THE TOXIC EFFECT OF SIX COMMONLY OCCURRING PLANTS IN NIGERIAN PASTURES

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ABSTRACT

The toxicology of six suspected poisonous plants Leucaena leucocephala Benth, Tribulus terrestris Linn Eugenia uniflora Linn, Dichapetalum madagascasience Poir, Lantana camara Linn, and Solanum torvum Benth); were evaluated in Sprague Dawley rats weighing between 200-300 grams. Two of these plants, Lantana camara and D. madagascasience were re-evaluated in West African dwarf goats. Spectrophotometric and titrimetric methods were also used to determine some inorganic and metallic contents of the plant.

The extracts of all the poisonous plants except S. torvum and D. madagascasiense produced dose dependent effects on rat serum proteins. The extracts of these plants which produced statistically significant changes ($P < 0.05$) in serum proteins of the rat include the 400 and 600mg/kg doses of the extrct of L. leucocephala; 400 mg/kg dose level for extract of T. terrestris; 200 mg/kg dose level for the extract of E. uniflora; 400 and 600 mg/kg dose levels extract of L. camara. The globulin fraction did not show any significant changes in all the rats treated with the poisonous plants. No changes were also observed in the serum dectrolytes analysed.

T.terrestris, L. Camara and E. uniflora produced dose related increases in activities of serum enzymes, alanine aminotransferase ALT (EC 2:6.1.2), aspartate aminotransferase (AST) (EC.2.6.1.1.), and alkaline phosphatase (ALP) (EC.3.1.3.1.). All the plants

produced significant increases in ALT except L. Leucocephala and S. torvum whereas E. uniflora, L. camara, and D. madagascasie use produced significant changes in AST (P<.0.05).

An evaluation of the haematological parameters of the treated rats, revealed that only extracts of T. terrestris, and L. camara produced dose dependent decreases in the total red cell blood count. These decreases were significant for, all the doses of the extracts of T. terrestris; the 400 and 600 mg/kg dose levels of L. camara ($P < 0.05$). The decreases in erythrocyte counts were associated with decreases in haemoglobin concentration.

The piosonous plants did not cause changes in the blood coagulation time. Only the extract of T. terrestris produced decreases in total leucocyte count.

Similarly, only the extract of T. terrestris caused clinical symptoms in the rats which included depression and inappetence. However, all the plant extracts except that of S. torvum produced gross pathologic lesions in the liver including pin point or ecchymotic hemorrhages. Histopathologic lesions include necrotic lesions of the hepatic tissues and vacuolation of some hepatic lobules. T. terrestris, L.camara, E. uniflora and L. leucocephala produced gross lesions of the renal tissue. Histologically the affected renal parenchyma was hyperemic and the tubular epithelium showed degeneration. The extracts of D.madag-gascasience and L. camara produced gastrointestinal lesions including superficial

necrosis of intestinal epithelial lining and desquamation of patches of the gastric and ileal mucosa.

In the West African Dwarf goats, L. camara and D.madagascasience produced increases in the serum activities of ALT, AST and ALP. Furthermore, these plants also produced increased total blood protein level and blood urea nitrogen. Lantana toxicity in the goats also produced decreased total RBC counts, decreases hematocrit and increased haemoglobin concentration. Animals with Lantana toxicity also showed anorexia and were emaciated. D. madagascasience did not produce changes in haematological parameters.

Phytochemical analysis showed different levels of cyanide, (CN-), nitrate (NO3-) nitrite (NO2-), oxalate and elements such as lead)Pb+), zinc+), iron (Fe++), manganese (Mn+), and copper (Cu++) in all the plants

These studies therefore shows that T. terrestris E. unifora, L. leucocephala, L. camara but particularly L. camara and T. terrestris are hepatotoxic, produce renal lesions and also affect hemopoiesis. Thus these plants are toxic to livestock and their growth among normal pasture could post potential risk to livestock production in Nigeria.

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CERTIFICATION BY SUPERVISORS

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

The human race and his animals depend on plants as a renewable source of many foodstuff. However, the harmful effects produced by certain constituents of these plants in many parts of the world each year, are not so widely appreciated (Kinghorn, 1979, Habermehl, 1987, Fernando 1987, Wee, 1987).

Plants constitute the third largest category of poisoning (Woodward 1985, Fernando 1987). Such plant poisoning are attributed to the fact that house plant have become more popular and many of these are unfortunately poisonous. Such poisonous plants are all around us as potted plants, imported exotics grown in green house, cultivated hybrids, decorating the gardens and even as everyday foodstuffs on the larder shelf. Moreover families are outdoors more with the renewed interest in the environment. There is more camping, picnicking, hiking and gardening therefore man and his animals are constantly in contact with poisonous plants (Woodward, 1985).

Conditions which favor the occurence of plant poisoning in livestock are prevalent in Nigeria. They include nomadism, luxuriant growth of pasture with poisonous plants, movements to obtain grazing on hoof from the north to the south. (Nwude and Prason 1977). During the annual dry season and particularly during

the periodic droughts animals are exposed by changing from one environment to the other to strange vegetation. Such cattle are usually unfamiliar with the plants in the new environment and are liable to be poisoned. The natural vegetation is in most instances relatively undisturbed in many areas so that poisonous plants could still constitute a significant part of the flora. In some areas, much of the land could be grazed, resulting in- sufficient palatable forage even during the rainy season (Ewer and Hall 1978).

Poisoning by plants in animals could arise from the following:-

(i) the seasonal nature of the growth of the poisonous plants or parts of it such as the seeds flowers or leave, which invariably are normally poisonous. For example Sclerocarpus africanus is found as a contaminant of hay and causes a lot of poisoning (Evans, Evans and Hughes 1954, Rajendran, Chennakasavalu, Rao Viraraghavan and Damodaran 1983). Both the leaves rhizomes contain the toxic principle the concentration of which vary with season (Thomas 1963).

(ii) Climatic conditions such as high humidity, dull or bright weather and temperature are known to favor the proliferation of poisonous plants and even the elaboration of the toxic constituents.

(iii) Location of the poisonous plants play a vital role in terms of how readily accessible they may be to the grazing animals. Poisonous plants which can only be found in the high rising forest will definitely not be a source of worry for livestock owners. However if these plants can be found in the grazing areas of the animals then these plants can be grazed along with the normally grazed plants which will cause toxicity. For example Holarrhena floribunda and Adenum obesum are trees or high shrubs and animals may not reach the leaves (Irvine 1961).

(iv) Most poisonous plants are unpalatable and may therefore not be grazed. The unpalatability may be associated with bitterness, presence of spines e.g. Encephalatos bateri, Euphorbia camerunica; the woodiness of the plant e.g. Solanum dulcamara (Woody nightshade); the presence of a repulsive aromatic smell e.g. Lantana camara; or the ability of the plant to cause blistering or rash around the mouth parts of the animal, e.g. Asclepias (milkweed) or Calotropis procera (Hutchinson and Dalziel 1954, Ewer and Hall 1978; Azariah, Azariah, Hall, Kesan, Sumathi and Sundararaj 1987). Animals will normally not graze such plants assuming that there were more nutritious species of plants around. Animals during drought or newly introduced animals will however be compelled by hunger to browse any available plant.

(v) The species and age of the animal affect plant poisoning since susceptibility or tolerance to poisoning is

species related sheep and goats are known to be more tolerant whereas young animals pigs and horses are easily poisoned (Hutchinson and Dalziel 1954).

(vi) Occasionally, the poisoning may not be due to a plant but to some fungi growing on it. This is especially common with certain fungi which attack cereal grain e.g. Aspergillus flavus which cause aflatoxicosis, and Claviceps purpurea which causes ergotism, by attaching themselves to grasses mainly Paspalum species and then render them toxic (Lim 1987).

(vii) Often animals which were born and grew in a locality become tolerant to some poisonous plants whereas animals which are newly introduced will quickly die because they are not able to tolerate the active principle. Furthermore, animals which may become familiar with the effect of certain poison plants will avoid it, whereas newly introduced animals will eat it and get poisoned. This situation frequently happen with normadism or transhumance.

(viii) Sometimes the degree of toxicity varies according to the soil type e.g many poisonous plants are much more toxic in limestone soil. High selenium soils may be very clearly marked by the selenium accumulators growing on them e.g. Astragalus species such as A. bisculcatus and A. pectinatus which may contain up to

300 ppm of Selenium (Avery, Nulo, Kramer, Johnson, Darcel and Leo, 1961).

Many plants have all their parts toxic e.g. P. aquilinum whereas some only in one or two parts such as the seeds or the flowers or the roots. This means that animals can only be poisoned if the poisonous parts e.g. the seeds are consumed e.g. Ricinus communis. If it is only the roots which are poisonous then animals like pigs which are notorious for their rooting habit will easily be poisoned e.g. Manihot escu- lenta, P. aquilinum (Evans, Widdop, and Harding (1972).

Sometimes the plants will change from the toxic to the non toxic state or vice versa for no apparent reason. This is as a result of variability in the toxic principle they contain.

Frequently the intervention of man may lead to poisoning of livestock by naturally occurring substances which are undou- btedly potentially dangerous but which are normaly avoided by animals. For example Equisetum species and P. aquilinum are refused by grazing animals if other herbage is accessible, but when the animals are housed and the dried plants are presented to them, in hay the unfortunate beast have no alternative but to eat them or starve (Clarke and Clarke 1975.

1.1 The Potentially harmful effects of toxic materials in plant foodstuffs.

Toxic contaminants can gain entry into plants by direct application of pesticides to plants that are eaten by animals, from contamination of the soil by pesticides applied to plants, from chemicals added to the soil or from the presence in soil of radionuclides.

Watt and Breyer-Brandwiyk (1962) observed that meat that had been roasted or grilled on wooden skewers derived from the ornamental shrub Nerium oleander has caused death from injestion of the cardioactive (digitalis glycoside) substance. A great variety of foreign substances have been demonstrated in cow milk and in human milk which might have been derived from injestion of plants, drugs or other chemicals (Sapeike 1969) Plant substances which gain entry into milk may produce minor or major unwanted effects. (Leiner 1974).

Goitrogenic substances such as goitrin in cabbage, Kale, turnips rapeseeds and other plants may pass into milk of cows that eat these plant. These may cause interference with the thyroid function in human, who drink such milk (Clement 1960). An infant that drunk milk from a cow that had eaten the foliage of Nerium oleander was observed to have died from this cause (Watt and Breyer-Brandwiyk 1962).

The juice from Solanum incanum had been used by the Transkeian Bantu in South Africa to curdle milk. There is high incidence of esophageal carcinoma in these group of people. Since dimethylnitrosamine (DMNA) has been identified in Solanum fruit, it is suggested that there may be a causative association between DMNA and nitrosamines and this neoplasm. (Du Pleiss, Nunn, and Roach 1969).

McBarron, Walker and Gardiner (1975) observed the death of twenty out of five hundred lambs who obtained water from a dam (ground tank) on a farm. The lambs were allowed access to the dam with concentration of bloom of Anabaena circinalis and Schizothrix calcicola.The feeding of the seeds of Lathyrus sativus (Moslehuddin, Hang, and Stoewsand 1987) or Abrus, precatorius as sole component of meal for poultry have shown to be deleterious (Rahman and Mia 1972).

In Nigeria, no extensive research effort can be said to have been done to determine whether or not most of the plants which are known in other part of the world to be very poisonous are actually equally poisonous also in Nigeria or otherwise.For example Lantana camara has been reported to be poisonous in other parts of the world.(Sharma et al 1981, Gujral and Vasudevan 1983, Arhiredy and Singh 1984) whereas the fruits of the same plant are reported to be edible (Gujral and Vasude- Van 1983) However there are no research evidence to confirm whether the varieties of this plant in this

country are poisonous (Nwude and Parsons 1977). This evidence is important since environmental factors are mentioned to influence toxic nature of plants. Furthermore in Nigeria, most of the reported cases of plant poisoning have been based on the observation of clinical symptoms after the animal might have consumed toxic quantities of the plant. Many toxic plants may not produce toxic symptoms until chronic consumption takes place. Prolonged exposure to such plants may not be possible in for example animals under normadism. The non observation of symptoms does not mean such plants are not toxic.

