Without deproteinization)

(Ref. Mod. acc. to Bartels, H. et al. (1971). Clin. Chim. Acta 32: 81)

Principle

When creatinine is mixed with picrate in an alkaline medium it forms a coloured complex and the rate of formation is measured.

Preparation of working solutions

Sodium hydroxide was diluted with distilled water at a rate of 1:4 then mixed with picric acid at 1:1 ratio. This reagent mixture was transferred into an amber bottle and stored at 25°C in a water bath for at least 30 minutes before use.

Method

The experiment was conducted as shown below:

Table 4.
Arrangement of creatinine assay.

	Standard	Sample
Reagent Mixture	2.0ml	2.0ml
Creatinine Standard	2.0ml	0.00ml
Serum	0.0ml	0.2ml

2.0ml of reagent mixture was pipetted into a cuvette followed by addition of 2.0ml of Creatinine standard and mixed. The mixture was then placed in the spectrophotometer and absorbency A_1 read after 30 seconds at 492 nm wavelength against air. Exactly 2 minutes later absorbency A_2 of the standard was read. Thus the absorbency standard ($A_{standard}$) was:

$A_2 - A_1 = A_{standard}$.

To 2.0ml of reagent mixture, 0.2ml of serum was added mixed and treated in a similar manner as the standard. The absorbency sample (A_{sample}) was: $A_2 - A_1 = A_{sample}$.

The concentration of creatinine was calculated using the formula:

$C = 2.0 \times A_{sample}/A_{standard}$ mg/dl.

3.4.2.5.2. Urea assay

Principle

Hydrosis of urea catalyzed by urease forms ammonium ions which react with salicylate and hypochlorite to give a green dye.

Preparation of working solutions

Buffer/Urease/Salicylate: One aluminum sachet containing phosphate buffer (120 mmol/l), urease (5 U/ml), sodium salicylate (62.5 mmol/l) sodium nitroprusside (5.00 mmol/l) and EDTA (1.48 mmol/l), was dissolved into 50ml distilled water, stirred vigorously and poured in an amber bottle and stored at 4°C as solution **1**.

Hypochlorite solution: The concentrated Hypochlorite (sodium hypochlorite: 6 mmol/l and sodium hydroxide: 150 mmol/l) was diluted with 450ml distilled water and stored in an amber bottle at 4°C as solution **2**.

Standard urea solution: This standard solution containing 30 mg/dl of urea was stored as above without diluting as solution **3**.

Method

The experiment was conducted as shown below:

Table 5.
Arrangement of Urea assay.

	Reagent Blank	Standard	Sample
Serum	0.00ml	0.00ml	0.02ml
Solution 3	0.00ml	0.02ml	0.00ml

Solution 1	2.50ml	2.50ml	2.50ml
Mix and incubate for 5 minutes at 25°C			
Solution 2	2.50ml	2.50ml	2.50ml

In the Reagent Blank, only 2.5ml of Solution 1 was pippeted onto; in the Standard tube 0.02ml of Standard Urea was mixed with 2.5ml of Solution 1 and in sample tubes 0.02ml of serum was mixed with 2.5ml of Solution 1. After mixing, the tubes were incubated in a water bath, at 25°C, for 5 minutes. Then 2.5ml of solution 2 was added to all the tubes, mixed and incubated into the water bath for 10 minutes. The absorbency A_{sample} and $A_{standard}$ was read at wavelength 578nm and 600nm filter against the blank within 60 minutes. The concentration was calculated thus: $C = 30 \times A_{sample}/A_{standard}$ mg/dl

3.4.2.5.3 Gamma Glutamyltransferase (GGT) assay

(Ref. Persijn, J.P., and W. van der Slik (1976) J. Clin. Chem. Clin. Biochem. 14: 421

Szasz, G., et al. (1974). Z. klin. Chem. u. klin. Biochem. 12: 228)

Principle

L-(-glutamyl-3-carboxy-4-nitroanilide {substrate}) mixed with glycylglycine (receptor) in the presence of GGT liberates L-(-glutamyl glycylcline and 5-amino-2-nitrobenzoate). The change in absorbency was measured.

Preparation of working solutions

Five GGT reagent tablets were dissolved respectively into five buffer bottles, closed firmly and incubated in a water bath at 25°C. Stable for five days at +15 to 25°C

Method

0.20ml of serum sample was pippeted into the reagent solution, mixed and then poured into a cuvette. Immediately an initial absorbency was read followed by others exactly 1, 2 and 3 minutes later. The absorbencies were read at wavelength 405nm using an open filter. The mean absorbency change per minute (A/min) was determined and was used for calculation. The GGT concentration was calculated thus:

International Units per Liter (IU/l) $= 1158$ x change of $A_{405\,nm}$ / min

3.4.2.5.4. Aspartate aminotransferase (AST) assay

(Ref. Bergmeyer, H.U. et al. (1986). J. Clin. Chem. Clin. Biochem. 24: 497)

Principle

AST in sera catalyses the convention of aminoacid L- aspartate, to a ketoacid, L- glutamate and changes α-oxoglutarate to oxaloacetate. In the presence of malate dehydrogenase oxaloacetate is converted to L-malate and during the process NADH is oxidised to a coenzyme NAD++ and the change in absorbency is measured by the spectrophotometer.

Preparation of working solutions

Five AST reagent tablets were dissolved respectively into five buffer bottles, closed firmly and incubated in a water bath at 25°C as reagent solution and can be kept for 5 days.

Method

0.20ml of serum sample was pippeted into the reagent solution, mixed and then poured into cuvette. After one minute, an initial absorbency was read followed by others exactly 1, 2 and

3 minutes later. The absorbencies were read at wavelength 365nm and 325 using an open filter of the Hitachi model spectrophotometer. The mean absorbency change per minute (A/min) was determined and was used for calculation. The concentration of AST was calculated thus:

International Units per Liter (IU/l) = 3235 x change of $A_{365\,nm}$ / min.

3.4.2.6. Postmortem examination

Postmortem examination was carried out immediately after exsanguination on day 14 of the experiment. Tissue specimens cut from three different areas of liver and kidney (also heart, spleen, brain and lung) were fixed in 10% buffered formalin for 36 hours after which they were refixed for another 24 hours using the same fixative. The tissues were then trimmed and processed to paraffin wax. The paraffin wax sections were cut at 4mm thickness and stained with haematoxyline and eosin.

3.4.3. Experiment no. 2

This was conducted two months after experiment 1. Six goats from same source (Kudu farm) aged between 8 and 18 months and weighing 14 to 17 kg were purchased and subjected to the same preparation and treatment as in experiment 1. However, in this experiment the goats were also given Praziquantel, 2.5%, m/v, (Cestocer, Bayer) to treat suspected *Stilesia hepatica* infestation. In this group there was no control. The duration of the experiment, was 40 days (days 0-9 dosing, 10-40 observation period and 40th day postmoterm). Initially, the experiment was arranged in such away that Goats 9, 10 and 11 were to receive a dosage of

20g/day for 10 consecutively days, observed for a period of 30 days post dosing and sacrificed on day 40 of the experiment. The other three goats (6, 7 and 8) were to receive a lesser dosage of 10g/Kg for 10 days, observed for 30 days and sacrificed on day 40 of the experiment. Unfortunately, goats 7 and 10 were withdrawn before dosing started as they were found to have an increase in GGT and AST. Goats 9 and 11 failed to consume the bulk volume of 20g/kg. In an attempt to force feed one of them (Goat 11), it collapsed and died. It was then decided to switch Goat 9 to a lesser dosage of 10g/kg of body weight. This left Goat 6, 8 and 9 for the experiment but unfortunately another goat (no.8) died suddenly on the seventh day of dosing. The second experiment was thus completed with only goat 6 and 9 which were dosed with 10g (of dry matter of the plant) per kilogram body weight for a period of 10 days, observed for 30 days and killed on the 40th day of the experiment. Dosage and quantities of material consumed are indicated in the table below.

Table 6.
Dosage rate of goats in experiment 2.

Goat no.	Wt of goat (Kg)	Dosage	Total amount given
6.	17	10g/kg DM	165g x 10 days = 1,700g
7.	16	10g/kg DM	Withdrawn
8.	14	10g/kg DM	144g x 7 days = 1,008g
9.	16	10g/kg DM	162g x 10 days = 1,600g
10.	14	20g/kg DM	Withdrawn
11.	14	20g/kg DM	Died first day of dosing

3.4.3.1. Clinical examination post dosing

Temperature, respiratory rate, heart rate, pulse rate and rumen activity were measured on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 18, 20, 30 and 40. During this period, signs of photosensitivity and jaundice were monitored.

3.4.3.2. Blood sampling post dosing

Blood samples were taken by vein puncture on days 0, 3, 6, 8, 10, 12, 14, 16, 18, 20 and 40.

3.4.3.3. Postmortem examination

The goats were sacrificed by exsanguination on day 40 of the experiment and liver and kidney tissues processed as in Experiment 1.

3.4.4. Experiment no. 3

This experiment was done two months after Experiment 2. In this experiment 3 goats (goat 12, 13, 14) were acquired from the same farm as in experiment 1 and 2 and were subjected to the same conditions as above. They were dosed at 5g/kg for 20 days, observed for 20 days post dosing and sacrificed on day 40 of the experiment. Goat 13 was withdrawn before dosing started due to high levels of AST while Goat 14 did not finish the course as it died on the 10th day of dosing. Thus the experiment was completed using only goat 12. Dosage and quantities of plant material consumed are indicated below.

Table 7.
Dosage rate of goats in experiment 3.

Goat no.	Wt of goat (Kg)	Dosage	Total amount given
12.	18	5g/kg DM	90g x 20 days = 1,800g
13.	13	5g/kg DM	Withdrawn
14*.	11	5g/kg DM	55g x 10 days = 550g

* Goat 14 died on 10th day of dosing

3.4.4.1. Clinical examination post dosing

Temperature, respiratory rate, heart rate, pulse rate and rumen activity were measured on days 0, 1, 2, 3, 4, 5, 7, 10, 15, 19, 25 and 29. Signs for photosensitivity and jaundice were monitored through out the experimental period.

3.4.4.2. Blood sampling post dosing

Blood samples were taken by vein puncture on days 0, 3, 6, 8, 10, 12, 14, 16, 18, 21, 24, 27, and 30.

3.4.4.3. Postmortem Examination

The goats were sacrificed by exsanguination on 40 of the day of experiment and liver and kidney tissues processed as in Experiment 1.

3.5 Statistical Analysis

A student t-test was applied to determine whether there was any significant difference ($P < 0.05$) between serum values before and after dosing using a C-stat for Windows version 1.0 statistical package (Holman and Kennedy, 1993).

CHAPTER FOUR

4. RESULTS

4.1. Retrospective study and survey

4.1.1. *Histopathological findings.*

Morester farm

Lesions seen in sheep liver sections from Morester farm showed focal hepatocyte necrosis and bile duct proliferation (Plate 3), periportal necrosis with destruction of normal architecture (Plate 4) and hepatic necrosis with infiltration of inflammatory cells in the portal area (Plate 5). The main lesion in goat liver from the same farm showed mild hemorrhage around portal area (Plate 6) and Kupffer cells infiltration of portal areas (Plate 7). There were no significant lesions in the kidneys.
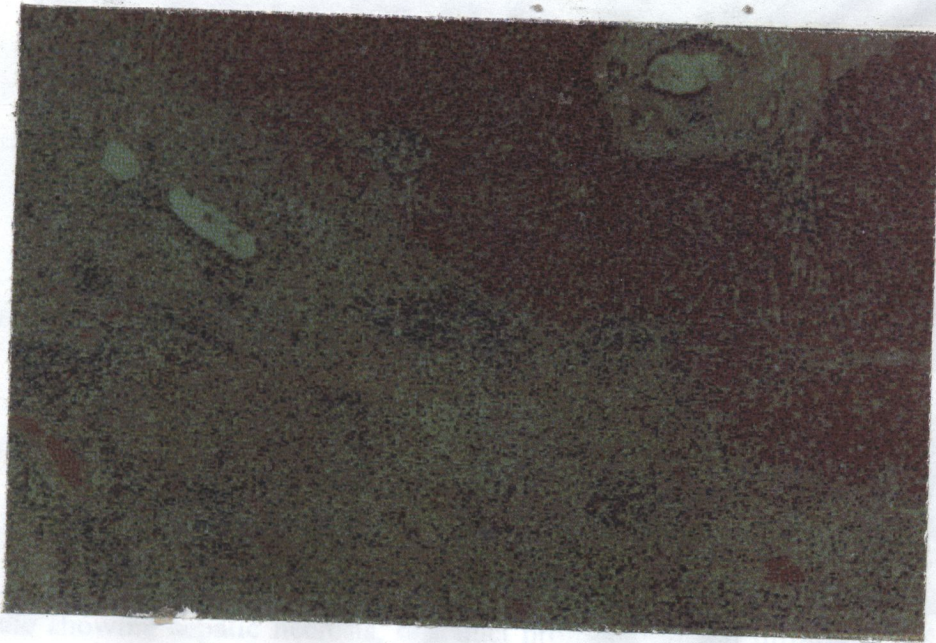
Plate 3. Liver tissue of a one year old sheep from Morester Farm suspected to have eaten *Sida cordifolia* showing hepatic necrosis and bile duct proliferation. HE x 4.
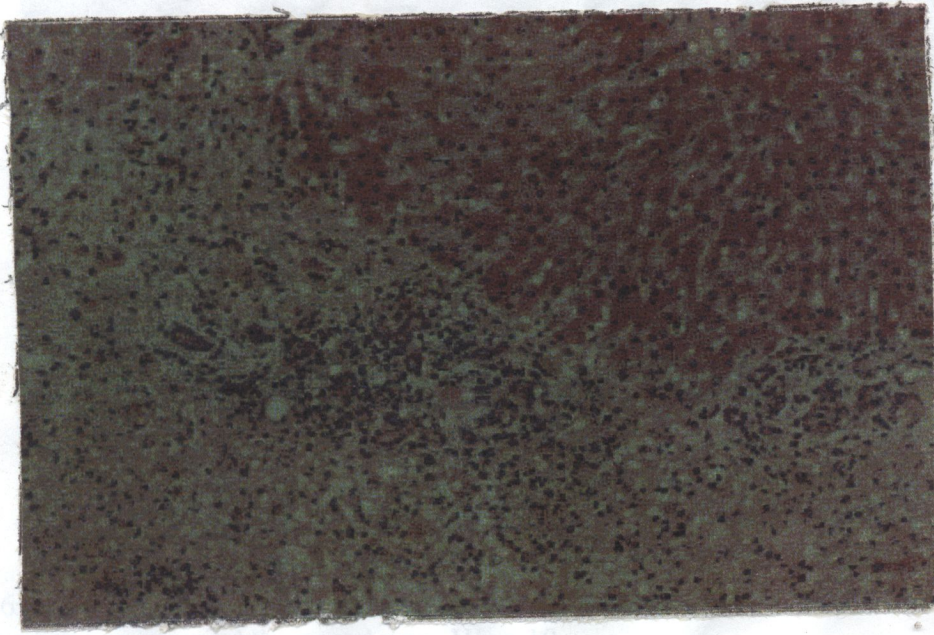
Plate 4. Sheep liver from Morester Farm showing periportal necrosis. HE x 10.
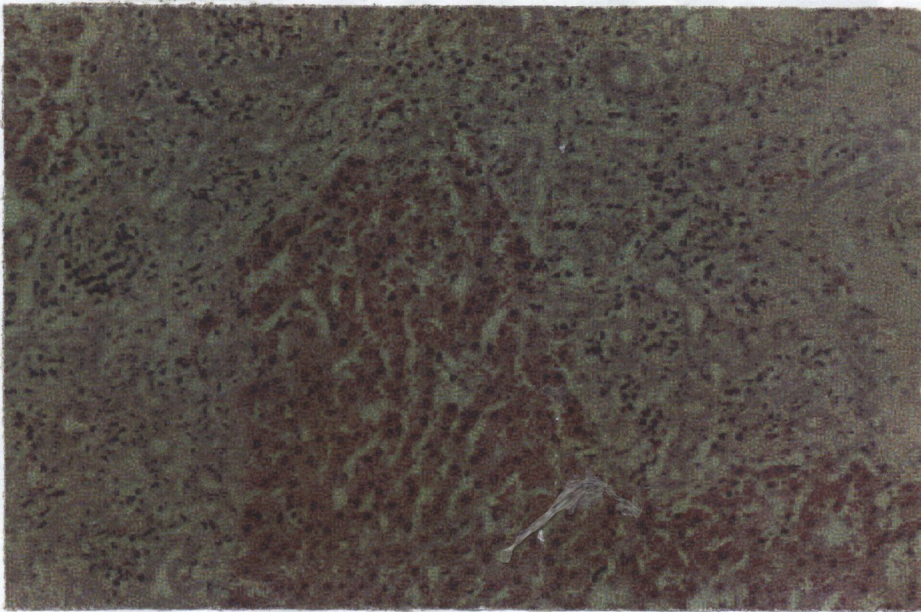
Plate 5. Liver tissue of a one year old sheep from Morester Farm suspected to have eaten *Sida cordifolia* showing hepatic necrosis, bile duct proliferation and infiltration of inflammatory cells in the portal area. HE x 10.
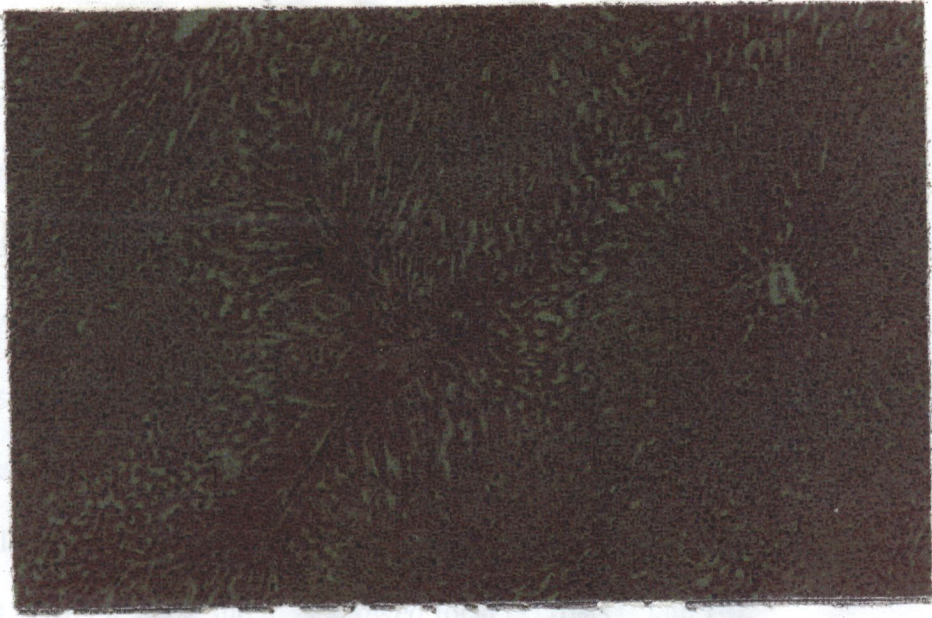
Plate 6. Liver of a goat from Morester Farm suspected to have eaten *Sida cordifolia* showing mild hemorrhage around the portal area. HE x 10.
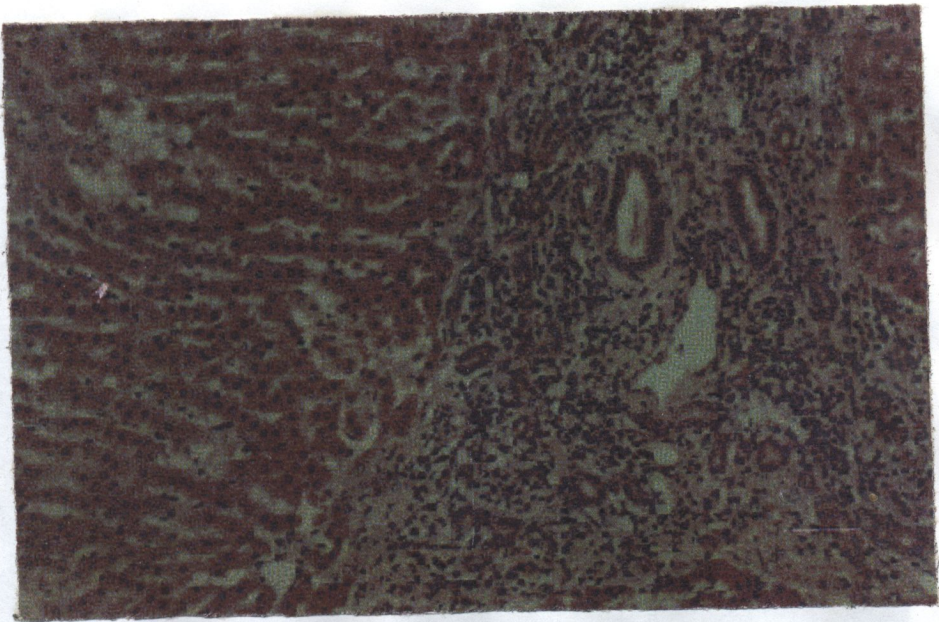
Plate 7. Liver of a two year old goat from Morester Farm suspected to have eaten *Sida cordifolia* showing Kupffer cell infiltration. HE x 10.

Other farms

The main characteristic of histological examination of sections of livers of sheep from other farms was fibrosis. The lesions were accentuated lobulation caused by marked fibrosis (Plate 8), massive fibrosis (Plate 9) centrilobular necrosis (Plate 7, 8 and 9), bridging fibrosis with hepatocellular unrest (Plate 10). Some of the liver specimen of cattle suspected to have died from plant poisoning showed fatty changes (Plate 12) and in others showed dilated sinusoid (Plate 13). In the horse the liver showed focal necrosis (Plate 14).
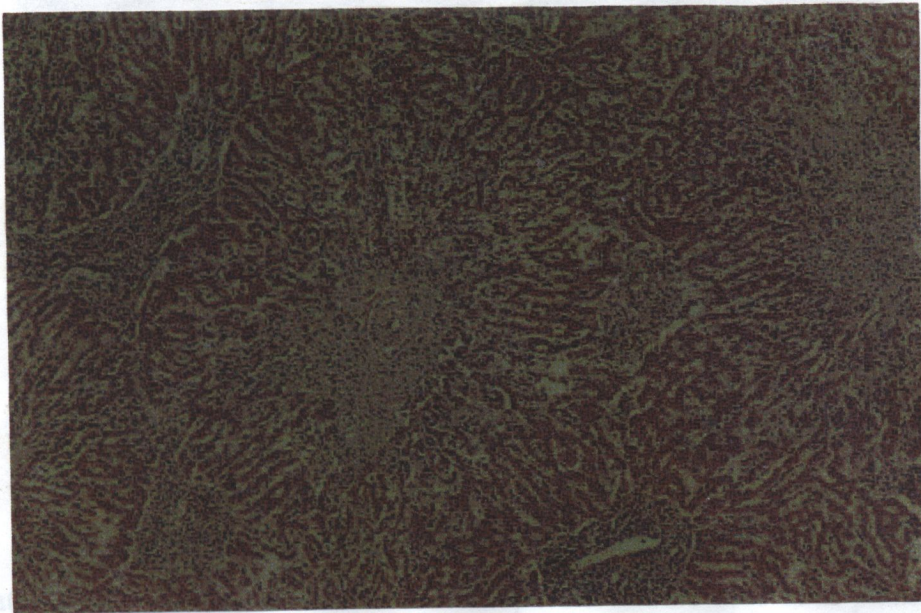
Plate 8. A four year old sheep from Goodhope Farm suspected to have died from plant poisoning showing accentuated lobulation due to marked fibrosis. HE x 10.
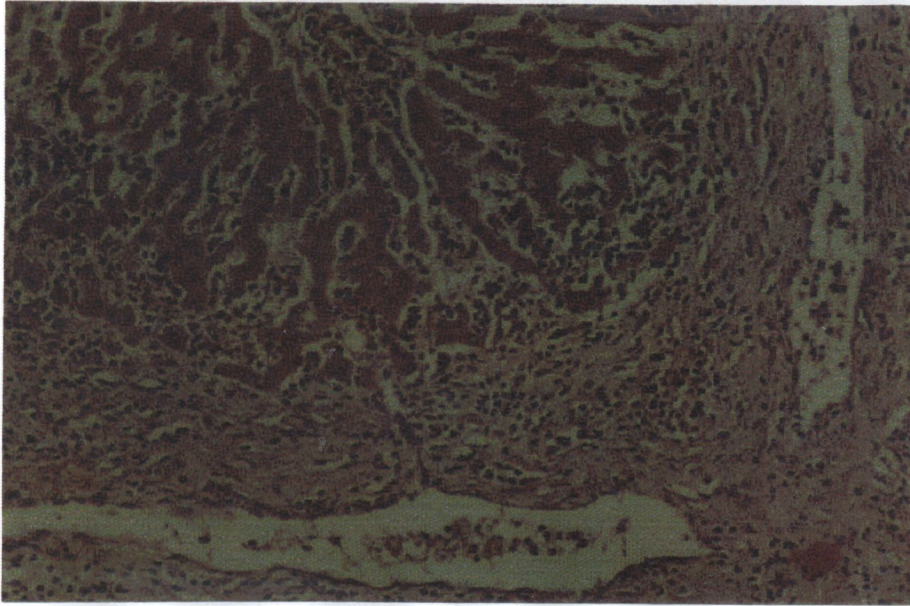
Plate 9. Higher magnification of Plate 8 showing massive fibrosis. HE x20
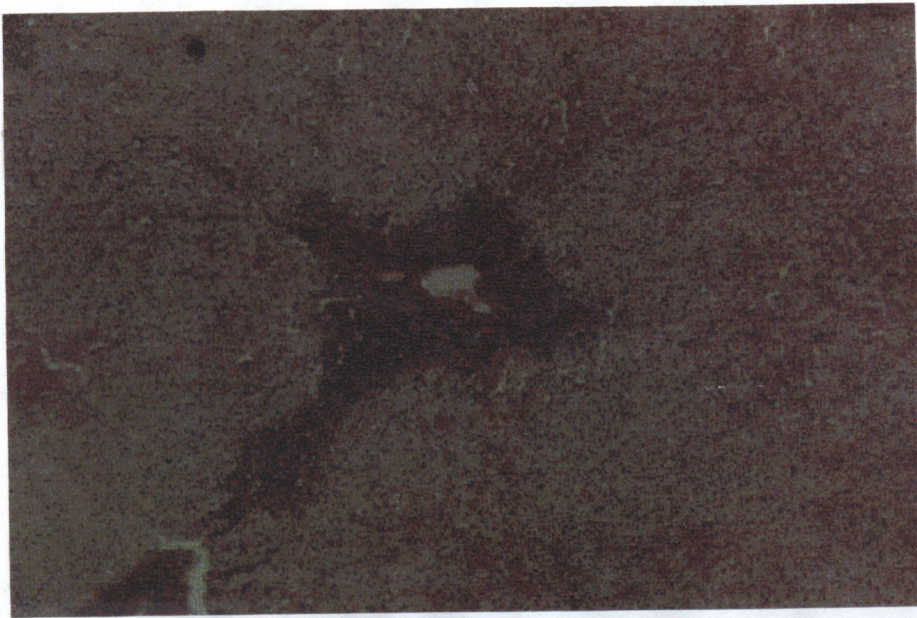
Plate 10. Sheep liver from Lakin farm suspected to have died from plant poisoning showing centrilobular necrosis. HE x 10.
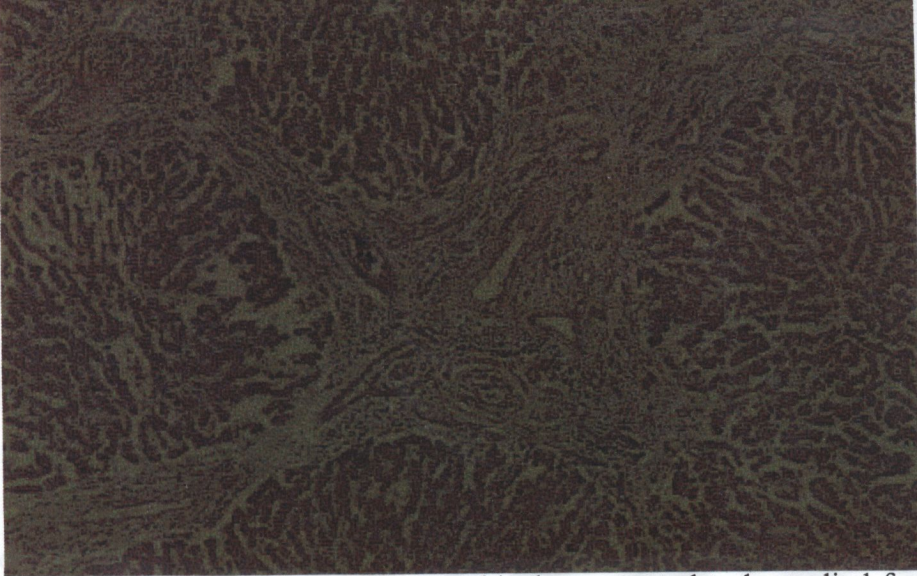
Plate 11. Liver of sheep from UNZA paddocks suspected to have died from plant poisoning showing bridging fibrosis and hepatocellular unrest. HE x 4.
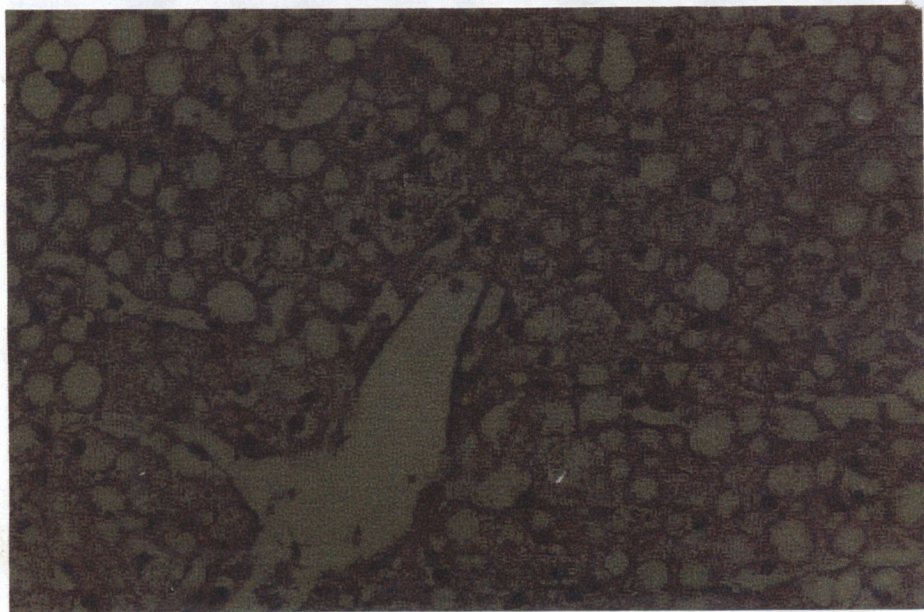
Plate 12. Liver of a nine month old heifer from Wangwe Farm suspected to have died from plant poisoning showing fatty changes. HE x 20.
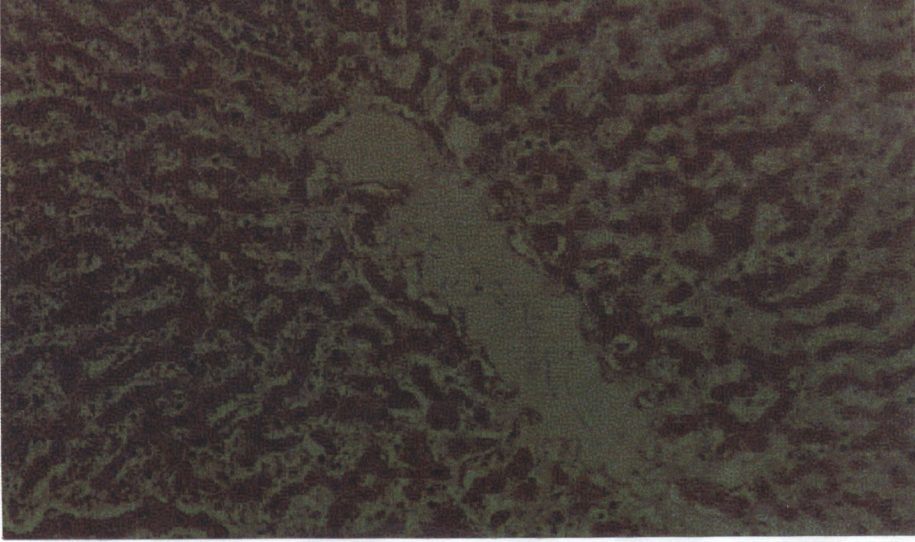
Plate 13. Liver of a four year old cow from Wangwe Farm suspected to have died from plant poisoning showing dilated sinusoid. HE x 20.
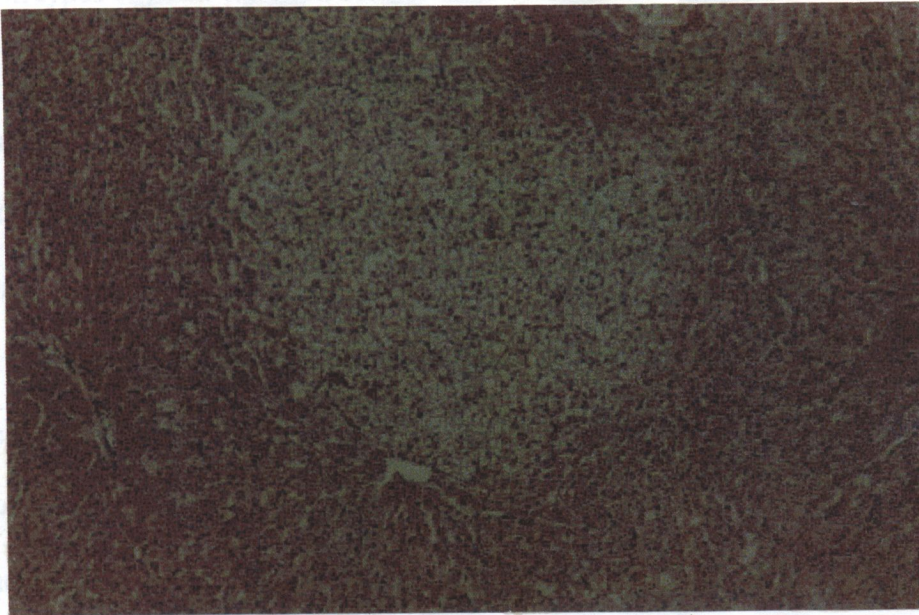
Plate 14. Liver of a Horse from Charcraft farm suspected to have died from plant poisoning showing focal necrosis. HE x 10.