These studies were therefore carried out to evaluate the haematological, biochemical, clinical symptoms and the gross and histopathological effects of the suspected poisonous plants, Solanum torvum Swart, (Plate 1) Tribulus terrestris Linn (Plate 2) Leucaena leucocephala Benth, (Plate 3) Eugenia uniflora Linn (Plate 4) Lantana camara Linn (Plate 5) and Dichapetalum madagascasiense Poir (Plate 6) in rats with a re-evaluation of the latter two plants in goats. Chemical analysis of the plants were conducted so as to determine some inorganic and metallic substances which are known to be found in poisonous plants.

1.2 LITERATURE REVIEW

1.2.1. Poisons and types of poisoning

Poison is defined as any substances which taken inwardly in a very small dose, or applied in any kind of manner to a living

body depraves health, or entirely destroys life (Clarke and Clarke, 1975, Woodward, 1985).

Poisoning can be subdivided into acute, (a sudden violent syndrome caused by single large dose of poison) and chronic forms (a persistent, lingering condition brought about by small repeated doses) with subacute poisoning coming in-between these two. This subdivision, according to Clarke and Clarke (1975) is not tenable, considering the fact that there are certain types of poisoning which would be difficult to fit into any of these categories. For instance, the symptoms produced by the plant Pteridium aquilinum (bracken fern) poisoning may not manifest until months after the plant has been ingested (Evans 1964). Furthermore, there are other symptoms which could arise from poisoning which cannot be categorized. Such signs are (i) allergy, an immunological response due to sensitization of the subject by a previous dose of the poison; (ii) carcinogenicity, in which the poison causes neoplasia formation e.g. bracken and cycads (Evans and Mason, 1965). (iii) teratogenicity in which the poison produces abnormalities in the offspring of an animal when ingested at a certain stage of pregnancy e.g. consumption of Veratrum californicum which produces a teratogenic effect in sheep (Keller and Binns, 1964; Clegg, 1971) or Conium maculatum (Edmonds et al., 1972).

Plants produce and present toxicity in a multitude of complex ways (Kingsbury, 1979). Although vertebrates have mechanical

and biochemical defenses against these toxins, few systems of the vertebrate body are immune to damage by some compounds from some plant source(Kingsbury, 1975). Toxicity, by and large, involves an interrelationship among dose, absorption, detoxicification and excretion (Cassarett and Doull, 1975).

1.2.2. Types of Plant Poisoning:

Poisonous plants as may be with other extraneous poisons have the propensity to cause the following symptoms:

(a) the plant may produce a local injury to tissues to which it may come directly in contact with e.g. the latex from Euphorbia camerunica is known to be esharotic and irritant (Smith, Jones, and Hunt 1974, Terblanche, Adelaar and Van Straten 1966.)

(b) some plants cause gastroenteritis as a predominant feature e.g. members of the genus Sinapsis and Pennisetum claudestinum (Kikuyu grass poisoning) Martinovich and Smith 1973.)

(c) poisonous plants which are hepatotoxic and may also invariably produce nephrotoxicity. Such plants include L.camara (Sharma et al 1982, Sharma et al., 1984 Phyllanthus abnormis, Heliotropium europaeum and members of the genus Lupinus (Gardiner 1966).

(d) plants which are predominantly nephrotoxic.These include oxalate bearing plants as Halogenton glomeratus (Littledike, James and Cook 1976) Amaranthus retroflexus (Marshall, Buck, and Bell 1967).

(e) poison plants causing death through cardiac insufficiency with consequent venous congestion, anoxia and petechiae and other changes commonly associated with anoxic conditions. Such plants include selenium accumulators such as Astragalus species, Datura stramonium, and Strophantus hispidus or S. sarmentosus.

(f) poison plants which produce extensive hemorrhages.These are plants which contain dicoumarin e.g. sweet clover and other plants such as bracken fern, plants of the genus Crotalaria (Rao, Joshi, and Kumar 1988, Singh et l, 1987).

(g) poison plants which produce anaemia or cause destruction of the red blood cells e.g. Allium cepa, Abrus precatorius (McPherson 1979).

(h) poison plants which cause the production of methemoglobin and chocolate colored blood. These are plants containing nitra- tes such as A. retrofelxus (Usher and Telling, 1975).

(i) plants which cause the production of carboxyhaemoglobin and cherry-red blood. Such plants include those which contain high levels of cyanide e.g. Cynodon plectostachyum, Manihot esculenta, Nerium oleander Phaseolus lunatus and Eleusine indica (Clarke and Clarke 1975, Liener, 1974).

(j) plant which produce marked and conspicuous pulmonary emphysema. These include plants of the genus Brassica (Greenhalgh, Sharman, and Aitken 1969).

(k) poison plants which may cause gangrene of the extremities including the limbs, tail and ears so that after several weeks, of ingestion of small amounts the most distal parts of the extremities may drop off . Such poisons are produced by fungus as Claviceps purpurea, and grasses Festuca arundinacea (Hore, 1961).

(l) poison plants which cause loss of hair or wool.Such plants include Leucaena leucocephala (Mullenax 1963).

(m) plants which cause degeneration of cardiac and skeletal muscle. The plants producing such lesions include Cassia occidentalis L. (O Hara, Pierce and Read, 1969).

(n) plants which are teratogenic or produce fetal developmental abnormalities and include plants as Veratrum californicum (Edmonds et al 1972).

(o) Miscellaneous or unclassified poisonous plants.Poisoning by such plants in animals is infrequent.They include phytoestro- gens in plants as Medicago sativa (Kelly, Allison and Shirley, 1976).

1.3. Toxic Chemical Constituents of Plants

A number of compounds that occur in plant have been isolated and suggested as having toxic or antinuitritional effects. Such substances are either organic or inorganic. Some of such compounds include organic substances as haemagglutinins, trypsin inhibitor, saponins, goitrogens, glycosides, isoflavones, tannins and others referred to as antivitamins and antimetals. The inorganic substances include oxalate, cyanide, nitrate and nitrite, copper,

lead magnesium, manganese, cobalt and selenium. However, some of the inorganic substances when they occur in trace amounts are essential for plant growth.

1.3.1 Cyanogenic Glycosides

It is generally believed that the toxic properties associated with cyanogenic glycosides, such as linamarin are due to the hydrocyanic acid released from the glycosides by the activity of an enzyme complex. For example, linamarin is glucoside of acetone cyanhydrin. It is readily hydrolysd by heat labile B-glucosidase linamarase to yield acetone cyanhydrin, which can be split by a hydroxynitrile lyase non enzymatically into acetone and hydrocyanic acid. The enzyme complex is present in the plant tissue and is released when cells are broken down. Conn (1969) illustrated the pathway for the enzymatic degradation of linamarin.

Hydrocyanic acid or prussic acid (HCN) is one of the most toxic and rapidly acting of the common poisons; its sodium and potassium salts are only slightly less toxic. The complex of cyanides e.g. ferrocyanides and thiocyanates, are also practically harmless (Clarke and Clarke, 1975).

1.3.1.1. Plants containing cyanogenetic glycoside:

By far the most important source of hydrocyanic acid to toxicity animals is plant material. Many species of plants contain hydrocyanic acid either free or, more usually, in the form of a cyanogenetic glycoside, an organic compound containing a sugar, and capable of yielding cyanide on hydrolysis.

Some species of Brassicae (cabbage) contain relatively large quantities of 3-butenyl-isothiocyanate. Sudden death in cattle receiving Brassicae seed cake which was later found to contain 0.33% of volatile isothiocyanate has been reported (Van Etten, 1969) Van Kampen (1970) considers that all the conditions associated with S. sudanense (Sudan grass) represents a lathyrogen disease. The HCN in the grass is said to combine with L-cystine to form B-cyanoalanine, a precursor of the lathyroyan, L-glutamyl-B-cyanoalanine.

Bracken (Pteridium aquilinum contains several potentially harmful agents, among which are a cyanogenetic glycoside,a thiaminase, an aplastic anaemia factor, a factor causing hematuria and a carcinogen (Thomas, 1963).

Linum cathárticum both cultivated and purging flax contain a cyanogenetic glycoside, linamarin from which HCN is released under the action of the enzyme linamarase. The processes used in the extraction of the oil from linseed and in the preparation of linseed cake, from the residue, do not destroy the enzyme; boiling

for at least 10 minutes is observed to be required. The available HCN content of fresh linseed meal appears to be of the order of 0.25 to 0.6 g/kg,). The level is said to drop on storage. Under normal conditions of feeding insufficient HCN is released to be harmful. However, should the meal be eaten rapidly, and voraciously, as by starving animals, there is definite danger of poisoning (Franklin and Reid, 1944). It is also believed that the rate of liberation of HCN is the significant factor in determining the toxicity of the case when fed as a cattle food.

The rose family include many of the most commonly grown fruit trees.The kernels and leaves of these contain many cyanogenetic glycosides.Amygdalin is the common but prunasin and prulaurasin also occur.The leaves may contain over 0.2% HCN which is considered as ten times the amount usually seen as dangerous (Stauffer 1970).

1.3.1.2. Tissue response to cyanide

Cyanide is believed to exert its toxic effect by inhibition of the respiratory enzyme cytochrome oxidase. Concentration of cyanide as low as 3x10-8 M have been shown to produse complete inhibition of cytochrome oxidase invitro, and lethal doses of cyanide have been reported to inhibit this respiratory enzyme invivo. (Hazzard,Kowanish,and Caughey 1982, Tewe and Maner 1982, Tewe and Pesu 1982. In cyanide poisoning, oxygen transport and oxygen tension are usually adequate and only cellular utilization of O2 is

depressed. As a consequence, this would mean that the administration of O2 would not subserve any useful purpose in antagonizing cyanide intoxication (Way, Gibbon and Shedy 1966).

Significant amounts of cyanide can be detoxified in the body. Routes of detoxication proposed include conversion to thiocyanate ion (SCN-), incorporation into the 1-carbon metabolic pool through interaction with vitamin B12, and conversion to 2-imino-4-thiozolidine carboxylic acid (Casarrett and Doull 1975, Gilman and Goodman and Gilman, 1980).

Lang (1933) proposed that an enzyme from the kidney and liver called rhodonase catalyses the reaction of thiosulphate with HCN to give this cyanate, a relatively less toxic substance which can be rapidly excreted. The properties of the enzyme rhodanase, now known as thiosulphate s-transferase and of a second enzyme mercaptopyruvate s-transferase, also capable of forming thiocyanate by sulphur transfer to cyanide have been reviewed by Sorbo (1975).

Wokes and Picard (1955) also suggested the involvement of vitamin B12 in cyanide detoxification.In this pathway of vitamin B12 in the form of cobalamine (B12a) reacts with cyanide to form cyanocobalamine (B12).The latter then loses some of the cyanide to form 1-carbon fragments for the synthesis of methylgroups and the resulting hydroxocobalamine returns to the liver to repeat the cycle.Also the hydroxocobalamine can combine with the thiocyanate

formed via the thiosulphate or mercaptopyruvic acid and proceed to form cyanocobalamine.

The extreme toxicity of cyanide to most species is a well known phenomenon. Montgomery (1969) observed that the lethal dose for human of potassium cyanide taken by mouth is estimated to be between 1.0 and 70.0 mg/kg body weight and will cause death in few minutes. Acute toxicity and death from consumption of cyanide containing food in human e.g. cassava and of plant materials in animals is a common occurrence. However it is pertinent to note that chronic effects from consumption of sublethal amounts of cyanide over long periods has been associated with occurence of human ataxic neuropathy and also goitre in Nigeria. This has been observed in areas where consumption of a staple food is cassava (Osuntokun 1973). It is suggested that the neuropathy is caused by or aggravated by the cyanide or derivative acting on the CNS and the thyroid enlarged by thiocyanate which is a well known goitrogen derived from metabolism of ingested cyanide (Osuntokun 1973 and Wilson 1973).