4.1.2. *Survey*

Questionnaire results from 15 farms (12 commercial, 3 small scale) from where suspected cases of plant poisoning were reported.

Farm description

Q 1. Soil type: There was hardly any variation in soil type because most farms visited are located around Lusaka town which has a dominant sandy/loamy soil. No farmer reported any soil analysis. This question was included because soil type can influence concentration of certain types of plant e.g. *Sida cordifolia* is common in sandy soils.

Q 2 and Q 3. Vegetation and application of fertilizer: All farmers interviewed relied on natural vegetation for grazing their livestock though a few (5) cultivated stargrass (*Cynodon plectostachyus*) for dry season grazing. This grass was watered but not fertilized. The dominant vegetation being grasses of *Hyparrhemia* species.

Q 4. Which poisonous plants did farmers know that existed on their farms: The plants which farmers were aware of (in order of importance) are *Lantana camara (12 farms)* , *Solanum spp (10), Ricinus communis* (Castor bean) (8), *Datura spp (5), Albizia spp.(2)*. Five farmers included aflatoxin and cobrot as other toxicants that existed on their farms. Thirteen farmers interviewed had no idea of the existence of *Sida cordifolia* on their farms. One farmers was aware of the plant but claimed to have no problem with it.

Q 5. Water source: Pollution of water sources with chemicals or plants such as algae can lead to animal intoxication, hence the inclusion of this question. In this study most farmers (13) used underground water (borehole) which was pumped into troughs. This type of water was supplied through out the year. However, during rain season the animals could drink from any collecting spot as they graze. One farmer relied on water from a stream and had no

problem while another had used sewage water for his dairy cattle during the dry season and experienced 4 deaths.

Q 6. Crops grown: This question was included in case some farmers grew toxic crops like sorghum or sunhemp which animals may eat by accident. The majority of the farms did not grow crops (10) on their affected farms (for some farmers had more than two farms). Five farmers grew crops like soybean, wheat, maize, paprika, and coffee.

Q 7. Herbicide/insecticides used: Two farmers used Bromicine + diuron. Three farmers used Atrazine.

Q 8, Q 9 and Q 10. No. of sheep, goats and other animal species. See table 8.

Table 8.
Livestock affected by suspected poisoning and grazing pattern on surveyed farms

Farm	Type of livestock on the farm	Grazing pattern	Spp affected
1	Cattle(100), Sheep(350) and Goats(90)	Cattle separated from S&G	Sheep and Goa
2	Cattle(200) and Sheep(120)	Graze together	Sheep
3	Cattle (900, Dairy) and Horse(50)	Graze together	Cattle
4	Cattle (700, Dairy) and sheep(600)	Separate	Sheep
5	Cattle (7, beef) and sheep (120)	Together	Cattle and she
6	Cattle (800, Dairy)	Alone	Dairy
7	Cattle (20), Sheep (8) and Goats (6)	Separate in rotation	Cattle
8	Horses (51)	Alone	Horse
9	Cattle(297), Sheep(8) and Goats (22)	Separate	Cattle
10	Cattle (87, Dairy)	Alone	Dairy
11	Cattle (250) and Goats (70)	Together	Cattle and Goa
12	Cattle (77, Dairy and 96 beef)	Together	Dairy
13	Cattle (46 Dairy and 88 beef)	Together	Dairy
14	Cattle (187) and Goats (37)	Together	Cattle
15	Cattle (66) and Goats (30)	Together	Cattle

S & G = Sheep and Goats

Management (Sheep and Goats)

Q 11. Drought problem: Drought conditions can lead animals to eating poisonous plants. Three farmers mentioned drought problem during the period of 1991-1992 which had affected water supply. Another two farmers said they experienced drought in 1993-94 season which affected availability of pasture on their farms such that they were forced to increase supplementation. The rest of the farms (10) though not affected by drought had enough natural grazing pasture for goats and sheep to survive.

Q 12. Grazing regime: From the nine farms that kept small ruminants, five claimed to have grazed the animals in designated paddocks unherded and experienced death in their stock. Four farmers herded their animals wherever there was vegetation. This means all farmers interviewed practiced free range grazing and whether the animals were herded or not mortality still occurred.

Table 9.
Summary of type of livestock and supplement used on the surveyed farm

Farm	Livestock on the farm	Livestock and type of supplement given	Period given
1	Cattle, Sheep and Goats	To cattle only; Maize bran, salt and blocklicks	Dry season
2	Cattle and Sheep	To both; Sunflower cake, Hay and Dicalcium	Dry season
3	Cattle (Dairy) and Horse	To both; Maize bran, Hay and Mineral licks	Daily basis
4	Cattle (Dairy) and sheep	To cattle only; Calf and heifer meal, salt and minerals	Daily basis
5	Cattle (beef) and sheep	To both; Hay and Poultry manure	Dry season
6	Cattle (Dairy) only	Dicalcium phosphate, silage and hay	Dairy
7	Cattle, Sheep and Goats	To all; Hay and Dicalcium phosphate	Dry season
8	Horses only	Maize and wheat bran, licks and molasses	Daily basis
9	Cattle, Sheep and Goats	To all specie given, salt, hay and silage	Daily basis
10	Cattle (Dairy)	Hay and Dicalcium phosphate	Daily basis
11	Cattle and Goats	No supplement given	
12	Cattle (Dairy and beef)	Only to dairy cattle; Maize bran and salt	Daily basis
13	Cattle (Dairy and beef)	Only to dairy cattle, Maize bran and salt	Daily basis
14	Cattle and Goats	No supplement given	
15	Cattle and Goats	No supplement given	

Q 13, Q 14 and Q 15. Supplement: See Table 9. Type of supplement varied from farm to farm but the major feed stuffs mentioned were maize bran, silage, hay and blocklicks which consisted of minerals, salt and concentrates. Only farmers keeping dairy cattle and horses were supplemented on daily basis through out the year. Three farmers who kept sheep and goats said they supplemented only during dry season. One farmer supplemented sheep when about to slaughter or when the ram was introduced for mating and another gave supplement when resources were available. Three farmers did not give any supplement to their livestock. Out of the 15 farms visited only one farmer prepared his own supplement (chicken manure mixed with salt and lime) and according to him he stopped the practice after he lost 40 sheep. Another farmer was supplementing his dairy herd with sunflower cake but after a month, 4 of his cattle died. Samples of the feed were sent to UK for analysis and were found to contain aflatoxins.

Q 16. Grazing together of different species: See Table 8. Only one farmer grazed in rotation, three had separate permanent grazing areas for cattle and for sheep and goats. The rest could graze anywhere.

Q 17 and Q 18. Excluded as it was found to be irrelevant.

Q 19. When was weaning done: On seven farms goats and sheep were allowed to wean themselves thus making it difficult to tell exactly when it was done. Two farmers weaned goats and sheep at 6 month of age.

Q 20. Were weaners separated from adults: In this study only farmers keeping dairy cattle and horses separated weaners from adults.

Q 21. Were animals housed at night: All the farmers housed the animals at night.

Q 22. Common diseases seen on the farm: Riftvalley fever (1), Pulp kidney (0), Corynebacteria (1), Mange (0), Mineral deficiency (1), Coccidiosis (0), Heartwater (1) and Worms (15).

Q 23. Suspected Poison: Chemical (2), Plant (8), not sure (5).

Q 24. Group affected: Six farmers said the young (lambs and kids) were the most affected.

Q 25. Clinical signs observed: Farmers who suspected chemical poisoning described observing bloat, salivation and nervous signs. On the other hand signs of suspected plant poisoning were described as paralysis, frothing, diarrhea, lethargy and salivation.

Q 26. Postmortem lesions: No farmer answered this question. They could not remember any important lesions on postmortem examination.

Q 27. Has any unknown disease occurred on your farm (Sheep and Goats): All farmers (9) who kept small ruminants responded experiencing a loss of livestock but not necessarily an outbreak. Except for one farmer who lost sheep after supplementing with poultry manure all unknown cases were not diagnosed by anyone hence they suspected toxicosis. Six farmers did not answer the questionnaire because they did not keep small ruminants.

Q 28. How often does the unknown disease occur on your farm and during which month: Six farmers responded that all their cases were acute, sporadic and occurred regardless of the season. Thus the pattern of occurrence of the unknown disease on these farms were not clearly identified.

Q 29. Describe the symptoms of the unknown disease: See Q 25. Some of the animals were found dead.

Disease control measures (Sheep and Goats)

Q 30. Vaccination (indicate type used and frequency): There was one common vaccine mentioned at all the farms visited, Heptavac (for Clostridia). Riftvalley fever vaccination was conducted only on two farms.

Q 31. Dipping (indicate type of dip used and frequency): All farmers interviewed carried out dipping though frequency varied. During rain season six farmers claimed to dip their animals on weekly bases and once per month during dry season. Three farmers dipped only in the rain season. Chemicals used were: Super dip (Chlorfenvinphos 10%, w/v), Paracide (Alphamenthrin 70% w/v) and Barricade (Cypermethrine 15% w/v).

Q 32. Deworming (indicate type of dewormer used and frequency): The antihelmintics frequently mentioned were Ivomec (Ivermectin), Panacur (Fenbendazole), Valbazene (Albendazole) and Lavisan (Levamisole). Frequency of application varied from farm to farm depending on the season. Six farmers dewormed every month during the rain season and only once in the dry season. Three farmers dewormed once per year.

Major disease outbreak seen in other animal species

Q 33. Which animals were affected: The animals involved were cattle, horses and one pig. One farmer lost 6 horses at the rate of one horse per month. Another farmer lost 4 dairy cattle at once and 8 became sick for two weeks but recovered. Salt poisoning was suspected in one pig.

Q 34. Which age group was affected: Adult

Q.35. Which disease did you suspect: Tickborne(3), Rabies (2), Trypanosomiasis (1), Botulism (1) Streptothricosis (1), Blackleg (2) and poisoning (14).

Q 36. How long was the duration of the disease: This varied from farm to farm. The common answer given was that the cases were acute.

Q 37. In which month did the disease occur: The answers were varied depending on the animal species involved. Cattle cases were noticed during dry season while horse cases could occur any time of the year.

Q 38. Which plants were the animals seen eating during the outbreak: There was no farmer who gave a satisfactory answer. All the farmers interviewed claimed their animals were on natural plants usually grasses.

Q 39. Was the pasture overgrazed during the outbreak: One farmer who lost 4 dairy cattle claimed the pasture was overgrazed when the animals died. Another farmer who lost 3 beef cattle claimed the paddock in which the animals were kept was completely denuded of pasture. Three other farmers claimed that even if their farms had grass it was so unpalatable due to dryness. The rest of the farms visited mentioned having enough pasture in the affected paddocks.

Q 40. Did the disease affect all the paddocks: All the farmers said only selected paddocks were affected.

Q 41. What was the difference between the affected paddock/s and the unaffected. Only one farmer could pinpoint the difference between the affected paddock and those which were not. He mentioned of the area being overgrazed on one part of the paddock there was a hedge of *Lantana camara* and a few *Datura* spp.

Q 42. What was the clinical presentation of the disease: In horses the major clinical sign described was mucopurulent discharge, in cattle it was paralysis of hind legs and in pigs the animals were just found dead

4.3. Results from Experimental Studies

4.3.1. Clinical signs

Experiment 1. One of the noticeable clinical signs seen in the dosed goats was the reduction in body weight (1-3kg loss), water and feed intake during dosing. The animals picked up when dosing ended. Goat 2 showed a weak heart beat a day after dosing ended (day 6). Goat 3 looked less active and had a slight elevated temperature (40.2°C) on the 5th day of dosing. On the sixth day the same goat started wheezing and coughing which lasted for 2 days. Goat 4 had irregular breathing on day 6 and 7 of the experiment. Respiratory rate increased from 40 (first day of dosing) to 54 (respiration per minute) on day sixth and dropped back to 40 on the 8th day. Goat 1 (serve for loss of body weight) and 5 remained unaffected through out the experiment.

Experiment 2. Out of the three goats (no.6, 8, and 9), only Goat 9 showed some change in heath status. Immediately after dosing the goat started coughing until the next day. On the 8th day of dosing the same goat had a slight elevation of temperature(40.5°C) and experienced a transient diarrhea for 10 days which made it lose 3.5Kg of body weight. Goat 8 did not show any clinical signs until it died on the 7th day of dosing.

Experiment 3. Goat 12 showed high pulse and heart rates (150 and 130 per minute respectively) on day 12 of dosing but these dropped to normal levels the next day signifying that the rise may have been caused by other factor such as fright.

Since in all the experimental animals there was no such significant change in temperature, respiratory rate and heart rate the results are given as pooled means and all the parameters fall within the normal range for goats (Table 10). Note that the pooled means are from goats that completed the course of experiment. The results from dead goats were not included to avoid bias. Individual values of means of the measured parameters are in Table 11. As shown there

was no significant difference (P > 0.05) between values before and after dosing. There was also no sign of jaundice or photosensitivity in all dosed goats.

Table 10.
Pooled means for parameters of goats (1,2,3,4,6,9 and 12) fed with *Sida cordifolia*

Parameter	Mean	SD	Range	Reference
Temperature (°C)	39.1	0.4	38.6-39.6 38.8-39.4	Merck Manual 1991* Witola (1997)**
Resp. Rate (per min)	34	5.6	25-35 32-35	Radostis (1994) Witola (1997)
Heart Rate (Beats/min)	101	5.3	70-135 80-90	Merck Manual 1991 Witola (1997)
Pulse Rate (per min)	104	5.0	70-90 80-90	Radostis (1994) Witola (1997)

* According to The Veterinary Merck Manual (1991).
**Values of Zambian goats (local breed) Witola (1997).

Table 11.
Individual mean and standard deviations of Temperature, Respiratory rate, Heart rate and Pulse rate for goat 1,2,3,4, 5, 6, 8, 9, 12 and 14

Goat No.	Status	Temperature °C	Respiratory rate per minute	Heart rate Beats/min	Pulse rate per minute
1.	Before	38.7 ± 0.34	32 ± 3.1	109 ± 7.4	112 ± 7.9
	After	38.8 ± 0.46	33 ± 4.4	1080 ± 8.5	111 ± 9.6
2.	Before	39.0 ± 0.54	33 ± 4.3	108 ± 9.4	110 ± 9.2
	After	38.9 ± 0.56	37 ± 8.3	105 ± 9.6	108 ± 8.1
3.	Before	38.7 ± 0.35	28 ± 3.2	100 ± 6.5	102 ± 7.3
	After	38.8 ± 0.52	32 ± 6.1	93 ± 9.5	102 ± 9.7
4.	Before	38.6 ± 0.59	42 ± 3.6	90 ± 5.3	97 ± 8.1
	After	38.6 ± 0.31	45 ± 6.3	95 ± 8.6	97 ± 9.1
5*	Before	39.1.± 0.53	39 ± 6.4	114 ± 8.2	115 ± 11.0
	After	38.7 ± 0.43	40 ± 6.4	108 ± 7.2	107 ± 8.8
6	Before	39.0 ± 0.76	29 ± 2.3	97 ± 7.0	97 ± 5.9
	After	39.6 ± 0.49	30 ± 1.3	101 ± 4.0	100 ± 3.5
8**	Before	38.5 ± 0.58	34 ± 2.5	113 ± 10.3	112 ± 9.5
	After	39.3 ± 0.54	30 ± 1.8	100 ± 5.8	99 ± 3.5
9	Before	38.3 ± 0.31	33 ± 2.3	104 ± 3.6	106 ± 3.8
	After	39.4 ± 0.49	30 ± 1.3	101 ± 4.1	100 ± 3.2
12	Before	38.8 ± 0.46	35 ± 3.0	112 ± 8.3	112 ± 8.4
	After	39.4 ± 0.59	31 ± 1.8	104± 9.7	107 ± 15.0
14***	Before	38.6 ± 0.45	30 ± 1.4	104 ± 5.2	106 ± 6.4
	After	38.4 ± 1.07	31 ± 2.8	103 ± 5.7	105 ± 6.0

*Goat No. 5 was control.
**Goat 8 died on the 7th day of dosing
***Goat 14 died on the 10th day of dosing

4.3.2. Changes in Serum Metabolites of experimental goats

Summaries of the results are given in Tables 12 and 13. With the exception of Goat 1 where GGT rose from 44 to 78 U/l, there were no significant changes (P > 0.05) between values of before and after dosing. As earlier mentioned the rise of the GGT was probably due to the presence of the parasite (*Stilesia hepatica* found lodged in the liver) and could have contributed to the abnormally high standard deviation (Table 12).

Table 12.
Pooled means for serum metabolites of goats (2,3,4,6,9 and 12) fed with *Sida cordifolia*

Metabolites	Mean	SD	Range	Reference
Urea (mg/dl)	27	2.6	10 - 60	Mitruka and Rawnsley (1977)
			25 - 42	Takahashi and Nawa (1997)*
Creatinine (mg/dl)	1	0.12	0.7 - 1.5	Vet. Merck Manual. (1991)
			1.0 - 2.0	Takahashi and Nawa (1997)
GGT (IU/l)	32	4	25 - 34	Mitruka and Rawnsley (1977)
			8 - 15	Takahashi and Nawa (1997)
AST (IU/l)	61	4	30-122	Mitruka and Rawnsley (1977)
			15-30	Takahashi and Nawa (1997)

* Takahashi and Nawa (1997) unpublished data, University of Zambia.

Table 13.
Individual mean and standard deviations of serum metabolites for goat 1, 2, 3, 4, 5 6, 8, 9, 12, and 14

Goat No.	Status	Urea (mg/dl)	Creatinine (mg/dl)	GGT (IU/l)	AST (IU/l)
1.	Before	18 ± 4.1	1 ± 0.2	44 ± 0.7	61 ± 11
	After	17 ± 7.9	1 ± 0.2	78 ± 21	79 ± 11
2.	Before	19 ± 4.5	1 ± 0.3	₊47 ± 2.9	72 ± 12
	After	20 ± 6.3	1 ± 0.3	46 ± 1.2	64 ± 6.4
3.	Before	24 ± 12	1 ± 0.2	33 ± 2.4	83 ± 31
	After	27 ± 6.8	1 ± 0.3	35 ± 2.7	77 ± 28
4.	Before	26 ± 9.2	1 ± 0.5	34± 3.9	62 ± 12
	After	28 ± 3.0	1 ± 0.3	40 ± 2.7	62 ± 9.0
5*	Before	20 ± 3.9	1 ± 0.3	60 ± 9.6	55 ± 13
	After	21± 6.7	1 ± 0.3	46 ± 3.3	47 ± 5.7
6.	Before	29 ± 2.9	1 ± 0.9	27 ± 1.5	51 ± 5.5
	After	27 ± 1.9	2 ± 0.3	21 ± 2.6	55 ± 4.3
8**.	Before	28 ± 9.4	not done	28 ± 2.6	49 ± 2.4
	After	28 ± 2.5	not done	27 ± 4.2	51 ± 0.5
9.	Before	34 ± 1.7	2 ± 0.3	19 ± 1.0	47 ± 2.3
	After	39 ± 3.6	2 ± 0.3	20 ± 2.1	47 ± 2.8
12.	Before	26 ± 0.7	1 ± 0.3	35 ± 3.2	68 ± 7.7
	After	26 ± 2.3	2 ± 0.9	35 ± 3.1	63 ± 6.2
14***	Before	26 ± 3.1	1 ± 0.1	44 ± 8.2	60 ± 5.0
	After	24 ± 2.3	1.1 ± 0.1	55 ± 0.8	57 ± 2.0

*Goat No. 5 was control. **Goat 8 died on day 7 of dosing. ***Goat 14 died on the 10th day of dosing

The following graphs show changes in serum metabolites concentrations for selected goats (nos. 1, 2, 3, 4, 5, 6, 9, and 12).

(a)
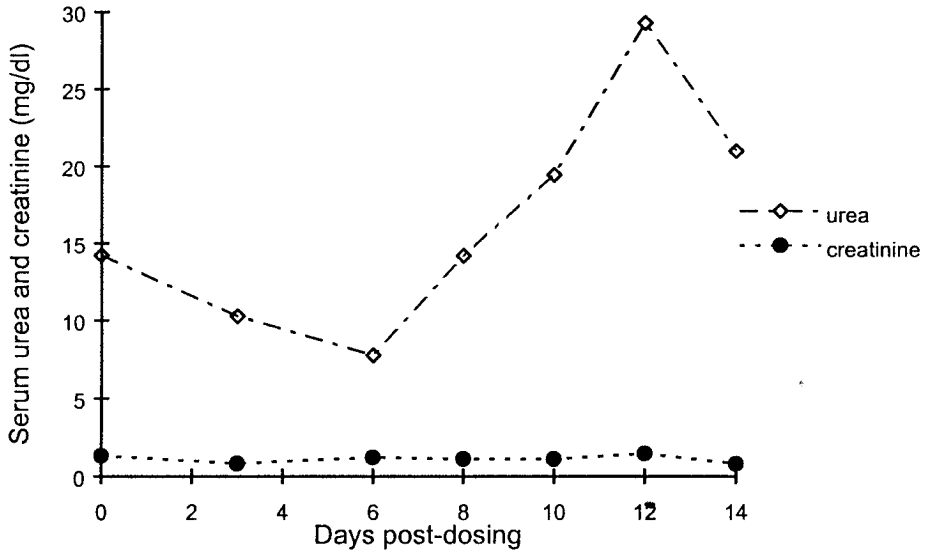
(b)
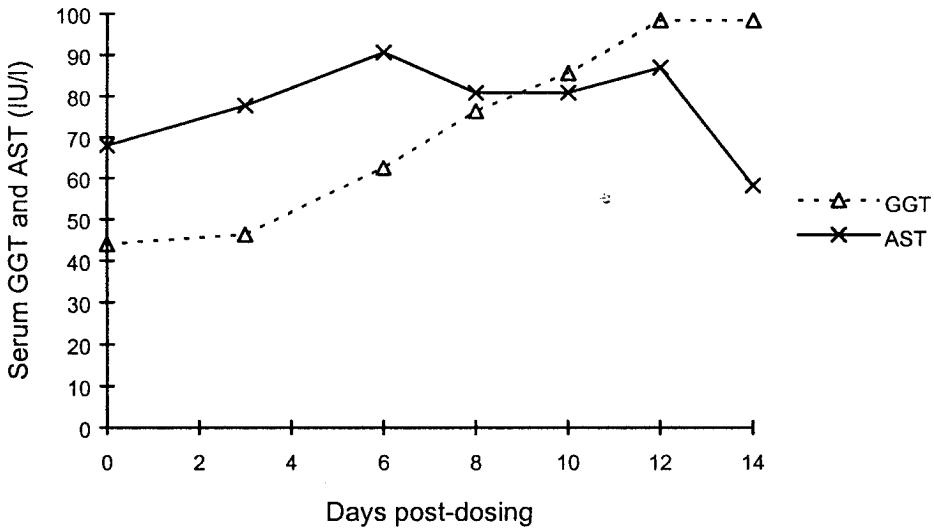
Fig 4.(a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat 1 of first experiment. Dosed with 10g/kg of S. cordifolia daily for 5 days (from day 0-5).