Animal experiments give credence to such theories. Lesions in the CNS were observed when rats were injected repeatedly with sublethal amounts of cyanide over a period of 22 weeks with some suggestions of demyelination (Smith et al., 1963). Similarly, the injection daily for 5 weeks of KCN in rats produced degeneration of neurons in the cortex and the degeneration of the corpus callosum

(Smith and Puckett 1965) Demyelination of the optic nerves and retina were observed in rats given sublethal doses of CN over periods of 3 weeks (Wessel 1971).

1.3.2. PROTEASE INHIBITORS

The first observation that there existed an inhibitor of trypsin in plant material is credited to Read,and Hass (1938) who reported that an aqueous extract of a soya been flour inhibited the ability of trypsin to liquify gelatin. The existence of an inhibitor of trypsin in Soyabean which could be inactivated by heat gave a reasonable explanation to the work of Osborne and Mende (1917) who had observed that heat treatment of soyabean increased the nutritive value of their proteins.

This observation of protease inhibitors which acted as antinuitritional factors in plant foodstuffs, particularly in such an important dietary source of protein stimulated search for other such factors. The protease inhibitors have now been observed to be present in all parts of some plants examined. In Ipomoea batatas (sweet potato) a trypsin inhibitor has been observed to occur in the leaves as well as the tuber (Honavar and Sohnie, 1955). Similarly the young leaves as well as the tuber of Solanum tuberosum (white potato)have been observed to be rich in chymotrypsin inhibitor (Ryan and Huisman, 1967). The leaves and cotyledons of Phaseolus aureus (mungbean are fairly high in trypsin inhibitor activity (Honavar and Sohnie, 1955).

The presence of such factors in plants results in growth depression in animals consuming them. Such growth depression according to Lyman and Lepkovsky (1957); Hall (1977), Sharma et al. (1987) may be as a result of an endogenous loss of essential amino acids derived from a hyperactive pancreas which is responding in a compensatory fashion to the effect of the trypsin inhibitor. The mechanism by which the trypsin inhibitor stimulates the enlargement of the pancreas is unclear. However Khayambashi and Lyman (1966) in their experiment suggested that trypsin inhibitors may liberate a hormone like agent which stimulates the secretory function of the pancreas. These authors observed that a rat pancreas when perfused with blood plasma obtained from a rat fed with trypsin inhibitor secreted increased amounts of the enzyme.

1.3.3. The Lectins

Extracts of many plants which have the property to agglutinate red blood cells have been shown to be caused by some remarkable protein called phytohaemagglutinin or lectins. They exist in leaves, bark, roots tubers, latex, seeds etc.

1.3.3.1 Agglutinins

Agglutinin-containing plants have been found in many botanical groups including monocotyledons, dicotyledons, mould, and lichens but most frequently they have been detected in legumino- sae and Euphobiaceae, (Tobiaska, 1964;Liener, 1974). Stilmark (1859) is reported to have first observed the effect of phytohaemagglutinin.

In observing the toxicity of castor oil he concluded that the toxicity of Ricinus communis was due to protein fraction which is called ricin which showed the ability to agglutinate the RBC from human and animal blood. The agglutinins are said to differ widely from each other in that they show considerable specificity. Thus they could be different molecular species with different chemical and biological characteristics. Orally ingested, ricin is known to be more toxic for horses and rabbits than for chicks (Bierbaum, 1966). Lethal doses of highly active ricin preparation injected intramuscularly ranged from 0.0001mg/kg for rabbits to 0.03mg/kg for goats.Toxic-ity of ricin for mice was observed to be influenced by strain, age, nutritional status and individual susceptibility.

There exist very large differences in toxicity between the various plant agglutinins and there is reason to believe that so-me of them may well be devoid of any toxic action (Liener,1974). For example Phaseolus vulgaris (kidney bean) contains a hemagglutinin which is toxic to chicks (Honavar et al.1962) and rats (Jaffe and Vegalette, 1968). It is detoxicated by heating and certain cultivars, such as haricot beans may contain negligi-ble amounts.

Lesions arising from intoxication with lectins whether ricin, abrin, crotin are similar and include macroscopic, and microscopic lesions. Most notable lesions are extensive inflammation with destruction of epithelial cells, oedema, hyperaemia and hemorrhages in the lymphatic tissue.The liver presents fatty degeneration

and necrosis. The myocardium may show degenerative lesions and the capillaries of all organs may be extended and filled with blood clots. Local hemorrhages are frequently observed at the site of injection (Brocg-Roussen and Fabre, 1977).

Thomson (1950) observed changes in the quantitative composition of plasma, liver and urine of rats acutely poisoned with ricin and a reduction of the respiratory quotient of liver slices from the same animals. He therefore concluded that toxic action of ricin may be due to its interference with toxic metabolic processes in the liver possibly the Krebs cycle. In the studies of Ikegwuonu and Bassir (1977) they observed rise in the blood values of urea, bilirubin, transmaminases, lactic dehydrogenase with ricin poisoned rats.

A hypothesis relating to hemagglutinating and toxic action of lectins was advanced by Jaffe (1960) who reasoned that reaction between the agglutinins and the cell membrane is the resulting alteration of the cell function and the production of the toxic effect. Only those cells bearing the specific receptor groups for the respective lectins would be affected. Thus the reduced intestinal absorption produced by orally ingested lectin could result from the combinations of the lectins with the cell lining the intestinal wall which thus interfere with normal activity.

According to Lansteiner (1954) the specificity of the action of the lectins on blood cells from various animals is comparable to

that of antibodies. The hemagglutinating activity of many plant agglutinins can be inhibited by some sugars and oligosacch-arides in a similar manner as certain hemagglutinating antibodies are inhibited. This suggest a structural relationship between these inhibitors and the receptors groups for both agglutinating agents located on the surface of R.B.C.

The black and red seeds of Abrus precatorius (Jequirity pea) a perennial vine found throughout the tropics contain the very poisonous phytotoxin, abrin, a substance very similar to ricin. The powdered seed is toxic by mouth to all species though much less poisonous than parenteral injections of abrin (Rahman and Mia, 1972). Ricin is a protein with molecular weight of 77,000 and its solutions are coagulated by heat, the coagulation causing complete loss of toxicity but not of antigenicity. An animal rendered immune by injection of gradually increasing doses of the toxin can tolerate amounts of up 800 times the normal lethal dose.

1.3.4. Polyphenolics

Although tree leaves have a high protein content, tannins, and other secondary compounds may bind this proteins, thus rendering it unavailable to the animal. Tannins and related polyphenolics may have negative effects in palatability and digestibility and many are also poisonous (Woodward and Reed, 1989).

The term tannin refers to a heterogeneous group of pheno- lic compounds which precipitate protein to varying degrees, depending on the type of phenolic and protein presents as well as the chemical environment. Some tannins may bind proteins in the neutral pH of the rumen and release them in the acidic abomasum (Barry and Forss, 1983).

Acacia decurrens and A. salicina may be toxic on account of their high tannin content of 35 per cent and 16 per cent respect- ively (McCosker and Hunt, 1966). The clinical signs nclude salivation, inappetence and ataxia of the hind limbs.The acorns and leaves of Quercus species are known to contain large amounts of tannic acid (acorns may contain 7 to 9 per cent) together with small amounts of a volatile oils. It is generally considered that it is this high tannic acid content which is responsible for the toxic properties of acorns, but this is by no means established (Clarke and Clarke, 1975.Clarke and Cotchin (1956) conclude that, although the chief, if not the only toxic factor in acorns is tannin, something apart from mere oral ingestion of tannin is concerned in acorn poisoning. Dollahite, Pigeon and Camp (1962) found that the lesions produced in the rabbit by Q. havardii are very similar to those produced by multiple doses of tannic acid. Supplejack (Ventilago viminalis) serves as a useful fodder, but can cause poisoning in sheep owing to its high tannic acid content if

it forms the major part of the diet for too long a period (Pryor et al., 1972).

1.3.5 Alkaloids

These are basically alkali substances which are mainly nitrogenous in nature. Certain alkaloids found in lupines have been identified as the cause of illness in animals.The following alkaloids have been isolated from lupine species : lupinidine or l-sparteine, d-lupanine, hydroxy-(oxy) lupanide and spathulatine. The toxic effects of these lupine alkaloids, have been grouped under the term lupine poisoning, alkaloidal poisoning or lupine madness (Gardiner, 1967, Smith, Jones and Hunt, 1974). Gardiner (1967) proposed that the term lupine poisoning be used to differentiate the syndrome associated with other, unidentified, toxins in lupines which produced injury to the liver.

The agents responsible for the teratogenic action by Veratum californicum are the steroidal alkaloids cyclopamine,jervine and veratrosine. Fed at other period of embryogenesis, the plant gives rise to other malformations for which different alkaloids including possibly veratranine may also be responsible (Binns et al., 1965; Keller, 1973).

Argemone mexicana (Mexican prickly poppy) though a native of Mexico and North America is observed to be almost a cosmopolitan weed. It contains the alkaloid sanguinarine, which in addition to being a local irritant, causes depression of pyruvate oxidation by

brain tissue. This alkaloid is also found in other genera of the family Papaveraceae (Hakim et al., 1961a). A. mexicana is said to be of some importance in human toxicology as the ingestion of argemone oil has been shown to produce epidemic dropsy associated with glaucoma. Since sanguinaine is secreted in milk it has been suggested that, the grazing of this weed may give rise to endemic primary glaucoma in man (Hakim etal.,1961b).

Laburnum anagyroides (laburnum) is one of the most poisonous trees.All the parts of this plant are toxic especially the flowers and seeds with the active principle being the alkaloid cytisine which has a similar action as nicotine (Clarke et al., 1971). Lethal doses of the seeds by mouth are in the horse (the most susceptible animal) 0.5 g/kg and in the dog 6 g/kg.Cytisus scoparius is observed to contain small amounts of cytisine together with another alkaloid, sparteine. Sparteine has similar physiological effects to coniine but it is less poisonous.

The bark of the African Erythrophloeum guineense (sassybark) is well known as ordeal poison and an ingredient of arrow poisons. It contains cassaine and other alkaloids and has been used for malicious poisoning of animals (Hall, 1974).

All the parts of the plant Colchicum autumnale have been shown to contain alkaloids, colchicine and colchicrine. Both are able to withstand drying, storage and boiling. Colchicine is the most toxic and according to Clarke and Clarke (1975) the seeds contain 0.2 to

0.4 per cent, the bulbs, 0.8 to 0.2%; the leaves 0.01 to 0.03%. In doses of 0.25mg/kg body weight, colchicine exerts a purgative effect. The average lethal dose for most species is shown to be of the order 1mg/kg. Colchicine is absorbed only slowly, and exerts its toxic effects only after several hours and is also slowly excreted mainly in the milk and urine so that there is a cumulative effects from small doses.

Lilies of Gloriosa species which are native to Africa contain colchicine type alkaloids and are extremely poisonous, giving rise among other symptoms to generalized alopecia (Gooneratne,1966).Heliotropium europaeum contains the hepatotoxic pyrrolizidine alkaloids heliotrine and lasiocarpine and their N-oxides, has caused large-scale poisoning, particularly of sheep (Bull et al., 1961). Echium lycopsis which also contains the pyrrolizidine alkaloids echiumidine and echimidine cause a similar syndrome as H.europaeum, with symptoms of jaundice and hemoglobinuria; in sheep. On post-mortem the livers are found to be enlarged and yellow, and to have a copper content of 900 to 1200ppm (St. George-Grambaur and Rao, 1962).