(a)
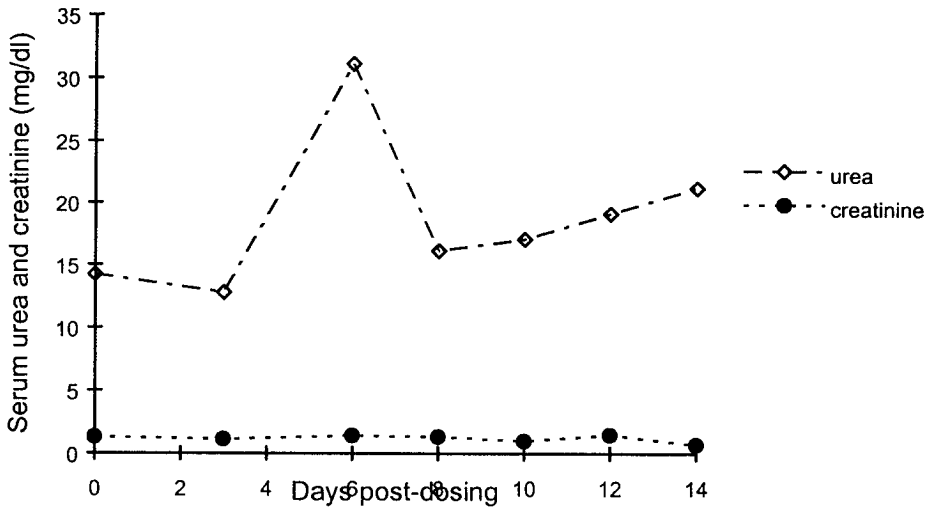
(b)
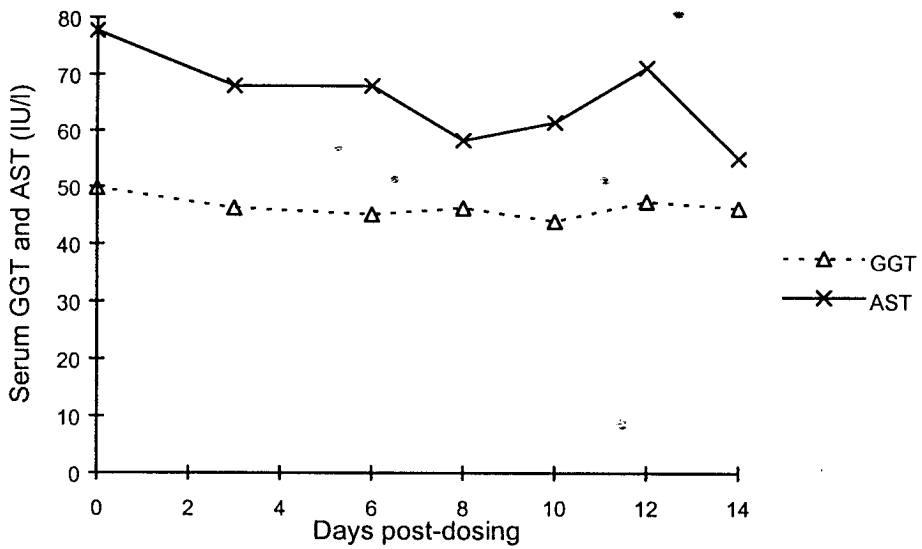
Fig 5. (a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat **2** of first experiment. Dosed with 10g/kg of *S. cordifolia* for 5 days (from day 0-5).

(a)
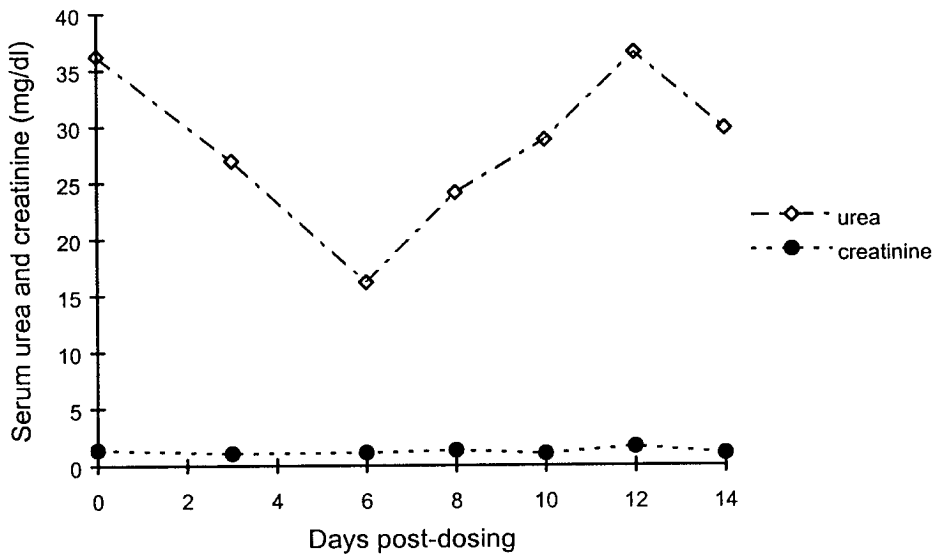
(b)
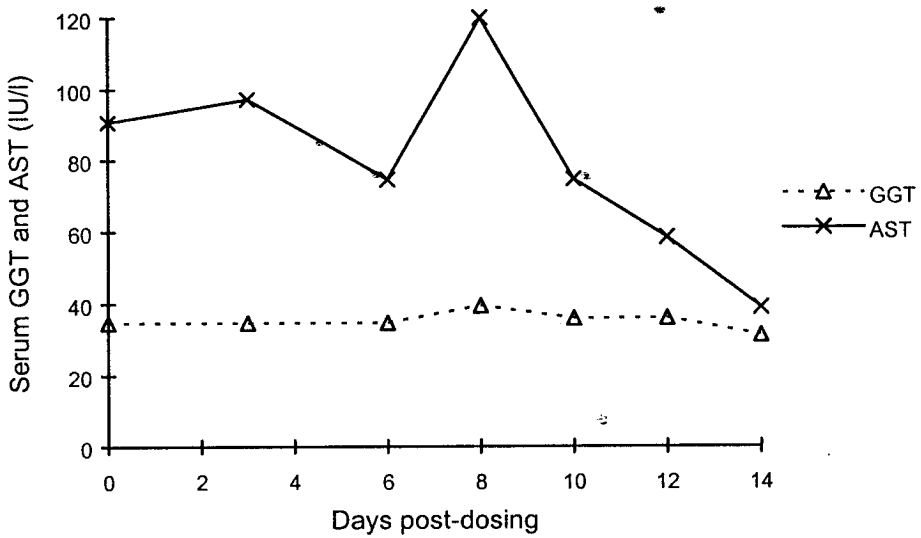
Fig 6. (a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat **3** of first experiment. Dosed with 5g/kg of *S. cordifolia* for 5 days (from day 0-5).

(a)
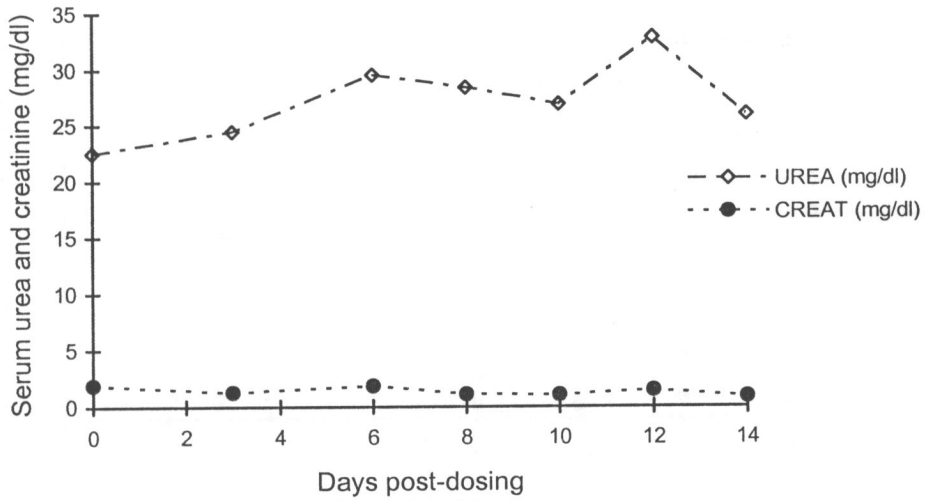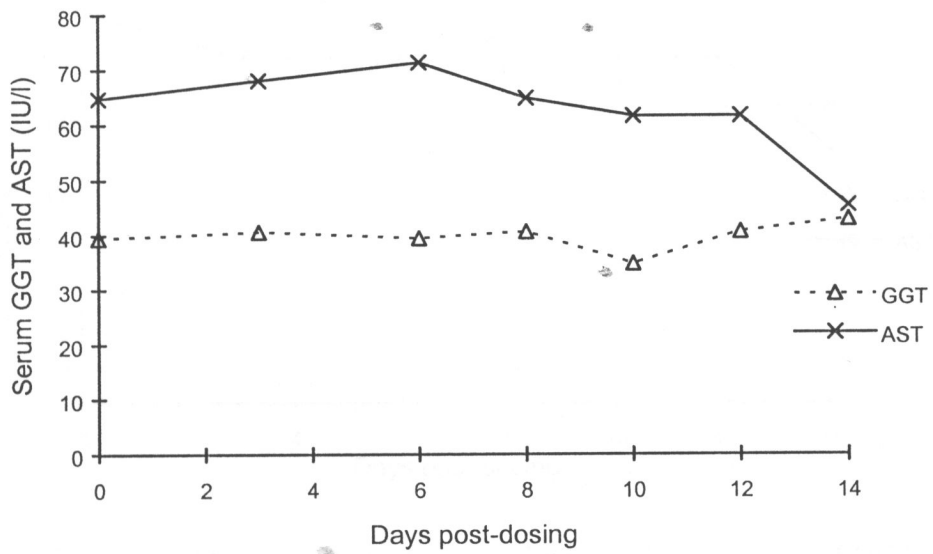
Fig 7. (a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat **4** of first experiment. Dosed with 5g/kg of *S. cordifolia* for 5 days (from day 0-5).
(a)
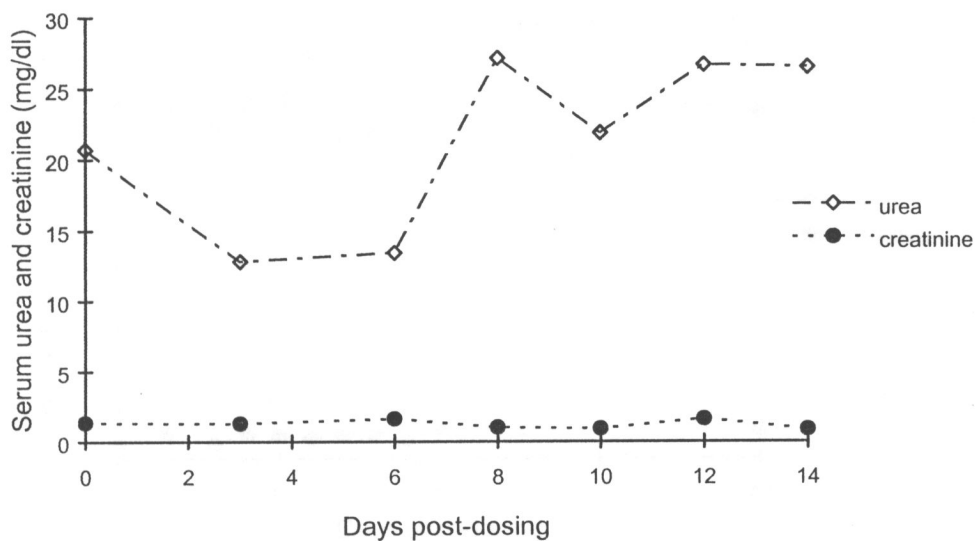
(b)
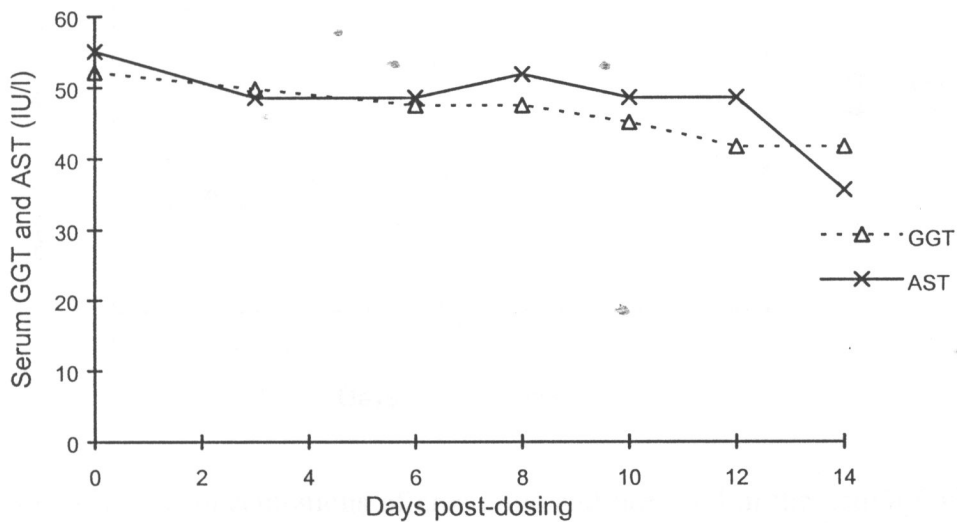
Fig 8. (a) Levels of concentrations of creatinine and urea and (b) activity of GGT and AST in the serum of a control Goat **5**

(a)
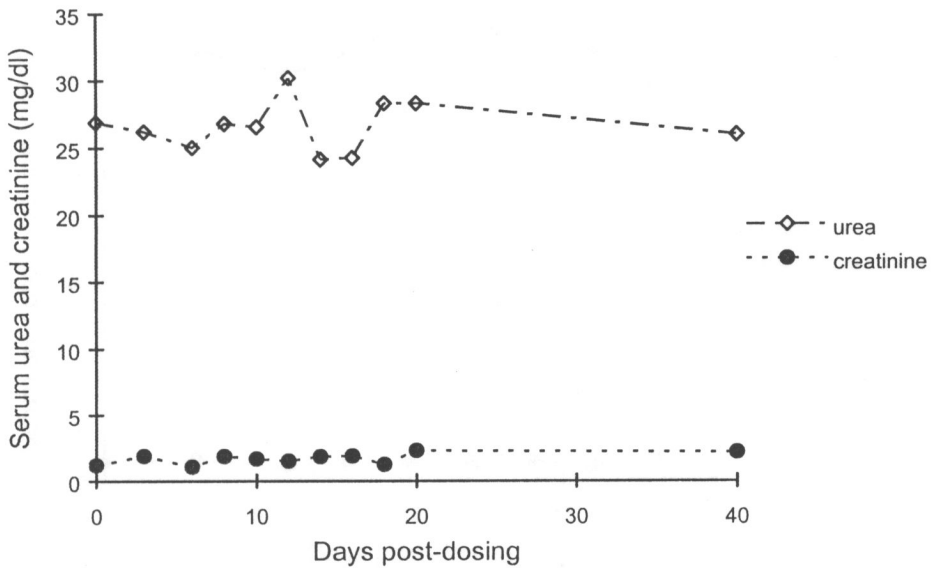
(b)
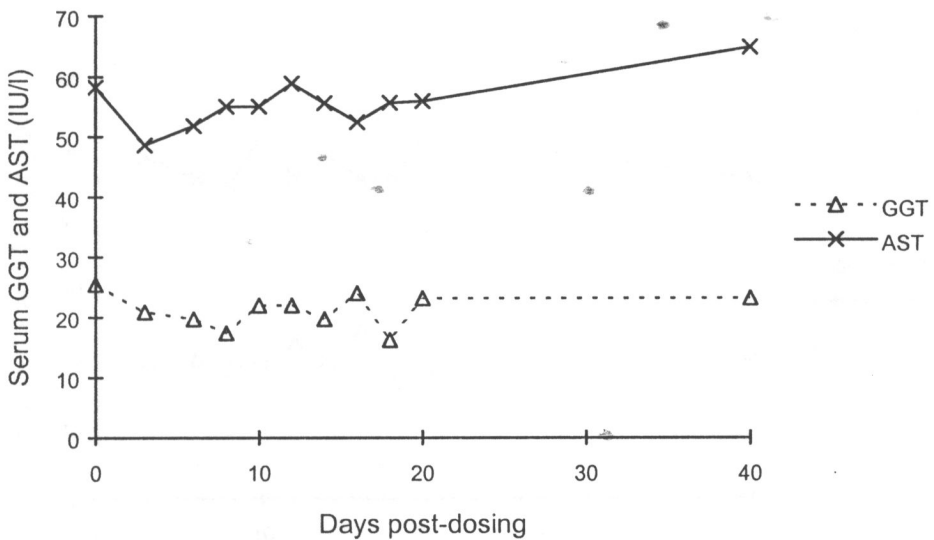
Fig 9 . Changes in the concentrations of creatinine and urea and in the activity of GGT and AST in the serum of Goat **6** of 2nd experiment. Dosed with 10g/kg of S. cordifolia for 10 days (from day 0-10).

(a)
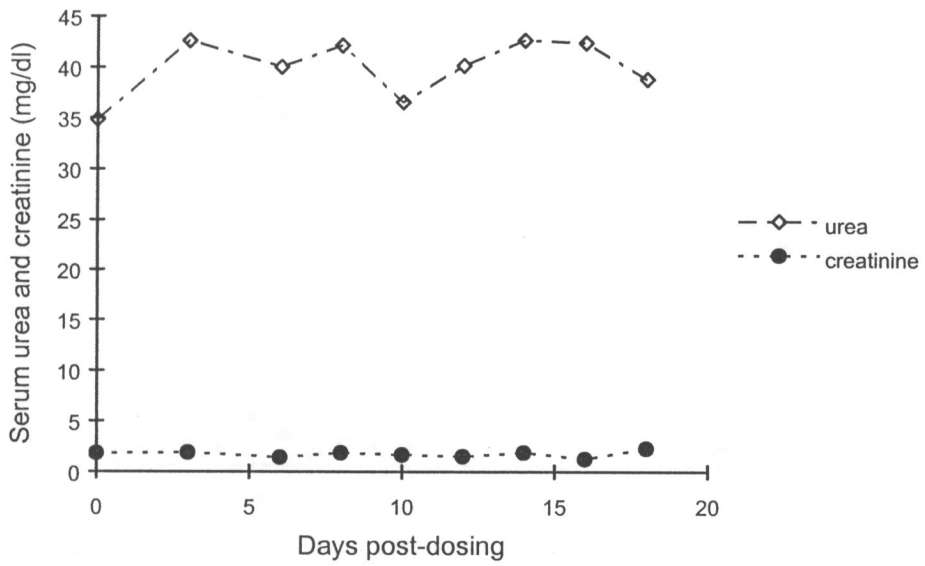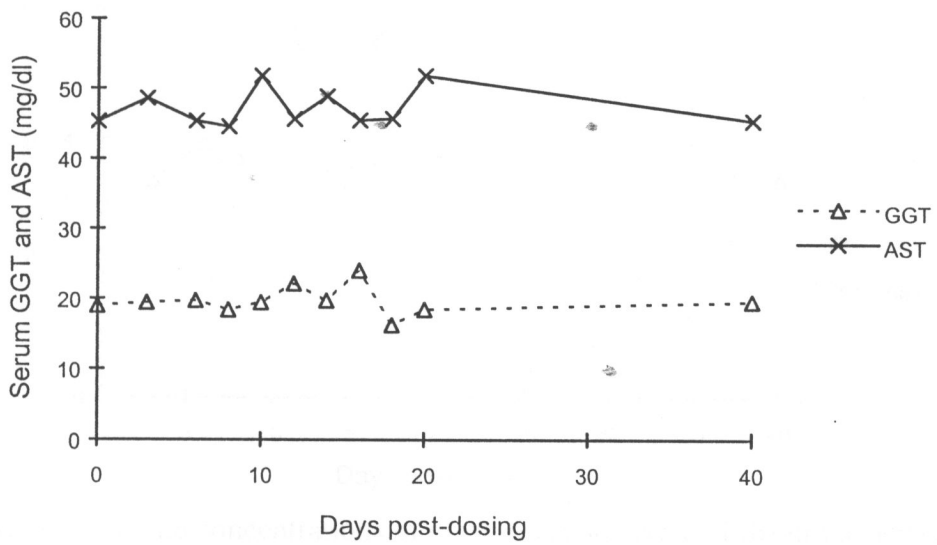
Fig 10.(a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat **9** of the 2nd experiment. Dosed with 10g/kg of S. cordifolia for 10 days (from day 0-10).

(a)
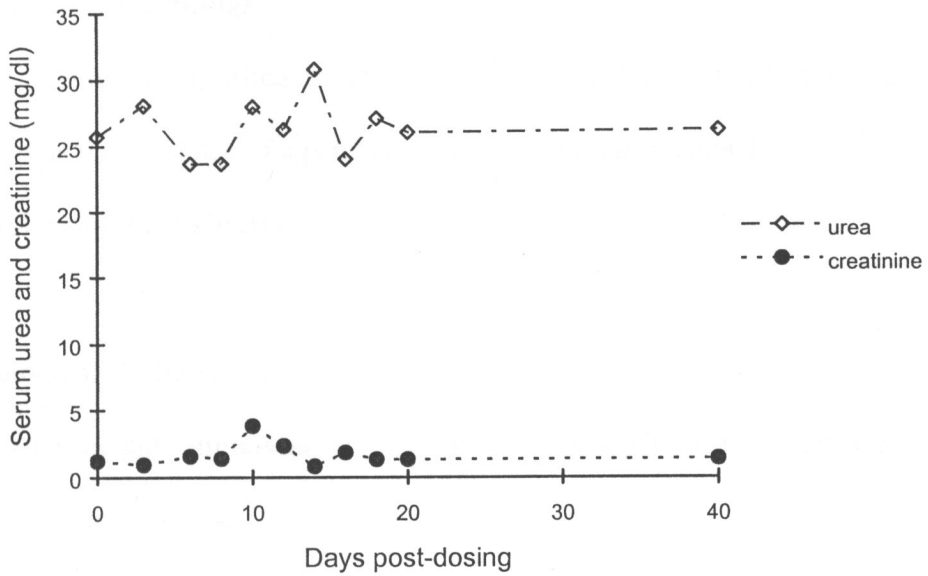
(b)
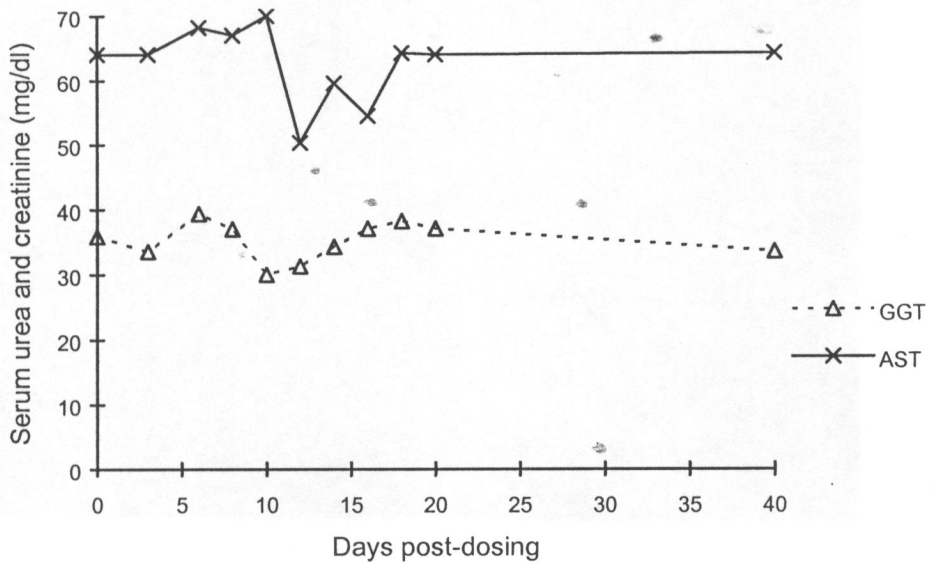
Fig 11.(a) Changes in the concentrations of creatinine and urea and (b) in the activity of GGT and AST in the serum of Goat **12** of the 3rd experiment. Dosed with 10g/kg of S. cordifolia for 20 days (from day 0-20).

4.3.3. Macroscopical findings

Liver: There were no significant gross lesions in the liver of all the goats. The only abnormality was the presence of a parasite, *Stilesia hepatica* in Goat 1.

Kidney: No lesions were observed.

4.3.4. Histological findings.

In all the experimental animals no significant lesion suggesting toxicosis was observed in both liver and kidney tissues.
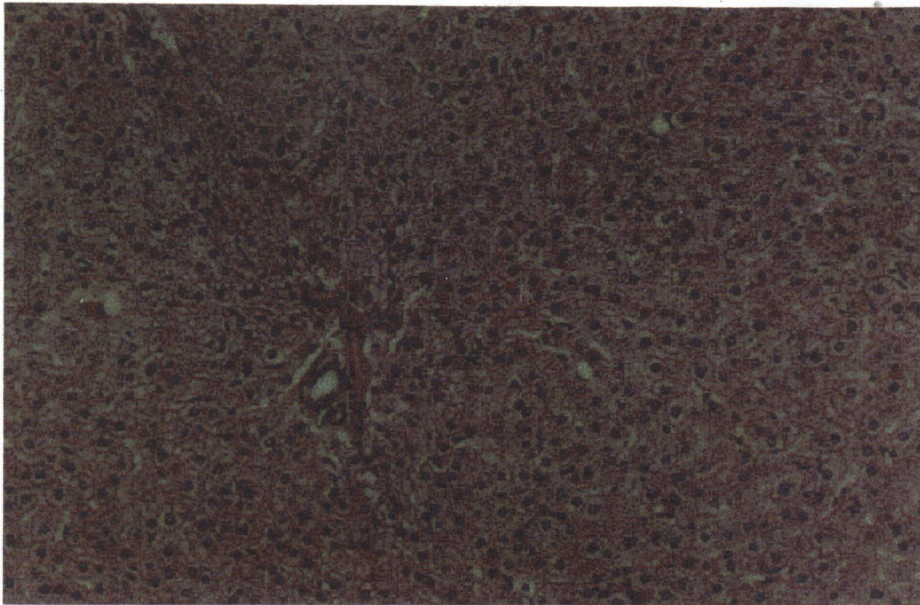
Plate 15. Liver from goat **1** dosed with 5g/kg of *Sida cordifolia* for 5 days. HE x 10.
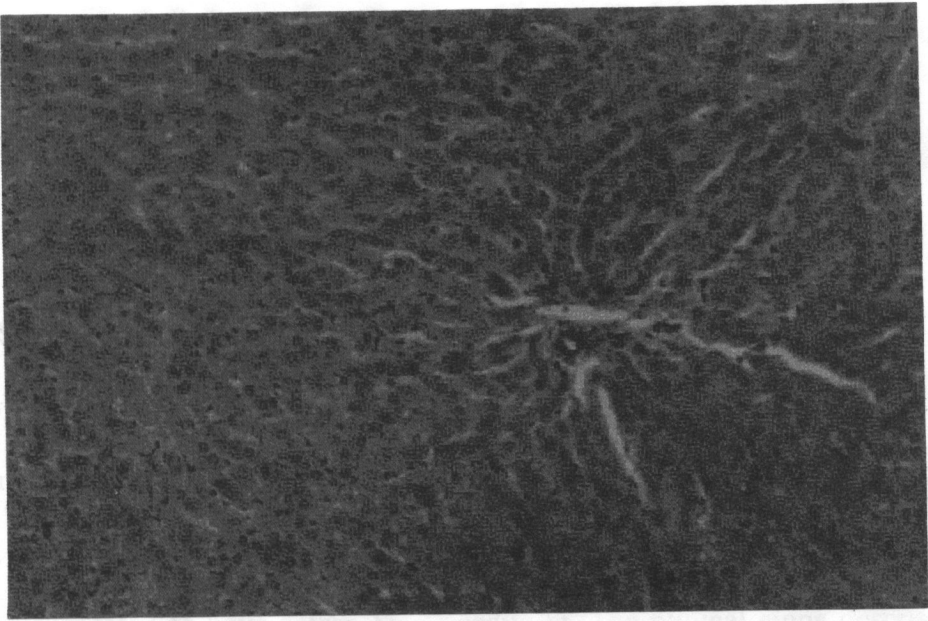
Plate 16. Liver from the control goat **5** showing normal liver cells. HE x 10.

CHAPTER FIVE

5. DISCUSSION AND CONCLUSION

The present study was planned to find out if the plant *Sida cordifolia* was toxic to small ruminants. A farmer (from Morester Farm) had reported mortalities in his goats and sheep which were suspected to have eatern *Sida cordifolia*. The clinical signs described from the affected farm were loss of weight, ascites, bloat and starring coat while histologically the lesions of livers were mild diffuse fibrosis and bile duct proliferation. To investigate if *Sida cordifolia* was involved at that farm, 14 female goats were drenched with various dosages of the milled plant to see whether signs and lesions described above could be reproduced. However, none of these signs were reproduced in all the experiments.