1.3.6. Toxic Amino Acids

Unusual free amino acids are particularly frequent in Lathyrus and Vicia such as lathyrine, homoarginine and hydroxy-arginine which may be poisonous in large doses.Mimosine in Leucaena leucocephala and Mimosa pudica causes hair loss in stock,

infertility and cataracts in rats and mice. It may interfere with metabolism of tyrosine, phenylalanine, and pyridoxal phosphate (Nowack, 1980). Other amino acids of legumes whose structure suggest that they may be toxins are wallardine and aldizzine in Acacia, though small amounts are also found in Pisum and Vicia as stizolobic and stizolobinic acid. Species of Mimosoideae contain the S- amino acid djenkolic acid (which causes kidney disorders in human) sometimes accompanied by an acetyl derivative (Nowacks,1980). Experimental poisoning of sheep has shown that mimosine is largely broken down in the rumen to 3,4- dihydroxy pyridine and excreted as such, (Hegarty et al., 1964).A certain degree of detoxication is brought about by moist heat (Mullenax, 1963).Linum usitatissimum (linseed) produces toxic effect if it is fed to chicken. The toxic effect have been shown to be due to linatine, a peptide formed from glutamic acid and L-amino-D-proline which has an antipyridoxine action (Liener, 1969).

1.3.7. Saponins

Both the vegetable parts and seeds of some legumes contain large amounts of heat stable sapogenic lycosides(Nowack, 1980). The best known are the soya sapogenols which are known to be bitter tasting and decreases intake of fodder.Medicago also contains the sapogenin medicagenic acid which is toxic. The growth of guinea pig, mice and Japanese quail is inversely correlated with the concentration of medicagenic acid. Some members of the

Caryophyllaceae family including Saponaria, Agrostemina, Arenaria and Stellaria are poisonous and known to contain saponin compounds widely distributed throughout the plant kingdom and which contains a sugar in combination with a steroid. Agrostemina githago which contains the sapogenin, githagenin is probably the most poisonous of these species. The growing plant is avoided by livestock but the seeds, if mixed with corn or meal can cause poisoning. Bierer and Rhodes (1960) reported that chicken fed 5% of corn-cockle seed in the ration showed no ill-effect but Kotz (1965) observed that a single dose of the ground seed of 2.5-8.0 g/kg will cause acute poisoning while continued feeding for seven weeks can cause chronic poisoning.

1.3.8. Flavones and Isoflavones

Flavones and isoflavones which have oestrogenic effects have been found in many genera of the Papilionoideae (Nowack, 1980). Some members of the family Gramineae including Lolium species (short rotation rye grass) and Lolium perenne (perennial rye grass) contain significant amounts of oestrogen-like compounds. There may be sufficient presence to cause sterility in cattle. Trifolium pratense has been shown to contain appreciable amounts of substances having oestrogenic activity (Cunningham and Hogan, 1954) and this has produced reproductive disturbances in sheep grazing it, (Chang,1958). T.subterraneum (subterranean clover) has been responsible for considerable economic loss to the sheep breeding

industry in Western Australia (Moule, 1961). The agent responsible for this loss was isolated to be genistein, an isoflavone possessing oestrogenic activity.

1.3.9 Oxalate-Bearing Plants

In animals cases of oxalate poisoning are encountered usually due to ingestion of large amounts of plant containing oxalates. Salts of oxalic acid are found in many plants.Clarke and Clarke (1975) gives a list of plants containing oxalates as: Atriplex spp., Beta vulgaris, Calandrinia spp., Emex australis, Enchylaena tomentosa, Halogeton glomeratus,Oxalis spp.Portulaca spp.Rheum rhaponticum Rumex spp.,Salsola kali,Sarcobatus vermiculatus, Sefaria sphocelata; Threlkeldia proceriflora and Trianthema spp.Salts of oxalic acid are found in many plants. Sodium and Potassium oxalates (and acid oxalates) are soluble, but calcium oxalate is insoluble and is not assimilated during digetion. The oxalate content of plants is observed to be highest at the leafy stage of growth.Another plant demonstrated by analysis (Marshall, Buck and Bell, 1967)to contain high levels of oxalate is Amaranthus retroflexus. The leaves of this plant may contain as much as 30 per cent of total oxalate on a dried weight basis. This plant is believed to be one of the causes of an entity called perirenal oedema disease of swine (Buck et al., 1966).

The occurrence of nutritional secondary hyperparathyroidism (NSH) or oseteodystrophia fibrosia in horses grazing a number of

grasses which causes lameness, debility is suggested to be due to the oxalate present in these grasses, Walthall and Mckenzie, 1976). Mckenzie et al.(1981) also demonstrated that potassium oxalate (2.6 and 4.3%) added to a diet of oaten and lucerne chaff produced negative calcium and phosphorus balances in horses but still allowed some calcium to be absorbed.Many of the known haza-rdous grasses (those on which NSH has occurred) however contain less than 2 per cent total oxalate (Blaney et al., 1981).The same authors however noted in a different experiment that all grasses associated with field cases of NSH produced negative calcium balances with the main loss of calcium being in the faeces. The ratio of Ca:P in the grasses (minimum of 0.69) indicated the little likelihood that absorption of Calcium was depressed by a large excess of dietary phosphorus. The pangola, green panic, kikuyi and buffel grasses are observed to supply a calcium intake equivalent to or greater than the requirement of 4.5 mg/kg live weight per day. However they were observed to produce negative calcium balances consistent with the effects of oxalate (Mckenzie et al., 1981). Other grasses, parabuffel and the setarias with more than 0.5 per cent oxalate content were however seen to supply less calcium but the resulting negative calcium balances were greater than the effects that could be reasonably attributed to a primary calcium deficiency alone.

Mofatt (1974) reports of hepatopathy with renal oxalosis in bovine fetuses with a suggestion that the ingestion of toxic plants could result in the fetal lesions. Acute oxalate toxicity has been recorded in many herbivores and is principally due to excessive ingestion of either oxalate rich plants or food cont- aminated with oxalate producing fungi, (Andrews, 1971). Large amounts of oxalic acid have been shown to be formed on moist straw infected with the fungus Aspergillus niger. Such straw which is indistinguishable from unspotted straw was suggested to be dangerous to horses and other animals. Wilson and Wilson (1961) have shown that A.flavus can also produce large quantities of oxalate. Silage infected with oxalate producing fungi has been responsible for progressive nephrosclerosis in horses(Andrews, 1971).Nutritional status,the ration of calcium to oxalate in the diet as well as the level of oxalate ingested can be important factors in producing oxalate toxicity,(Blaney et al,1981).Oxalo- sis with resultant hypocalcaemia can lead to coagulation defects and neuromuscular dysfunction with the calcium oxalate crystals due to their deposition in the kidney tubules which can lead also to renal failure.Death has been observed in a Loala Phacolarctos cinereus) with the typical lesions of oxalate poisoning which was associated with its consumption of eucalyptus leaves and the leaves of certain other plants with oxalates (Canfield and Dickens, 1982).

1.3.10 Plants Which Contain Dangerous Levels of Nitrate

The principal hazard to livestock from nitrates arises from the fact that certain plants, grown on soils containing excess of nitrate salts, may take up sufficient to render them toxic to animals eating them. Clarke and Clarke (1975) gives a list of 88 species of plants which may contain dangerous concentrations of nitrate. It has been observed that the use of the herbicide 2,4-D(2,4-dichlorophenoxyacetic acid) sublethal doses of which, like periods of drought, may result in accumulation of amounts of nitrate toxic to animals. Air-dried leaves from plants sprayed with 2,4-D were found to contain 4.5 per cent potassium nitrate on a dry matter basis, an amount considered to be well above the minimum lethal concentration in animal fodder (Stahler and Whitehead, 1950) whereas the untreated plants contained 0.22 per cent.Weather has also been incriminated to affect the level of nitrates in plants. Harris and Rhodes (1969) observed that on dull days or at night, the nitrate level is higher than it is in sunlight as conversion to amino acids does not take place.

Subacute and chronic nitrate toxicity in livestock produces signs of anorexia, dyspnea, grinding of teeth, restlessness, vasodilatation, reduced blood pressure, abortion and decreased milk excretion, possible avitaminosis A and possible thyroid malfunction (Blood and Henderson, 1974). The potential influence of nitrate toxicity on thyroid dysfunction has been studied in several animal

species. In rats a definite correlation is observed to exit by thyroid hypofunction and nitrate poisoning (Lee et al., 1970) and it appears that nitrate is an antithyroid substance in this species. This effect is probably the result of nitrate interference with the thyroidal iodide-concentrating mec-hanism (Lee et al.,1970).When nitrate is present higher levels of iodine are needed for the maintenance of normal thyroid glands. Abortion in swine,sheep and cattle have been attributed to nitra-te and nitrite poisoning.Schmitz (1961) postulated a relationship between high maternal methemoglobin levels and abortion in pregnant women.Stuart et al.(1968) using guinea pigs observed reproductive disturbances in the female. Abortion, fetal absorption, fetal mummification and maternal death were important manifeta-tion.Hypoxia of fetus due to maternal methemogloin was hypothesized as a cause of fetal death.

Nitrates per se are relatively non-toxic; their importance as a cause of poisoning is due to their conversion either in the foodstuff or within the alimentary tract into nitrite. Nitrite converts the haemoglobin of the blood into methemoglobin, which is unable to act as an oxygen carrier (Mcilwain and Schipper, 1963; Sinclair and Jones, 1964; Smith et al., 1974; Clarke and Clarke, 1975; Blood and Henderson, 1976). There has been many reports of livestock poisoning by nitrate and nitrites. Andrade et al. (1971a, b). Hill and Blaney (1980) Rogers and Hopekawd (1980)

reported that fatal intoxication was observed in cattle and yearlings pastured in Brachiaria species which have high contents of nitrate. Signs of poisoning reported were hematuria, semi-pasty faeces, weakness, and pale or yellowish mucous membranes retraction and intermittent urination with incoordination when the animals were forced to move. There were liver and kidney damage. The total nitrate in the serum of affected cows was 0.468 mg per cent which was more than three times that in control animals (0.148 mg %).The Brachiaria plants from the pasture had high nitrate content (0.6%) which was the cause of intoxication.Miyazaki and Kawachinia (1975) observed that when as much as 50 per cent of the total haemoglobin in the sheep was converted to methemoglobin by poisoning with sodium nitrite, biochemical estimations showed that as the methemoglobin increased, serum ammonia also increased and serum urea inecreased. These authors suggested that nitrite was probably being reduced to ammonia in the rumen and its synthesis to urea in the liver may not be so efficient when methemoglobin is pronounced. A decrease occurred in bilirubin could not be explained. Blood glucose increased as methemoglobin increased. Methemoglobin analyses in sheep fed with various Astragalus species, indicated that nitro-compounds in A.diversifoluis,A.convallarius,and A.plerocarpus resembled 3-nitro-1-propanol in toxicity and the rate of absorption from the digestive tract (Williams and James, 1975). A.

ptercarpus, A.convallaris,and A.diversifolius produced acute poisoning in sheep at 100 mg nitrite per kilogram body weight and are the species most likely to cause livestock losses on the range.

1.3.10.1 Nitrates/nitrites and Nitrosamine formation

Nitrosamines can result from an interaction between nitrite ion and existing secondary amines. For example N- nitrososcosine is readily produced from creatine and nitrite ion (Archer et al., 1971). Creatine is present in the muscular tissue of vertebrates and is a normal constituent of meat. In vitro studies involving nitrite and secondary amine have shown that gastric juices of mammals provides an excellent medium for the production of nitroso derivatives (Dombrowski and Pratt, 1972). Indeed investigations revealed the presence of simple alkyl nitrosamines in food products which had been treated with nitrites, (Fazio et al., 1971). Their presence in food products has been demonstrated to be detrimental to human health. The situation becomes more complicated when it is observed that nitrite and secondary or tertiary amines in food can combine to form nitrosamines which are potent carcinogens.

Magee and Barnes (1956) first reported the carcinogenicity of dimethylnitrosamine (DMN) in rats and it has since been established that many other nitrosamines are carcinogenic to a wide range of species causing cancer at different sites of the body. Some can cause cancer after only one dose, and some are powerful mutagens (Liener, 1974). The first evidence that nitrosamines could be

present in food was provided by Norwegian workers who were investigating a liver disease in fur-bearing animals. A similar hepatic disorder was later observed in cattle and sheep (Liener, 1974).

R1 and R2 can either be alkyl or aryl group or in some cases alicyclic.

The nitrosation of amines by nitrite leads to nitrosamines which are then metabolized to alkylating agents. Thus in vivo nitrosation of dietary dimethylamine (DMA) will lead to the formation of the carcinogen dimethylnitrosamine (DMNA) which causes the methylation of liver DNA Bratchi et al. (1980) observed this by administration of some doses of potassium nitrate and 14C-DM hydrochloride in rats. After 6 hours, the liver DNA was isolated and its specific radioactivity was compared with that obtained after administration of either labelled DMA or DMNA. It was observed that about 2-3% of the dose of DMA had been nitrosated to DMNA.

1.4 TOXIC EFFECTS ON CELLULAR ELEMENTS OF BLOOD

1.4.1 Physiologic Properties of Constituents of Blood

The blood in an animal serves as a transport medium.It carries nutrients from the digestive tract to the tissues, end-products of metabolism from the cells to the organ of excretion, oxygen from the lungs to the tissues, carbon-dioxide from the tissues to the lungs and secretions of the endocrine glands. Additionally the blood plays a role in regulation of body temperature, maintain a constant concentration of water and electrolytes in the cells, as well as the body,s hydrogen ion concentration and defend against micro-organisms (Schalm, Jain, and Sarrol, 1975).Three classes of blood cells or corpuscles are recognized, namely: the erythrocytes, or red cells,the leucocytes or white cells and the thrombocytes or platelets.The leukocytes defend the body; the erythrocytes transport oxygen,and carbon-dioxide and the thrombocytes assist in the coagulation process. The non-cellular portions of the blood include water electrolytes, proteins, glucose, amino acids, enzymes and hormonal compounds, (Jain, 1986).

1.4.2 Hematopoiesis

Hematopoiesis or hemopoiesis, means making blood, particularly blood cells.The haematopoietic system is widely distributed and includes organs having functions other than contributing to blood formation such as the bone marrow, thymus,

lymph nodes, and follicles, spleen, mono-nuclear phagocyte system (reticuloen- dothelial system) liver,stomach and intestine and the kidney (Jain, 1986).

The mass of circulating red cells and erythropoietic tissue in the bone marrow constitute a functional unit,called the erythron.Physiologic and pathologic states may affect the erythron, resulting in an absolute or relative change above or below normal level. The erythron in health remains in delicate balance, for the daily production of erythrocytes, equal the daily loss from destruction of senescent cells. The rate of erythrocyte replacement in normal individuals is incredibly fast; almost 1 million red cells are replaced every second in a healthy 15 kg dog for example (Jain, 1986). In response to anaemia, the normal haematopoietic tissue can undergo a six to eight fold increase in erythrocyte production.

1.4.3 Haematopathies associated with Poisons

The blood is susceptible to a number of diseases brought about by infectious and non-infectious agents. The RBC for example is designed to circulate in the blood stream for a number of days. After this period presumably because its metabolic machinery or stroma wears outs, the cell is removed from the circulation. However certain untoward events can abruptly shorten the life span of RBC. Prominent among these is the administration of drugs, chemicals or plant substances. Such substances may produce

disturbances of blood circulation such as coagulation defects, hemorrhages or anaemia and agranulocytosis (Schalm et al., 1975).

1.4.3.1 Anaemia

Anaemia is rarely a primary disease;rather it generally reflects a secondary development. It is a sign and more commonly only one of the results of a generalized disease process.Anaemia can be evaluated and classified on the basis of erythrocyte morphology,pathogenetic mechanisms and bone marrow erythroid responses (Smith,Hunt and Andrews,1974;Jain, 1986).

Chemical substances which produce anaemia are classified into those which cause anaemia in all subjects e.g. plants containing agglutinins (Liener, 1974) and those which produce anaemia in only the occasional unusually susceptible individuals such as occurs with the plant Vicia fava (favism) (Bowman and Walker, 1961). Susceptibility to chemically induced anaemia may occur because the RBC of the vulnerable individual are abnormal or because abnormal plasma factors are formed in response to the administration of the chemical substance.

1.4.4. Classification of Anaemia

Anaemias can be classified according to Smith,Jones and Hunt (1974) under (1) pathophysiologic considerations into blood loss or hemorrhagic anaemias; hemolytic anaemias and; depressive or hypoproliferative anaemias (2) erythrocyte morphologic classification employing the two red cell indices, mean corpuscular volume (MCV) and mean corpuscuar haemoglobin concentration (MCHC). The size of the erythrocyte in the anaemic state is designated macrocytic, normocytic or microcytic and the mean haemoglobin concentration is designated either normochromic or hypochromic.The macrocytic anaemia can be hypochromic or normochromic and may be divided into true or transitory macrocytic anaemias.True macrocytic anaemias have normochromic erythrocytes whereas transitory macrocytic anaemias have below normal MCHC values.

Macrocytic normochromic anaemia is characteristic of Vitamin B12 and folate deficiency, administration of certain antimitotic drugs, severe liver disease, (as occurs with some plant poisoning) splenectomy, myeloproliferative disorder involving red cells, and some leukemias.Macrocytic hypochromic anaemia is observed during remission in acute blood loss or acute hemolytic anaemia.

Normocytic-normochromic anaemias occur when there is selec-

tive depression of erythrogenesis in chronic disease. The microcytic hypochromic anaemias occur when there is selective depression of erythrogenesis as in chronic disease, (Jain, 1986).

1.4.4.1 Hemolytic Anaemias associated with Plant Poisoning

Hemolytic anaemia results from excessive destruction of the circulating erythrocytes, occurring within the blood stream. Other observations which are noticed to accompany hemolytic anaemia are hemolytic icterus which may be detected by a high bilirubin level in the blood or the physical signs;hemoglobiuria in the acute forms an the presence of nucleated red cells and reticulocytes in blood when it is chronic (Smith et al., 1974).

The category of drugs and chemicals which can cause hemolytic anaemia includes copper, lead, phenothiazine, methylene blue, saponins, naphthalenes and drugs such as acetanilide, nitrfurantoin, neoarsphenamine, phenacetin and some sulfonamides.

Copper toxicity anaemia starts with the accumulation of copper in the liver of animals receiving copper or feeding on copper-contaminated pasture or certain plants having high copper content. For example damage to the liver caused by grazing Heliotropium europaeum can lead to abnormal accumulation of copper and death resulting from the hemolytic crisis of chronic copper poisoning (Clarke and Clarke, 1975). Some climatic conditions are known to favor the growth of non-grainineous plants which accumulate copper e.g. (Trifolium subterraneum) (Hogan et al.,

1968). The accumulation of the copper occurs over prolonged periods during which animals are usually asymptomatic. Under conditions of stress, copper is released to the blood stream to precipitate rapid RBC destruction (Pearson, 1956; Smith, Jones and Hunt, 1974).

In ruminants, the metabolism of copper,molybdenum and sulphate ions is inter-related.Copper-molybdenum complex in the presence of inorganic sulphate is believed to inhibit urinary excretion and metabolism of copper in tissues, particularly in the liver, leading to impaired synthesis of ceruloplasmin. Copper toxicosis is common when a copper: molybdenum ratio in forage is greater than 10:1 either because of excessive copper or deficiency of molybdenum (Jain, 1986).

Ricin the toxic principle of the plant R. conmunis when administered by various routes,will produce hemolytic anaemia (Geary, 1950). The anaemia which often accompanies poisoning by Brassica species is also believed to be hemolytic in origin (Greenhalgh et al., 1969).

Brassica aleracea has been reported to cause Heinz body anaemia in cattle, sheep, and goats(Dunbar and Chambers,1963; Greenhalgh et al., 1969). Sheep developed a relatively mild anaemia but sheep with low levels of glutathione (GSH) were more susceptible than those with high levels.Affected animals showed retarded growth, hemoglobinuria, icterus and hemosiderin deposits

in the liver. Brassica species feeding in goats has been reported to induce hemolytic anaemia but recovery followed even though the feeding of the plant continued (Greenhalgh et al., 1969).

Allium cepa or onions,(wild and domestic) contain n-propyl disulfide, which produces Heinz body anaemia in cattle (Hutchinson, 1977), sheep (Van Kampen, Lynn, James, and Johnson, 1970) and horses (Pierse, Joyce, England and Jones, 1972).Jain (1986) reported the observation of hemolytic anaemia and some deaths among steers being fed cannery offals, consisting of onions and tomato pulp and vines. Both symptoms and lesions can be summarized as hemolytic anaemia, hemolytic icterus, and hemoglobinuria.Anaemia as estimated by haemoglobin determination is said to be extreme.

Horses that ingested Acer ruburum leaves developed acute hemolytic anaemia from an oxidant present in the leaves (Divers, George, and George 1982; Tennant, Dill, Glickman, Mino et al., 1981). Clinical observation included weakness, polypnea, tachycardia,depression,icterus,cyanosis and brownish discoloration of the blood and urine.Hematologic findings included methemoglobinemia, free plasma haemoglobin decreased PCV, and Heinz bodies in erythrocytes.

In the case of Vicia faba hemolytic anaemia and hemoglo-

binuria are also symptoms (Bowman and Walker,1961).A specific hemolytic anaemia, favism in certain human subjects is said to be dependent upon a genetic factor and the ingestion of Vicia faba.

1.4.4.2. Hypoproliferative anaemia associated with Plants

The excessive ingestion of a wide variety of plants or their products, have been found to cause hypoproliferative anaemia. In this situation,the hematopoietic tissue of the bone marrow are injured in such a way that their ability to produce erythrocytes is impaired or destroyed.

Hypoproliferative anaemia may result from limited supply or defective utilization of nutrients essential for red cell production or from anatomic disruption, functional impairment or lack of stimulation of hematopoietic tissue. In anaemia secondary to chemicals, plants or drug toxicity, the bone marrow, ability to produce red cells is often suppressed or destroyed by the insulting agent so that anaemia ensues in the face of adequate essential nutrients. Cellular damage in such cases may be mild, and reversible upon removal of the primary cause, or it may be severe and permanent. In most cases of marrow suppression ,secondary to drugs, chemicals or plant poisoning, the anaemia is normocytic normochromic (Jain, 1986).

In Pteridium aquilinum poisoning, the clinical picture and hematological changes, are characteristic of an aplastic anaemia. The fundamental lesions appear to be a depression

of bone marrow activity leading to leucopenia,and thrombocytopenia(Rao,Joshi and Kumar,1988).There is increased capillary fragility, prolonged bleeding time and defective clot retraction. Numerous petechial hemorrhages can be seen on the visible mucous membrane and there may be bleeding from the nostrils and intestinal and urogenital tract especially at the latter stages. Postmortem reveals extensive hemorrhages throughout the tissues of the body. Blood smears are found to streak, a phenomenon associated with abnormal fibrinogen components and increase in circulating heparin-like substances (Evans, 1964; Singh et al., 1987; Rao et al., 1988).

1.4.4.3. Sensitivity to Drug-Induced Hemolytic Anaemia due to Intra-erythrocytic defects

This may be due to metabolic abnormalities or unstable haemoglobin.The erythrocyte is a living, metabolically active cell. It must maintain a gradient of sodium and potassium across its membranes reduce methemoglobin that has been formed by metabolic activities (Beutler, 1969).