In the first experiment two different dosages (5g D.M./kg and 10g D.M./kg of body weight) with exposure period of 5 days and an observation period of 10 days were employed. This acute toxicity test (Timbrell, 1995) was meant to correspond with the disease entity in those animals which died without showing clinical signs. The two different dosages were used to check if there was a difference in the dose - response relationship. To monitor the development of any pathological lesion which could be detected on postmortem, serum metabolites for liver damage (GGT and AST) and for kidney damage (creatinine and urea) were measured. GGT and AST were chosen as indicators for hepatotoxicity because using GGT it is possible to detect lower grades of early acute hepatotoxicity while AST gives the best information on the course of development of severe acute hepatocellular damage (Malherbe *et al.*, 1975). In addition, GGT can not be suspected to have come from somewhere else because in the blood, this enzyme is known to be hepatic in origin (Malherbe *et al.*,

1975). The goats were sacrificed by exsanguination instead of using euthanasia in order to avoid unnecessary rise and fall of GGT by such drugs (Malherbe *et al.*, 1975). Creatinine and urea were chosen because they are good indicators of kidney damage (Braun *et al.*, 1978). The liver and kidneys were chosen as target organs because the liver being the major organ in handling xenobiotics is at high risk of damage considering that 20% of cardiac out put passes through it (Kahl, 1992). Similarly the kidney being the major excretory organ is also prone to many toxic agents (Kumaravelu, 1994).

In the present study the results of serum metabolites (GGT and AST) were not statistically significant (P >0.05). See Table 12 and 13. Takahashi and Nawa, 1997, (unpublished data, University of Zambia) found normal levels of GGT and AST in indigenous Zambian goats (Gwembe dwarf breed), ranged between 15-30 IU/Liter for AST and 8-15 IU/L for GGT. These values are half of what was found in this study (GGT, 32±4 IU/L : AST, 61±4 IU/L). Probably the difference could be attributed to different method used (Malherbe *et al.*, 1977). Generally there is always variation in the levels of these two enzymes in goats. For example, Mitruka and Rawnsley gave a range of 25-34 IU/L and 30-122 IU/L for GGT and AST respectively while Silanikove *et al.*, (1995) gave 29-33 IU/L for GGT and 36-44 IU/L for AST. An increase of 138 International Unit/Liter was recorded as high by Marzni et al., (1985). Therefore, values by Takahashi and Nawa, 1997, (unpublished data) and what has been found in this study fall within the normal range. The rise of GGT in Goat 1 (dosed with 10g/kg) was probably due to presence of the parasite *Stilesia. hepatica*. This assumption is supported by the fact that GGT is reported to rise in bile duct abnormalities (Muchili, 1975) caused by liver parasites (Cheeke, 1998). The parasite was discovered at postmortem examination as the only abnormality seen in the liver tissue. Lack of necrosis in the liver despite the presence of the parasite confirm the findings of Verster and Marincowitz, (1980)

who states that *S. hepatica* is not pathogenic. This parasite has also been reported not to respond to antihelmintic treatment (Hanson, 1990) except Praziquantel (Verster and Marincowitz, 1980). This may explain why in experiment 1 it was not possible to completely eliminate the parasite from all the goats because Praziquantel was not used. A temperature rise in goat 3 (Experiment 1) from 39°C to 40.2°C was not enough to indicate a pathological process because a diurnal variation of up to 1°C exist in most animals (Radostits *et al.*, 1994). Diarrhea seen in goat 4 was probably due to the high dosage of plant material (Van der Vyver *et al.*, 1985). Changes in body weight, food and water intake could mean adverse effects (Timbrell, 1995) but in this study this was not collaborated with changes in the serum metabolites or postmortem examination findings.

In the second experiment a dosage of 10g D.M./kg was used with an extended dosing period of 10 days and a prolonged observable period of 30 days to accommodate the morbidity period of those animals which died after a month of illness. The same reason was used to choose the dosing range in the third and final experiment where a reduced dosage of 5g/kg but with a longer dosing period of 20 days and an observation period of 20 days were employed. The extended dosing period and observation range in both experiments were long enough for the experiment to provide information on the target organs affected by the plant and the major toxic effects as well as accommodate any slow onset of the toxicity (Timbrell, 1995). In both experiments (2 and 3) there were no statistically significant changes (P > 0.05) in the serum metabolites before and after dosing. There were also no lesions in both the liver and kidneys to suggest any toxicity (Photo 15 and 16). Thus experiment 2 and 3 failed to induce toxicity syndrome in goats.

A toxicity test may fail to yield results for various reasons. Wrong identification of a plant is one of them (Seawright, 1982). Under dosing is another. Some factors described earlier in the literature review, i.e. animal, environmental and plant factors may also play a role. An example of animal factor is species difference. As Seawright (1982) puts it, failure of a toxicity test may be due to non susceptibility of test animals. Or indeed they may be of the same species but if the test animals were foreign there might be some genetic variation in the gastrointestinal tract of such animals such that they may not respond to a poisonous plant. An example is of Indonesian goats which can eat *Leucaena leucocephala* without any problem while the same plant can not be eaten by goats in Australia without toxic effects (Cheeke, 1998). This is because the rumen of Indonesian goats have developed a mimosine (toxin in *Leucaena leucocephala*) degrading microorganism known as *Synergistes jonesii,* which is not the case with Australian goats (Gregg, 1995). In such a situation a toxicity test with *Leucaena leucocephala* using Indonesian goats is likely to fail. Environmental influence like season and climate can also make a difference between a positive and negative toxicity test. Certain plants become toxic only at a certain time of a year such that if picked for toxicity test when it is harmless the results will be negative. *Lolium* species is known to be toxic only in winter, at least in South Africa (Kellerma *et al.*, 1988) thus if picked at any other season other than winter the results will be negative. The arrowgrass (*Triglochin maritima*) can produce negative results if picked from an area with enough moisture than from a hot and dry locality (Radeleff, 1970). Plant factor such as growth stage is of paramount importance. If picked when it less or non at all toxic the results will be negative. Similarly, the level of toxic principle in the plant can also influence a toxicity test. A test plant picked from an area with low levels of toxic principle can produce negative results. Henrici (1952) discovered that samples of *Tribulus terrestris* contained more saponins in one geographical area where the disease geeldikkop occurred than in another area where the disease did not exist. Thus if the

plant was picked from the latter area the results could have been negative for saponin toxicity. Another factor which can influence a toxicity test is management. A harmless plant can be rendered toxic if sprayed with insecticides, herbicides or fertilizer (Klaassen *et al.*, 1986) and thus give false results. On the other hand a toxicity test may fail to yield positive results because of use of a small sample size of test animals. This point was proved by Van der Vyer (1985) in his attempt to reproduce valsiekte poisoning. The plant used in the toxicity test, *Chrysocoma tenifolia* is known to cause low morbidity in sheep and as such it was difficult to reproduce the disease syndrome due to the use of a small size sample.

In this study the failure to reproduce the disease syndrome as described from Morester Farm could mean that the plant, *Sida cordifolia,* was truly harmless or that the negative results were due to some of the above mentioned factors. The plant was identified by an experienced plant taxonomist from Department of Biology, UNZA hence regarded as the real *Sida cordifolia.* Various dosages were used to accommodate any suspicion of under dosing. The experimental animals used were the true representative of the animals exposed in the field. Genetic variation in the gastrointestinal tract between field goats and experimental ones can be ruled out because the same breed (local) of goats just like those at Morester farm were used in the experiment. The animals were of the same sex, almost same size, they were put on a standard diet and these were of the right age group (6 to 18 month old) thus these factors could not influence the negativity of the experiment. The sample size of the experimental animals were more or less the same as those with other researchers who had positive results albeit different plants (Schneider *et al.*, 1987; Fourie *et al.*, 1989).

Available literature does not state weather *S. cordifolia* is toxic to livestock or poultry. Therefore, it is not known at which growth stage, fresh or dried does the plant becomes toxic, if at all. Similarly the level of active constituents were not isolated, characterized or

quantified. What is known in *S. cordifolia* is that it has very low sympathomimetic alkaloid content, about 0.085% (Watt and Breyer -Brandwijk, 1962) and these alkaloids considerably declines in older plants (Ghosal *et al.*, 1975). In this study the plant was picked at flowering stage and only tender aerial leaves together with flowers and seeds were used albeit dried. The presence of ephedrine in the plant should have at least elicited CNS signs or to stimulates α and β receptors thereby increasing the heart rate and reduce the breathing rate as it relaxes bronchiole muscles (Bowman and Rand, 1980 : Brander et al., 1991). Equally the presence of choline could also have stimulated signs like hindquarter paralysis, dyspnea and imperceptible pulse (Bowman and Rand, 1980). However, these signs did not occur either because of the use of dried plant or it was due to the fact that ephedrine is a relatively low psychostimulant especially if taken orally (Bowman and Rand, 1980) as done in the present study. The dried plant material was used just as Fourie *et al.*, (1989) did in his toxicity test of *Pachystigma latifolium* and Ibrahim *et al.*, (1992) when he fed *Azadirachta indica* to chicks. In any case, the use of the dried plant material in the present study was part of investigation and it is now known that dried *Sida cordifolia* failed to elicit toxicosis in goats. Further experiments are suggested to determine if the plant can cause toxicosis in fresh form.

Knowledge of active constituents of the plant is not necessary for the toxicity test. Ibrahim *et al.*, (1992) did not do know the level of the toxic principle in *Azadirachta indica* but still his results were positive. The toxic principle of *Cestrum laevigatum* remains unknown but was proved experimentally to be toxic to cattle (Kellerman *et al.*, 1988). This shows that a toxicity test may be conducted without necessarily knowing the toxic principle or measuring the level of active constituents. This is true for a plant you suspect to have caused death at a particular area. But if it is just to find out whether the plant was toxic or not then it is justified to pick the plant from different geographical areas in case in one area the plant has more active

constituents than other areas. At Morester Farm the emphasis was to test *Sida cordifolia* if it was toxic or not hence there was no need to measure the active constituents. At the moment it can be stated that dried *Sida cordifolia,* picked at flowering stage failed to induce toxicity in goats at a dosage of 5g D.M./kg and 10g D.M./kg of body weight.

From the retrospective study it shows that the goats and sheep of Morester Farm had liver problem. The main characteristics of the liver lesions were hepatic necrosis with bile duct proliferation (Plate 3), periportal necrosis (Plate 4), mild hemorrhage around the portal areas (Plate 6) and Kupffer cell infiltration (Plate 7). In comparison the liver lesions seen in small ruminants from other farms, the liver lesions from Morester Farm seemed unique and most of them were acute rather than chronic (no fibrosis was seen) a feature which was very prominent in liver tissues of goats and sheep from other farms (Plates 8-11). Unlike other farms the pattern of distribution of the above lesions was focal and the lesions were more in the portal area than centrilobular. However, at this stage it is still difficult to pin point the causative agents. Radostits *et al.,* (1994) tries to separate lesions seen in toxic liver against those caused by other factors. He states that in hepatotoxicity the lesions are centrilobular and may be extensive or may appear as a cloudy swelling while in infectious liver the lesions are likely to be patchy and even focal in their distribution. Parasitic lesions (e. g. flukes) show focal hemorrhages under the capsule with necrosis and traumatic injury identified by tracts. Congestive lesions (due to heart failure) are marked by an increase in blood, accentuation of the lobules and fatty infiltration of the parenchyma. Nutritional lesions are characterized by massive necrosis. Lesions caused by endotoxins (e. g. in mastitis) are multifocal hepatocellular necrosis. Some researchers have even attempted to pin point microscopic lesions caused by poisonous plants. For example, Kellerman *et al* (1988) grouped all plants that produce zonally distributed hepatic lesions as belonging to Asteraceae (e. g.

Lasiospermum bipinnatum and *Athanasia trifurcata*) and that fungus and algae are responsible for fatty change. Carlton and McGavin (1995) reported that most poisonous plants (*Xanthium* Species, *Cassia* Species, *Trema aspera, Cestrum pergui, Microcystis* and *Cycadoles*) cause centrilobular necrosis.

Going by the lesions seen at Morester Farm and what has been described by Radostits et al., (1994) there are more chances that the outbreak at the affected farm could have been caused by an infectious agent. As seen from the above most toxicants poisonous plants cause lesions in the centrilobular area probably because this is the area where detoxification by enzymes of mixed function oxidases (MFO) take place (Calton and McGavin, 1995). However, there are other plants that can cause lesions elsewhere in the liver tissue: *Lasiospermum bipinnatum* causes periportal necrosis (Jubb *et al,.* 1993). Thus the possibility of plant poisoning still stands though the distribution of the lesions weighs more toward an infection. This is where information from the survey becomes useful.

Looking at the possibility of other poisonous plants causing hepatic lesions seen at the affected farm, the offending plant should be one that causes acute hepatotoxicity because histological findings of liver tissues from dead sheep and goats from Morester Farm did not show fibrotic lesions to suggest chronic hepatotoxicity (see Plate 3-7). The plant must not cause photosensitivity because according to the history of the disease, there was no photosensitivity in animals which died after a month of illness. The plant must affect both sheep and goats but not cattle as the latter was not affected despite grazing together. Indeed the offending plant must grow in loamy/sandy soils (as this was the type of soil at the affected farm) and should be present in Zambia. According to Jubb *et al.,* (1993) plants that are likely to cause acute hepatotoxicity are Blue green algae (*Microcystis*), *Cestrum* Species, *Xanthium*

pugens and others belonging to Cycadales family. Kellerman *et al.*, (1988) grouped plants that affect the liver without causing photosensitivity as *Aspergillus flavus, Senecio* Species., *Crotalaria, Cestrum* species, *Hertial pallens, Pteronia* species, *Galenia africana* and *Xanthium* species. From the first group, members of Cycadales family are not known in Zambia though they have been reported elsewhere to cause acute hepatotoxicity in sheep (Jubb *et al*, 1993). Blue green algae can cause photosensitivity if taken subacutely and the hepatic lesions are usually massive which was not the case with the retrospective findings of lesions from Morester Farm. *Aspergillus flavus, Senecio* specie and *Crotalaria* have been known to cause chronic toxicity. *Pteronia* Species, and *Hertial pallens* affect only sheep (Kellerman *et al*, 1988) but at the study farm, goats were also affected. Hence the above plant may be ruled out. This leaves us with two plants, *Cestrum* and *Xanthium* Species as suspects. Although these two plants are wide spread in the country very few farmers know their existence or that they are toxic (from the survey farmers had listed *Lantana camara, Solanum Ricinus communis, Datura* Species, *Albizia* , aflatoxins* and *Fusarium graminearum* as common toxicants). *Xanthium pugens* usually causes toxicity in pigs though it is possible for goats and sheep to be affected (Radeleff, 1970; Kellerman *et al.*, 1988). Prominent microscopic lesions found in the liver of animals poisoned by *Xanthium pugens* have been described by Kellerman *et al.*, (1988) as ranging from diffuse necrosis of the centrilobular and midzonal hepatocytes to fatty changes. These lesions were not found at the affected farm. Even clinical signs caused by *Xanthium* poisoning (muscle weakness, opisthotonus, convulsions and GIT pains) did not tally with those described at the affected farm. This leaves us with *Cestrum* a plant known to cause toxicity in the month of June and July (Southern Africa) just as it was reported from Morester Farm that animals were dying in these two months. According to Kellerman *et al.*, (1988) microscopic lesions of *Cestrum* toxicity are centrilobular necrosis and hemorrhages sometimes accompanied by infiltration of neutrophils

and mild bile ductular proliferation just as in some of the lesions seen from Morester Farm (Plate 3-7). However, *Cestrum* does affect cattle something that did not occur at the affected farm despite cattle grazing together with sheep and goats. Generally sheep and goats are more resistant to plant poisoning than cattle (Cheeke, 1998) a fact which was noticed during the survey. The majority of farms that grazed cattle and goats together experienced more death in cattle than the small ruminants (see table 8). This could be attributed to cattle eating unselectively than sheep and goats which are browsers (they tend to select short grasses, fobs and shrubs). It is therefore, very likely that if the plant existed at the Morester Farm it could have been eaten by cattle. Thus *Cestrum* and the above suspected plants may be ruled out. More over the history given by the visiting clinician and herdsmen that the small ruminants were seen eating the plant was not collaborated by any findings of plant fragment in the stool or in the GIT of those animals that died suddenly without showing symptoms.