In red cell metabolism, there are three pathways for energy in form of ATP formation. When glucose is phosphorylated to glucose-6-phosphate in the hexokinase reaction, further metabolism takes place by the (1) Embden-Meyerhof way which is the major way in which glucose is metabolized anaerobically to pyruvate or lactic acid. This pathway provides energy in the form of ATP

and can reduce nicotinamide adenine dinucleotide (NAD) to NADH for methemoglobin reduction. (2) the hexose monophosphate shunt in which no ATP is formed but NADP is reduced to NADPH. The main function of this route of metabolism appears to be to provide reduced NADP for the reduction of oxidized glutathione. Glutathione plays a role in maintaining sulphydryl enzymes within the red cell in active form and in detoxifying small quantities of hydrogen peroxides. The levels of hydrogen peroxides that may appear when certain types of drugs react with oxyhaemoglobin may be so slow that catalase represents a relatively inefficient means of their disposal, (Cohen and Hochstein, 1963).Another system the glutathione peroxidase system appears to be effective in detoxifying such small amounts of peroxides.

The glutathione that is oxidized (to oxidized glutathione) in the glutathione peroxidase reaction, that which may be oxidized in the process of reducing mixed disulfides, and possibly mixed disulfides themselves, can be reduced to glutathione through the mediation of the enzyme glutathione reductase with NADPH as substrate.Thus the complete pathway requires three groups of enzymes, the glucose-6-phosphate, dehydrogenase-phosphogluconic dehydrogenase system, to reduce NADP to NADPH; the glutathione reductase system, to oxidize NADPH to reduce oxidized glutathione to glutathione, and finally the glutathione peroxidase system that oxidizes glutathione for the reduction of peroxides. The red cell

becomes excessively susceptible to drug induced hemolysis when any of these three enzyme systems is not functioning properly (Beutler, 1969).

1.4.4.4. Deficiency of Glucose-6-Phosphate Dehydrogenase

Hemolytic anaemia associated with the administration of 8-aminoquinoline antimalarial compounds such as pamaquine napthoate, primaquine, isopentaquine has been recognized since shortly after the introduction of these compounds into medical practice, (Hockwald et al., 1952). Primaquine was very effective antimalarial compound, but it produced hemolytic anaemia in susceptible persons.

Hemolysis of individuals with deficient erythrocyte levels of glucose-6-phosphate dehydrogenase is known to occur upon exposure to various drugs.It has been suggested that the oxidative changes observed during hemolysis e.g. loss of glutathione, (GSH) oxidation of haemoglobin,are manifestations of the presence of hydrogen peroxides (Cohen and Hochstein, 1963). Glucose-6-phosphate deficient red cells are sensitive to hydrogen peroxides by virtue of diminished generation of NADPH.

Specific hemolytic anaemia,favism in certain human subjects is said to be dependent upon a genetic factor and the ingestion of the broad bean Vicia faba (Bowman and Walker, 1961).The defect is noted to result in the deficiency of the enzyme Glucose-6-phosphate dehydrogenase.The deficiency in this enzyme (G-6-PD) is reflected

in reduced glutathione in the red blood cells. A similar hemolytic anaemia is observed in horses and cattle which have been fed the same type of plant (Panciera, Johnson and Osburn, 1966).

Earlier studies by Dern et al.(1955) had observed that not only do other 8-aminoquinoline derivatives produce lysis of primaquine-sensitive cells in vivo, a variety of other compounds such as sulfanilamide (Prontosil(R) album) acetanilid (Antifebrin), phenacetin and small doses of phenylhydrazine, diphenylsulfon (Dapsone(R))), furazolidone, nitrofurazone, do so as well.

These drugs have been shown to produce selective destruction of the older red blood cells while the young cells appeared to have a capacity to protect themselves against destruction. Later observations show that in some types of G-6-PD deficiency even quite young cells are destroyed and hemolysis was therefore not self limited (Salvido, Pannacciulli, Tizianetho and Ajmar, 1967).

1.4.4.5. Glutathione reductase Deficiency

Reports have been made of cases with suffoxone-induced hemolytic anaemia whose red cells G-6-PD activity was normal, (Beutler, 1969). Deficiency of glutathione reductase of the red cells has been associated with pancytopenia,with non-spherocytic, hemolytic anaemia with or without thrombocytopenia and leukopenia with thrombocytopenia and even with isolated instances of

leukaemia and hemophilia B.

1.4.4.6. Glutathione deficiency

A marked diminution of the content of both reduced and oxidized glutathione in red cells was first described by Oort and associates (Beutler, 1969). The condition is said to be very rare. Glutathione (GSH) deficiency is associated with a mild non-spherocytic hemolytic anaemia. Ashby survival studies showed that the mean life span of GSH deficient red cell was only one-third to one-fourth of the normal red cells. Some evidence suggest that the plant Vicia faba might induced hemolysis in glutathione deficient patients (Prins, Oort, Loos, Zurcher and Berkers, 1966).

1.4.4.7. Phosphogluconic dehydrogenase (PGD) deficiency

There can be the partial as well as the complete absence of phosphogluconic dehydrogenase(Parr and Fitch 1964).Phosphoglu-conic dehydrogenase is the second enzyme in the hexose monophosp-hate shunt.Decrease in its activity would limit reduction of NADP to NADPH and could also result in the accumulation of 6-phosphog-luconic acid in red cells.If drug induced hemolysis does occur in PGD deficiency, the mechanism would be essentially the same as that in the G-6-PD deficiency (Beutler, 1969).

1.4.4.8 The Unstable Haemoglobin

Abnormalities in the globin portion of haemoglobin molecule affecting either the alpha or beta chain near the

site of attachment of heme gives rise to instability of haemoglobin and results in sensitivity to the hemolytic effect of some substance.

Hemoglobinopathy has resulted in the production of hemolytic anaemia in patients treated with, for example Sulfisomidine (Elkosm). The feeding of the plant Brassica oleracea (Kale) to cattle was shown to produce reduced haemoglobin with relatively severe anaemia and the production of haemoglobin C by sheep with haemoglobin genotype AB and AA (Grant et al., 1968; Tucker, 1969). The plant also produced increased numbers of Heinz bodies in the red blood cells of sheep with haemoglobin genotypes BB, AB and AA.

In these hemoglobinopathies,the RBC usually contained inclusion bodies, but investigation of the G-6-PD activity, glutathione concentration and glutathione stability all resulted in normal values. Unstable haemoglobin apparently readily becomes denatured into insoluble aggregates. This process appears to be hastened by substances that catalyze the oxidation of haemoglobin.

Oxidative denaturation of haemoglobin is prevented by methemoglobin reductase,superoxide dismutase(SOD) glutathione peroxidase, and catalase. Certain oxidants perhaps those with a mild to moderate oxidative action produce only methemoglobinaemia, whereas strong oxidants also react with globin chains to induce denaturation and precipitation of haemoglobin in the form of

Heinz bodies.For example, it has been observed that substances as acetaminophen in cats produces both methemoglobinemia and Heinz body formation, while nitrite poisoning in cow and pigs produces only methemoglobinemia (Jain, 1986). Development of significant methemoglobinemia has been observed in an occasional cat anaesthetized with ketamine hydrochloride. Cats develop Heinz bodies without metahaemoglobinaemia under natural condition and after methylene blue administration. Horses feeding on the leaves of Acer rubrum were observed to also develop acute hemolytic anaemia from methemoglobinemia and Heinz body formation (Divers et al., 1982; and Tennant et al., 1981).

1.4.4.9. Drug-dependent immune Hemolytic Anaemia

The first case studied to implicate immunologic factors in drug-induced hemolytic anaemia involved stibophen (Fuadin) (Harris,1956).It was found out that an antibody in the serum was bound to the red cells in the presence of the drug or in one of its derivatives. A different type of drug-induced hemolytic anaemia was reported by Ley et al. (1958) after the administration of massive amounts of penicillin. In their studies, the antibody was found to be effective against red cells that merely had been treated with a drug.

Though cases have not been associated with plant poisonings to produce immune hemolytic anaemia, this by no means does not mean

the situation does not exist since many plant poisonings cause hemolytic anaemia.

1.4.5. Hemorrhage

Hemorrhage is the escape of blood from a vessel whether it be to the outside of the body, into a body cavity or into adjacent tissues. Toxic injury to capillary endothelium culminating in transient openings of punctiform size is chiefly responsible for the petechiae and ecchymoses seen on serous and mucous membranes, as well as within the depths of the tissues.

1.4.5.1. Poisonous Plants producing hemorrhages

Poisonous plants such as Melilotus alba (sweet clover) contain the compound dicoumarin, a potent anticoagulant for the blood (Fraser and Nelson,1959). Cattle which consume hay containing M.alba for a month are reported to show uncontrollable hemorrhage from accidental or operative wounds and slow internal hemorrhages as a result of bruises and minor injuries. Postmoterm lesions show hematomas in the subcutis, between the muscles or beneath the capsules of organs. Ecchymotic hemorrhages occur in many places, commonly beneath the endocardium. The liver lobule show petechiae (Prier and Derse, 1962; Smith et al., 1974).

The illness that is associated with Pteridium aquilinum is sudden hemorrhages from many and often several body openings, delayed clotting time, thrombocytopenia, neutropenia, anaemia and death. Autopsy lesions include widespread petechiae and

ecchymoses, especially on the heart and other serous surfaces, on mucous membranes and in muscles and the subcutaneous tissues. Abomasal ecchymoses in cattle is said to lead to ulceration (Evans, Patel, and Koohy, 1982;Rajendran, Chennakesavalu Vivaraghavan and Danodaran, 1983).

Poisoning by members of Crotalaria genus which are mainly leguminous plant, include hemorrhages which appear in the form of petechiae or large ecchymoses. The hemorrhages are noted to be characterized by a yet unexplained bright red color, involving the serous and mucous surfaces. All organs are said to be congested, many are edematous, especially the abomasum, omasum and gall bladder (Allen, Carstens, and Knezevic, 1965; Carstens, and Allen, 1970).

Abrus precatorius (Jeguirty pea) produces congestion of the visceral organs and petechial hemorrhages throughout the body.Anthoxanthum odoratum (sweet vernal) is known to produce hemorrhagic diathesis in cattle fed home produced hay (Clarke and Clarke, 1975).

1.4.6. Methemoglobinemia and Methemoglobin forming substances. A great variety of chemical substances are known to convert haemoglobin into methemoglobin and thereby impair the blood capacity to transport oxygen to the tissues. Nitrates and nitrites in food plants and water which are themselves innocuous may by the action of intestinal microflora be converted into nitrite and so

form methemoglobinemia when transported into the blood circulation (Miyazaki and Kawashima, 1975; Andrade et al., 1971). There are reported instances in which without obvious external cause, methemoglobin is formed from haemoglobin and constitutes a constant hazard to the well being of the animal.

Bodansky (1951) gives a review of some of the methemoglobin forming substances. The substances include nitrobenzene, aniline, p-aminotoluene (p-AT) paraquinoacetophenone (p-AAP) and para-aminopropiophenone (p- APP).

1.4.6.1. Formation of methaemoglobins

Methemoglobin is an oxidation product of the normal blood pigment haemoglobin. The prosthetic group of haemoglobin is variously known as heme, ferrohaeme (Pauling and Coryell, 1936) and is a complex of iron and protoporphyrin IX.In methemoglobin, iron is present in the ferric state. Methemoglobin is also known as haemoglobin and ferrihemoglobin.

Methemoglobin may be formed from haemoglobin in a variety of ways.Essentially the action is an oxidation of the ferrous to the ferric ion, which is brought about by any of these ways: (a) by the direct action of oxidants (b) the action of hydrogen donors in the presence of atmospheric oxygen and (c) autoxidation. The ferrous ion of haemoglobin may be oxidized directly by ferric tartrate, ferricyanide, bivalent copper, chlorate, nitrate, quinones,

alloxans and dyes of high oxidation-eduction potential (Bodansky, 1951).

Another mode of oxidation is that of the action of hydrogen donors in the presence of atmospheric oxygen. Chief representatives in this group are the dyes e.g phenolindophenol, and methylene blue. The formation of methemoglobin from oxyhemo-globin by substances such as aminophenols, phencyhydroxylamines, phenylhydrazines and hydrazobenzene may be formulated in a similar manner.