Looking at the possibility of chemical poisoning, Jubb *et al.*, (1993) short listed compounds that are likely to cause acute hepatotoxity as carbon tetrachloride, cresols, phosphorous and iron dextrans. Carbon tetrachloride cause fatty liver (Martin *et al.*, 1981) and phosphorous cause jaundice (Jubb *et al.*, 1993) which was not the case at the affected farm. Iron dextra poisoning is common in pigs than sheep and goats. Cresol poisoning occurs in animals kept in tarred walls and floor (Jubb *et al.* , 1993) which was not the case at the affected farm as the animals were kept in a barn without tarred floor. Therefore, it is very doubtful for these chemicals to have caused poisoning at the affected farm. Morester Farm used borehole water for their livestock and so did neighboring farms. Borehole water may cause fluorosis in livestock but such a possibility does not hold because during the survey a physical inspection of teeth in cattle and small ruminants by the author did no show any sign of fluoride toxicity nor did farmers complain of the disease despite the extensive usage of this type of water by

almost all the farmers interviewed. This shows that, at least in areas around Lusaka, fluorisis is not a problem. The only problem with water was with one farmer who used sewage water for his dairy cattle. The farmer was one of the three who had experienced drought on their farms. This was sewage poisoning though it has been claimed that there is no scientific foundation for the existence of such condition (Garner, 1961). Another possibility is poisoning by eating contaminated pasture. It was noticed during the survey that in most neighboring farms the dominant natural grass was *Hyparrhemia* species which the small ruminants used to graze. There were a few farmers though, who cultivated stargrass (*Cynodon plectostachyus*) for dry season grazing especially for dairy cattle. Stargrass if heavily fertilized and allowed to be grazed by full term ewes tends to cause prenatal losses in lambs (Kellerman and Naude, 1996). At Morester Farm the dominant pasture which small ruminants grazed was Kikuyu grass (*Pennisetum clandestinum*). Thus it is not possible that stargrass could have caused poisoning at this farm. Yet another possibility is poisoning from parasiticides. It has been reported that some of these chemicals (e.g. Chlorinated hydrocarbons) are not readily lost from treated forage especially the growing vegetation where it can persist for months (Radereff, 1970). However, at Morester Farm the history was that the sheep and goats used to frequent an uncultivated field where no application of fertilizer or herbicides was conducted. The acaricide used at the farm was Alphamenthrin. This dip has its effect on the neuromuscular system and besides young ones were not dipped with this acaricide until 6 month of age. This then rules out dip chemical as cause of poisoning and any chemical poisoning for that matter.

Looking at nutritional deficiency, especially mineral deficiency, in this country, it is a bigger problem of large animals (cattle and horses) than small ruminants. That is why the majority of farmers interviewed put much emphasis on supplementing large animals. Probably the small

ruminant characteristics (resistance to dehydration, preference for browsing and wide range feeding habits) enabled them to thrive without supplementation. However, during severe drought all animals can be affected. If the animals are not supplemented during drought they eat anything to survive, in the process increasing chances of eating poisonous plants. Therefore, supplementation may reduce chances of eating poisonous plants. In addition, minerals deficiencies and imbalance can lead to weakening of body immunity rendering the animal to an infection.

The soil at Morester Farm had very poor mineral content. Most of the important minerals i.e. copper, potassium, sodium and zinc were all low. Deficiency of minerals in the soil causes plants to be deficient also. Such plants will have low protein value, energy, low palatability and such plants have antinutritional factors (Low et al., 1993). It is very likely that these are the type of plants the small ruminants at Morester Farm were subjected to in the paddock they used to graze. Copper deficiency is the most serious mineral limitation of animal production in tropical areas (Abdelrahman and Kincaid, 1992). Clinical signs of copper deficiency include anemia, poor growth, bone disorders, scouring, infertility, depigmentation of hair and wool, gastrointestinal tract and lesion in the brain stem and the spinal cord. However, the problem at Morester Farm points to copper toxicity than deficiency. Copper toxicity causes liver necrosis (McDonald et al., 1987) but at this farm, mineral supplementation was not that adequate to cause toxicity. Potassium and Sodium deficiencies rarely occur in goats and sheep (McDonald et al., 1987) but if it happens the important clinical signs are paralysis and poor growth. Zinc deficiency mostly affects pigs and chickens and toxicity rarely occurs because most animals have a higher tolerance of Zinc (McDonald et al., 1987). All the above deficiency signs of mineral deficiency were not apparent at the farm despite the fact that supplementation was not regular. On the other hand supplementation can also cause problems. For example, bad preparation of supplement can also cause problems. One farmer had

contaminated his supplementary feed (sunflower cake) with aflatoxins and lost four dairy cows. Another wrongly prepared a supplement of chicken manure and lost 40 sheep. However, at Morester farm all the supplements were purchased from recognised dealers. Thus nutritional deficiency can not be linked to the cause of an outbreak at Morester farm.

This leaves us with the possibility of infectious agents being responsible for causing the liver lesions. Although poisoning is the common cause of liver disease in farm animals, bacterial infections like *Listeria monocytogenes*, *Campylobacter*, *Yersinia pseudotuberculosis*, *Pasteurella hemolytica*, *Haemophilus*, *Salmonella* and *Mycobacterium* have been implicated (Radostits *et al.*, 1994). Riftvalley fever, a viral disease, is also a suspect. Going by the survey, most farmers, including Morester Farm, paid more attention in the prevention of Clostridia than any other small ruminants diseases. Rift valley fever although prevalent in the country and in particular in the study area, Lusaka (52%) (Hussen et al., 1987) few farmers vaccinate their animals against this disease. Thus the possibility of an infection with this disease was there at the affected farm. However, the history from the affected farm does not mention any abortion and the disease was more pronounced in the young than adults. Thus Riftvalley infection may be ruled out. Similarly, *Listeria, Campylobacter, Yersinia* do cause abortions (The Veterinary Merck Manual, 1991) therefore these agents could not have been involved. *Pasteurella* and *Haemophilus* are more of respiratory diseases while it is rare for *Mycobacterium* to cause liver problem in goats and sheep (The Veterinary Merck Manual, 1991). This leaves us with *Salmonellosis* and *Corynebacterium* as the most probable cause. Salmonella may be ruled out because it usually cause enteric syndrome than liver lesions. Going by the distribution pattern of lesions and clinical signs in animals from Morester Farm, one would quickly conclude an infectious disease involving corynebacteria. What more with the isolation of this bacterium from specimen brought from the affected farm and the

introduction at the farm of a billy goat with "septic tick bites" before the outbreak. Corynebacterium is able to produce a toxin leading to toxemia (Radostis *et al.*, 1994) and could have been responsible for those animals that were dying without showing clinical signs. It may be argued that Corynebacteria vaccine was used at the farm but this was after the disease had occurred and moreover corynebacteria has a lipid cell barrier which makes it difficult to treat or maintain an effective vaccination (The Veterinary Merck Manual, 1991), hence the continuous out break. It may be further argued that there was no need to conduct a toxicity test after all the bacterium involved was already isolated and that the plant's extracts were well known that they were more sympathomimetic (Ghosal *et al.*, 1975) than hepatotoxic. The reason is that there are still some unknown factors that can cause a plant to be toxic. Some plants grown in certain localities may be analyzed and found to contain harmless properties but the same plant growing in different area may accumulate certain compounds like nitrites or fluoride and cause poisoning. For example, stargrass (*Cynodon nlemfuensis*) may appear harmless but can cause fluorosis in areas where the soil has too much of fluoride (Botha *et al.*, 1993)

The survey points to something else other than *Sida cordifolia* as the cause of the outbreak at Morester Farm. This was one of the aims of conducting the survey to see if there were other causes responsible for the outbreak. The other aim was to see if there were any similarity between the affected farm and its neighbors in the vegetation, farm management, husbandry, disease outbreaks and climate although the weather could change in some farms (some farms were badly hit by the drought than others despite being in the same locality i.e. Lusaka area. Morester Farm had so many similarities with the neighboring farms. The vegetation was similar, the dominant grass being *Hyparrhemia* spp, the soil sandy/loamy, water supply through boreholes and most did not grow crops on farms where animals were raised. Grazing

pattern was same - free range. Weaning of small ruminants and separating the young from adults was not practiced by most farms (serve for two). Large animals were always herded while small ruminants in some farms were not (Morester Farm included). The notion that herded animals may have less opportunity to avoid poisonous plants (Cheeke, 1998) was not apparent in this survey as all farms experienced mortalities from suspected poisonous plants. Probably the sample (15 farms) was so small to have any significance. Other similarities were that all farmers housed the small ruminants at night although the difference was in the structure of houses. Some farmers housed their stock in small wooden structures with a roof on (e.g. Morester Farm) or in tobacco barns like houses as seen on several other farms. It is known that housing can affect disease pattern on the farm; a warm wet barn can promote diseases like naval ill, mastitis, enteritis coccidiosis and corynebacteria (The Veterinary Merck Manual, 1991). At Morester Farm the use of small houses could have helped to quickly spread the disease. All of the commercial farmers visited observed similar methods of controlling common diseases both in large animals and small ruminants. They dewormed against local helminths, dipped their stock against tick-borne diseases, they vaccinated against common diseases and in small ruminants all of them put much emphasis on controlling Clostridia infection than any other disease. Since most of the commercial farmers in this country are educated and united they often share information on any abnormalities that may affect their farms. Hence they carried out similar control measures on their farms.

The noticeable differences between Morester Farm and other farms was that the former was the only farm that experienced a disease outbreak in goat and sheep only. Other farms had experienced mortalities in both large and small ruminants and that the cases were sporadic. The disease signs described by other farmers, diarrhea, bloat, dyspnea, frothing, salivation, paralysis and lethargy are slightly different from those of Morester Farm i.e. ascites, weight

loss, bloat and staring coat. Signs from other farms are typical of poisoning while those from Morester are difficult to judge off hand. Even if cases from other farms were not confirmed as caused by plant poisoning the pattern of liver lesions (in small ruminants) were centrilobular and chronic while Moresters' appeared periportal and acute. Although most other farmers claimed the diseases were acute the liver lesions seen in the small ruminants showed chronicity. Probably its due to the fact that the liver is an organ which has very large reserve of function until three quarters of hepatic dysfunction appears (Radostits *et al.*, 1994).

The similarities described above showed that Morester farm was not that different from others. The difference, especially in the pattern of the disease outbreak and liver lesions suggest the problem was unique at this farm. The fact that only two farmers knew the existence of *Sida cordifolia* on their farms (one even mentioned that he had no problem with it) it is a clear testimony that the plant hardly causes any hepatotoxicity. More so that the plant (*S. cordifolia*) failed to raise levels of serum metabolites in experimental goats or reproduce hepatic lesions as seen from the retrospective study just goes to that *Sida cordifolia* is not toxic to Zambian goats.

Foot note by the Main Supervisor

Had detailed gross pathology findings on all goats been included, a clearer conclusion would have been drawn regarding the main quest of this study, ie. toxicity of *Sida cordiforlia* to goats.

CHAPTER SIX

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CHAPTER SEVEN

7.0. ANNEX

7.1. FARMERS QUESTIONNAIRE

General information

Name of farm..

Owner..

District..

Altitude.....................Maximum Temp.....................Annual rainfall................

Hectarage..

Farm description

Q 1. Soil type (have you done any soil analysis)..

Q2. Vegetation..

Q 3. Do you apply fertilizer to your vegetation..

Q 4. What type of poisonous plants do you know which exsist at your farm..........

Q 5. Water source; dry season..

wet season............. ....

Q 6. Type of crops grown..

Q 7. Type of herbicide/insecticide used on the farm..

Q 8. No. of sheep.................breed..

Q 9. No. of Goats................breed..

Q 10. Other species available on the farm..

Q 11. Is there a drought problem at your farm? (If yes, which months)................

Management (Sheep and Goats)

Q 12. Grazing regime (free range or zero grazing)...

Q 13. Type of supplement given...

Q 14. When is supplement given (dry/wet season)...

Q 15. Is the supplement given prepared at your own farm?...............................

Q 16. Do animals of different species graze together or graze in rotation or are kept on separate pastures...

Q 17. Type of breeding control: castration, separation of female from male or others (please specify)...

Q 18. When is parturition...

Q 19. When is weaning...

Q 20. Are weaners separated from adults..

Q 21. Are the animals housed at night?..

Diseases seen on the farm (goats and sheep)

Insert: C = common, O = occasionally, R = rare, N = non

Q 22. Riftvalley......; Pulpy kidney..........Corynebacterial............Mange..................

Mineral deficiency...................Coccidiosis.....................Worms............................

Q 23. Did you suspect any poisoning; chemical or plant origin.................................

Q 24. Which age group was affected, young or old..

Q 25. Describe symptoms of suspected poisoning...

Q 26. Was postmortem conducted. If yes what was the main lesion seen................

Q 27. Has any unknown disease occured on your farm...

Q 28. How often does the unknown disease occur and in which month...................

Q 29. Describe the symptoms of the unknown disease..

Disease control measures(Sheep and Goats)

Q 30. Vaccination (indicate type used and frequency)...

Q 31. Dipping (indicate type used and frequency)...

Q 32. Deworming (indicate type used and frequency)...

Major disease outbreak seen in other species

Q 33. Which animals were affected...

Q 34. Which age group was affected..

Q.35. Which disease did you suspect...

Q 36. How long was the duration of the disease...

Q 37. Which month did the disease occur...

Q 38. Which plants were the animals eating during the outbreak?..............................

Q 39. Was the pasture overgrazed during the outbreak...

Q 40. Did it affect all the paddocks...

Q 41. What was the difference between the affected paddock/s and the unaffected.

....

Q 42. What was the symptom of the disease...

....