The third important mechanism in the formation of methhemo-globin from haemoglobin is autoxidation.Bodansky (1951) reported that various autoxidazable substances present in turpentine, linseed oil, the alcohol-soluble substances ofpotato juice and sterile pneumococcus and other bacterial filtrates were capable of accelerating the formation of methemoglobin. This type of methemoglobin formation which is greatly dependent upon the oxygen tension, is negligible at zero oxygen tension and maximal at relatively low tension where about half of the haemoglobin is in the form of oxyhaemoglobin. At higher oxygen tensions, where a greater proportion of the haemoglobin in the form of oxyhaemogolbin,the rate of formation of methemoglobin is again decreased.

1.4.6.2. Chemical interactions and properties of methemoglobin

Methemoglobin can combine with a great variety of substances.Most of these interactions consist of a combination of the ferric ion with other ions such as fluoride or cyanide, but changes in the globin part of the molecule are also possible:

1. Methemoglobin hydroxide-Methemoglobin interacts with hydroxyl ion by attaching to the sixth bond position of the iron atom. The addition of sodium hydroxide to methemoglobin may also result in the denaturation of the globin moiety.

2. Methemoglobin fluoride-Methemoglobin reacts with fluoride ion to form methemoglobin fluoride also known as fluoro-methemoglobin.

3. Cyanomethaemoglobin-the interaction of methaemoglobin with cyanide is of considerable interest since it is this compound which has been considered under the therapeutic effectiveness of methemoglobin in counteracting the toxic or even lethal action of cyanide.The formation of cyanmethaemoglobin is the basis of the therapeutic and prophylactic actions of certain compounds in cyanide poisoning (Chen et al., 1934)

4. Hydrogen peroxide methemoglobin - the addition of hydrogen peroxide to oxyhaemoglobin leads to succession of reactions in equilibrium, mainly the formation of methemoglobin, the combination of methemoglobin with hydrogen peroxide, the reversion to methemoglobin itself as well as the partial oxida-

tion of methemoglobin through peroxidase reaction to other compounds.Hydroperoxide methemoglobin is not a stable compound. The nature of the oxidation products of methemoglobin caused by hydrogen peroxide is of interest in connection with a study of the nature of bile pigment (Lemberg et al., 1941). The authors showed that the green pigment choleglobin can be formed by the action of hydrogen peroxide directly on methemoglobin or even indirectly from haemoglobin.

5. Methemoglobin azide-azide forms a definite compound with methemoglobin similar to that which cyanide ion forms.

6. Methemoglobin hydrosulfide-hydrogen sulfide reacts with methemoglobin at an acid pH, to form methaemo- globin hydrosulfide (Bodansky, 1951).

7. Other compounds of methemoglobin-methemoglobin has been shown to combine with a number of other substances such as fulminate, thiocyanate, cyanate, ethanol, ammonia, imidazole and oxidation products of aniline.

1.4.6.3. Effect of methemoglobinemia on blood oxygenation

When oxygen tension in the blood is plotted against the degree of oxygenation, (or percent of oxyhaemoglobin sigmoid curve is obtained.The affinity of oxygen for haemoglobin is influenced by temperature, pH, ionic strength, concentration of haemoglobin and by the presence of other blood pigments such as carboxyhemoglobin and methemoglobin.

Douglas et al. (1912) showed that the presence of carboxyhemoglobin shifted the dissociation curve of the residual haemoglobin to the left and the form of the curve even became more hyperbolic in the presence of carboxyhaemoglobin. It can therefore be observed that the effects of poisoning by carbon monoxide are two-fold. Some of the haemoglobin is combined with carbon monoxide and therefore becomes unavailable for transport of oxygen and also the residual oxyhaemoglobin becomes less capable of dissociation

Darling and Roughton (1942) demonstrated that methemoglobin produces a similar effect.Quantitatively,however, the effect of methemoglobin is said to be less than carboxyhemoglobin. For example, a content of 23% carboxyhemoglobin shifted the dissociation curve of the residual oxyhaemoglobin in human blood about as far to the left as did a content of 43% methemoglobin (Darling and Roughton, 1942). The effect of methemoglobin on the oxygen dissociation curve is wholly reversible.

The alteration of the shape and position of the oxygen dissociation curve in carboxyhaemoglobinaemia or methemoglobinemia implies that the tissues are liable to anoxia, not only because of loss of the oxygen-carrying capacity of the blood but also because of the residual oxyhaemoglobin is less capable of dissociating and therefore unloading oxygen in the tissue.

Smith and Gosselin (1964) observed that methemoglobin induced in mice and other animals protects against the lethal

effects of inhaled hydrogen sulfide or injected sodium sulfide. This protective effect was said to be due to a trapping and/or inactivation of free hydrosulfide anions by methemoglobin. Cyanmehaemoglobin prepared and injected intraperitoneally into mice protected the animals against injected sulfide. This suggests that the molecule must possess binding or inactivation sites. For sulfide in addition to the ferric heme site already occupied by cyanide.

The reaction of nitrite with haemoglobin therefore,appears to produce at least two kinds of changes: (a) oxidation of the heme of iron which produces one binding site/heme group available to a variety of ligands and (b) globin alterations which results in the production of 1 to 3 specific sulfide inactivation site/heme group (Smith,1967).

The usual source of nitrate and nitrite poisoning in vetrinnary practice is in plants, growing or cured, which have derived a large amount of nitrate. A review of plants containing dangerous levels of nitrate and nitrite has already been presented under this review. In presence of moisture and possibly with the aid of bacteria, the phytogenous nitrates are easily reduced to nitrites (and eventually to ammonia). This occurs within the stomach, and especially the rumen, or externally as in stacks of hay that have become wet (Miyazaki and Kawashima. 1975; Andrade et al., 1971).

The outstanding symptom from nitrate and nitrite poisoning is the dark, brownish color of the blood, the effect of methemoglobin (Andrade et al., 1971).Clotting is said to be normal but the mucous membranes are cyanotic except those of the stomach and intestines, which show more or less hyperaemia and inflammation.

1.4.7. Coagulation and Platelet interaction during Hemostasis

The process of coagulation is simply defined as the conversion of fibrinogen to fibrin by the action of an enzyme thrombin, the conversion being accelerated by the presence of calcium ions and heat labile accelerator. The coagulation process is one of four steps namely the contact reaction, thromboplastinogenesis thrombin formation and fibrin formation.

The hemostatic response is mediated by the cell surfaces and collagen exposed at the wound site. Exposure to collagen initiates activation of the intrinsic coagulation system beginning with conversion of factor XII (Hegeman factor) to XIIa. Prekallikrein and high molecular weight- kinninogen at the injury site enhances the activation of the intrinsic pathway. Ruptured endothelial cells smooth muscle cells and other damaged cells exude tissue factor initiating the extrinsic pathway beginning with factor VII. Simultaneously platelets coming in contact with exposed collagen or thrombin (factor IIa) become activated i.e. they become spherical and adherent. Platelets bind to collagen via their surface receptors form von Willebrands factor complexed to factor VII of

endothelial cells. Activated platelets then undergo the release reaction. Platelets release a variety of substance including ADP and thromboxane A2 both of which cause more platelets to adhere and aggregate thus enlarging the platelet plug. Availability of platelet factor 3 and V (Proaccelerin) on the surface of the activated platelets further potentiates the clotting process (Jain, 1986).

During the hemostatic phase, factor V molecules at the surface of platelets in the hemostatic plug serve as binding sites for factor Xa. The binding step localizes the clotting process and markedly enhances the speed of the reaction. In the fibrin generation phase the localized factor Xa molecules trigger formation of a path of cross-linked fibrin molecules in which red cells become trapped.The platelet surface membrane protects the attached factor Xa and possibly thrombin (factor IIa) from degradation by the antithrombin III - heparin complex or other serine protease inhibitors. Finally in the wound repair phase, thrombosthenin, within clotted platelets contracts, converting the bulky plug into a tidy patch.

1.4.7.1. Acquired Coagulopathies

Jain (1986) mentions that the acquired coagulopathies are more common than the inherited disorders and are usually associated with multiple coagulation abnormalities.The diagnosis of such disorders is often indicated by the associated clinical

features and by results of screening tests such as prothrombin time (which assays extrinsic (factor VII) and common (factors X, V, II and I) pathways; activated partial thromboplastin time (assays intrinsic (factors XII, XI, IX, and VII) and common factors X, V, II and I) pathways; thrombin time (assays fibrinogen (factor 1).

Chemical substances which produces coagulation abnormalities usually cause platelets disorders, vitamin K, deficiency and antagonism, liver disease dysproteinemia or fibrinolysis.

1.4.7.2. Destructive lesions of the liver and blood coagulation

Destructive lesions of the liver which affect the coagul-ation process occurs in severe forms of acute (e. g. Heliotropium europaeum, aflatoxicosis (Doerr, Wyatt, and Hamilton, 1976) or chronic hepatitis (cirrhosis) where there is failure of the blood to clot due to deficiency of prothrombin which is produced by the liver cells.

Aflatoxicosis which is associated with various hemorrhagic episodes causes the loss of one of the two active sites of tissue thromboplastin. For example in studies in chicken, it was observed that factors VII and V activities and fibrinogen were reduced at 2.5 ug/g and above of aflatoxin. Factor X activity was depressed at 5.0 and 10 ug/g. The clot retraction in the whole blood was found to increase with growth inhibitory levels of the aflatoxin. The series of studies suggested the severe impairment of extrinsic and common clotting pathway functions in chicken and that

prothrombin is particularly affected during this coagulopathy (Doerr et al., 1976). However in similar studies the elevation of prothrombin times and of leucocyte counts were observed within 24 hours of A. flavus intoxication.

1.4.7.3. Hypocalcaemia and blood coagulation

Hypocalcaemia may result in failure of the blood to clot if calcium levels required for the coagulation process is below normal. Oxalates are used routinely in hematology to prevent the clotting of blood sample by formation of insoluble calcium oxalate and thereby remove soluble Ca++ required for the clotting mechanism (Swenson, 1975).

It is to be expected that the same reaction occurs when an animal ingests large amounts of oxalate at a time as observed to occur in poisoning by plants such as Halogenton glomeratus (Shupe and James, 1969) or Amaranthus retroflexus (Buck et al., 1966; Marshall, Buck and Bell, 1967). The signs consist of depression, anorexia, slight to moderate bloating, weakness, in coordination, restlessness frequent attempts to urinate,occasional reddish brown urine and brownish black faeces, blood tinged nasal exudate, coma, followed by death. There is widespread hemorrhages especially in the rumen.

1.4.7.4. Platelet Disorders

Platelet disorders include thrombocytopenia thrombasth-

enia,thrombocytopathy (abnormal platelet function) thrombocytosis and thrombocythemia. Acquired functional disorders of platelets with or without hemorrhagic manifestation associated with toxicity have been reported in therapy with certain drugs particularly nonsteroidal anti-inflammatory drugs notably aspirin. Platelet abnormalities have also been observed to have some relationship to the level of blood urea nitrogen (BUN) and duration of uremia. Urea per se does not seem to be directly detrimental but its metabolites quanidinosuccinic acid and phenolic acids are believed to be involved. Decreased production of prostaglandin endoperxidases by platelets and increased production of prostacyclin (PGI2) an inhibitor of platelet adhesion and aggregation by blood vessels of uremic patients are thought to act synergistically and contribute to the bleeding tendency (Remuzzi and Cavenagh, 1978; Rao and Walsh, 1983).

The liver is the principal organ concerned with the production of most of the procoagulants and thus it is obvious that a bleeding diathesis would follow a significant decrease in this function of the liver. Additional contributory factors include thrombocytopenia, dysfibrinogenaemia, enhanced fibrinolysis and abnormalities of platelet function (Rao and Walsh, 1983).

A reduction in the number of circulating platelets results in purpura hemorrhagica owing to the prolongation of the bleeding time.Oedema and hemorrhages of the subcutaneous tissues,

mucous membranes and internal organs are the characteristic pathological and clinical findings. Plants such as P.aquilinum which produce aplastic anaemia are reported to produce their effect through affecting blood clotting (Rao et al., 1988). Anthoxantum odoratum which produces hemorrhagic diathesis in cattle causes increased prothrombin and partial thromboplastin times.

1.4.7.5. Vitamin K Deficiency and Antagonism

Vitamin K is essential in the formation of several coagulation proteins referred to as the vitamin K-dependent coagulation proteins namely factors II, VII, IX and X.

Vitamin K antagonism is encountered in animals upon ingestion of substances such as warfarin, indanediones, bromadialone, and brodifacoum whose mode of action depends on vitamin K antagonism (Mount and Feldman, 1983). These substances were observed to inhibit the enzyme system (epoxide reductase enzyme) essential for recycling of vitamin K necessary for activation of precursor forms of coagulation factors produced by the liver.

Poisoning by Mellilotus alba (sweet clover) is reported to produce serious coagulopathies because of its content of dicoumarin a potent vitamin K antagonists (Fraser and Nelson,1959; Prier and Derse, 1962). Infact Warfarin is a derivative of dicoumarin.

1.4.8. Leukocytes

The leucocyte system serves to defend the body against foreign organisms or extraneous material.They are of greatest numbers in the peripheral blood and are subdivided as neutrophils, lymphocytes, monocytes, eosinophils and basophils. Each subtype has a somewhat different function and each may behave as a separate but related system. Leukocytes differ from the other cell types in the blood because most of its functions are performed outside the vascular system where they defend the body by phagocytosis (neutrophils, monocytes, eosinophils and basophil) or antibody production as carried out by lymphocytes or their more mature forms, plasma cells (Swenson, 1975).

Only about half of the leukocytes in peripheral blood circulate freely with the remaining being attached to the walls of the blood vessels (or marginated). Upon tissue injury the marginated neutrophils pass between vascular epithelium cells by diapedesis into damaged area where they engage in phagocytosis and are found in inflammatory exudate.Toxic substances may cause leukopenia by suppressing granulocytopoiesis or lymphopoiesis.Toxic substances of plant origin, such as bracken fern,certain drugs as sulfonamides and similar chemicals which compete with folic acid utilization cause leucopenia (Jain, 1986).

1.4.8.1. The neutrophils

The neutrophils form the first line of cellular defence against microbial infection. Mature neutrophils have characteristically polymorphic segmented nucleus with undulated nuclear membrane and clumped chromatin. Defective bactericidal activity of neutrophils may be related to cellular dysfunction of phagocytosis, postphagocytic events and bactericidal action. Defective bactericidal activity of neutrophils has been found in various conditions such as ureamia and jaundice (Wardle and Williams, 1980); drug therapy (example amphotericin B, tetracycline, certain sulfa drugs, corticosteroids and some antineoplastic drugs such as methotrexate and Vinca alkaloids, (Mandell, 1982). Neutropenia and terminal anaemia accompany the thrombocytopenia in Pteridium aquilinum poisoning and are due to destruction of the early myeloid cells (Smith et al., 1974).

1.4.8.2. The eosinophils

The eosinophils have characteristically bright pinkish red uniformly stained cytoplasmic granules and a polymorphic nucleus that is smoother and less segmented than that in mature neutrophils. Eosinopenia of physical and emotional stress is attributed to elevated levels of catecholamines such as adrenaline and adrenocorticosterods.Corticosteroid-induced eosinopenia is attributed to migration of eosinophils into lymphoid organs namely the spleen,lymph nodes, and thymus while eosinophilolysis or

decreased production was not involved (Sabag et al., 1978). , Eosinophilia occurs in some allergic diseases and infestation with large parasites and with poisonous plants such as Lasiosiphon kraussianus (Nwude, 1976).

1.4.8.3. The basophils

The basophils are numerically insignificant but functionally important leukocytes. The blood basophils bear some morphologic resemblance to the tissue mast cell and is believed to share similar function. The question is often asked whether basophils and mast cells share a common origin or whether they are one and the same cells, acquiring different morphology under the influence of local microenvironment and different locations in the body (Swenson, 1975).

1.4.8.4. The Monocyte and Macrophages

The monocytes are known to be derived from hematopoietic stem cells in the bone marrow, and shortly after entering the circulation, they migrate into various tissues and body cavities and become fixed or free macrophages. The blood monocyte and tissue macrophages constituted what was called the reticuloendothelial system (RES) which was used to describe a collection of widely distributed cells capable of phagocytosis which, in addition to blood monocytes and tissue macrophages (histiocytes), included the

reticular cells of the spleen and lymph nodes, reticulo-endothelial cells of the lymph and blood sinuses, and the Kupffer cells of the liver, (Jain, 1986).

The blood monocytes, the promonocytes and their precursors in the bone marrow, and the tissue macrophages are considered components of the mononuclear phagocytes, system (MPS). Members of the MPS include histiocytes, in connective tissue, fixed and free macrophages in the lymph nodes, spleen, and bone marrow; pleural and peritoneal macrophages, Kupffer cells of the liver, alveolar macrophages, osteoclasts and microglial cells in the nervous system.

Corticosteroids depress bactericidal and fungicidal activities of monocytes (Thompson and van Furth, 1973). They affect functional competence of the MPS in a number of ways (Kimberly and Ralph, 1983). They inhibit proliferation and maturation of monocyte precursors in the bone marrow, exudation of monocytes in inflammatory foci and antibody and complement- mediated phagocytosis by the MPS. Corticosteriods inhibit activation of macrophages by rendering them unresponsive to lymphokines.

1.4.8.5. <u>The lymphocytes</u>

The lymphocytes play a pivotal role in the initiation and execution of the immune response. Lymphocytes are

a heterogenous group of cells both morphologically and functionally.

Morphologically, lymphocytes are classified as small medium and large or simply as small (6-9 um) and large (9-15 um). The cell size, the degree of cytoplasmic basophilia, and, to a certain extent, the nuclear chromatin pattern indicate the relative age or maturity (Garcia and Iorio, 1968).

Functionally lymphocytes are subclassed into those concerned with cell mediated immunity and immunoregulatory functions referred to as thymus dependent, thymus derived, thymus-processed or T-cells while those concerned with the formation of humoral antibody are thymus-independent, bursa-derived in birds and bursa-equivalent bone marrow-derived in mammals or B-cells. A minor, third population of mononuclear cells are referred to as "non-T, non B" or "null" cells (Ferrarini el al., 1980).

A decreases in lymphopoiesis is associated with lymphopenia such as after corticosteroid administration or radiation. Increased concentration of corticosteroids is known to cause lymphoid and thymic atrophy (Esteban, 1968). Corticosteroid-induced lymphopenia is associated with lympholysis in blood and lymphoid is associated with lympholysis in blood and lymphoid tissues (rabbit)

or altered distribution of lymphocytes out of the vascular pool into other body compartments such as the bone marrow (Cohen, 1972).

Nwude (1976) reported of lymphopenia associated with the consumption of L. kraussianus in goats, sheep and donkeys. Certain plant lectins have the capacity to release the development potential of specific resting or dormant cells in the circulatory system of man and transform them to an active growth state (Nowell, 1960). This subgroup of the lectins has been termed mitogen and the class of cells affected is primarily that of the immune responsive cells, or lymphocytes, Plants with such lectins include Phytolacca americana, Abrus precatorius, Hura crepitans and Robinia pseudoacacia, McPherson, 1979).

1.5 TOXIC EFFECTS ON NON-CELLULAR CONSTITUENTS OF BLOOD

This involves the study of pathologic changes at the molecular level. It concerns the changes which occur in the chemical constituents of the blood and tissues and it therefore provides for a better understanding of the disease process as well as supply information helpful in differential diagnosis, therapy and prognostication (Cornelius and Kaneko, 1965).

1.5.1. Nitrogenous constituents of the Blood

The blood contains a number of nitrogenous substances mainly proteins and nonprotein nitrogenous substances. The blood protein shares an intimate relation to protein metabolism in the liver as well as interacting with other tissues throughout the body, thus showing the central position of the blood proteins in the general metabolism of protein. Therefore changes that are observed in the qualitative and quantitative composition of blood protein is of significance to demonstrate the state of the subject under-study at a particular time.

The heterogeneity of the blood proteins is readily demonstrated in the ultracentrifuge. In addition to fibrinogen, albumin and a group of globulins sedimenting at a rate between these two, the ultracentrifugal analysis of blood reveals a group of very rapidly sedimenting globulins and lipoproteins, the sedimentation of which varies markedly with the density of the medium.

From isotopic and other evidence, it has been concluded that the liver is the chief, if not the sole site of formation of albumin, fibrinogen, prothrombin and & - and B-globulins (Cantarow and Trumper, 1956; Jain, 1986). The Y-globulins are believed to be synthesized mainly in plasma cells.

1.5.1.1. Blood Nonprotein Nitrogen

The nonprotein nitrogen (NPN) of the blood, that portion of the nitrogenous substances not precipitated by the usual protein precipitants e.g. trichloroacetic acid, includes uric acid, amino acids, creatine, creatinine, ammonia and a fraction designated undetermined nitrogen. The NPN constituents of blood are usually observed to be of greater clinical interest than the proteins since they represent the products of the intermediary metabolism of ingested and tissue protein.

1.5.1.2. Nitrogen Balance

Since most of the nitrogen of the diet represents protein, and most of the nitrogenous excretory products are derived from protein catabolism, it is apparent that the balance between the two will reveal significant features of protein metabolism. Nitrogen balance is defined as the quantitative difference between the nitrogen intake and the nitrogen output.

Positive nitrogen balance exists when intake exceeds output and this obtains whenever new tissues are being synthesized. In negative nitrogen balance, the output exceeds the intake.

1.5.1.3. Dysproteinaemia

The best method for the overall evaluation of pro-

degenerative, metabolic and regenerative processes occurring in specific liver disease. This therefore makes it difficult to define a typical plasma protein pattern in certain types of liver disease. However changes in certain types of patterns were observed by Kaneko (1980) to be characteristic of specific hepatopathies. Total serum protein values are observed to be rarely of aid in clinical interpretations unless their values fall below 5gm\dl which are usually observed only in late stages of disease processes.

The fall in serum albumin concentration attributable to failure of hepatic parenchymal synthesis is usually not an early change, and is common in chronic conditions such as diffuse fibrosis as is observed with plants of the genus Senecio which are distributed world-wide. It is believed that there exists a correlation between the severity of the liver lesions associated with Senecio poisoning and the extent of changes that may be observed in serum protein and albumin levels (Fowler, 1968; Thorpe and Ford 1968). Similar observations were made with the plant Lupinus (Gardiner, 1965; Shape er al., 1968). Aspergillus flavus (Gumbmann and Williams, 1969; Cysewski et al., 1968).

In portal fibrosis, the characteristic change is usually diminution in the serum albumin level and in elevation in the

gamma-globulin level. The changes in albumin level are less conspicuous in acute hepatitis, but an elevation in the gamma-globulins is a consistent finding. Low albumin levels are also observed in animals with nephritis, nephrosis, malnutrition and other chronic diseases causing cachexia.

1.5.1.5. Proteinuria

The urinary loss of protein in excessive quanti ties is proteinuria. Pathological proteinuria is an anomally intrinsic to renal disease, but may have pre-renal or postrenal causation. Ordinarily, proteinuria of renal origin is associated with defects in either glomerular or tubular apparatus giving rise to excretory dysfunction. In glomerulonephritis, proteinuria arises through abnormal glomerular filtration. Proteinuria is marked whenever renal membrane function is affected. Proteinuria is a common occurrence in poisoning with nephrotoxic plants such as H. glomeratus (Shupe and James, 1969). Amaranthus retroflexus which is reported to produce peri-renal oedema syndrome (Buck et al., 1966; Marshall, Buck, and Bell, 1967).

The accompanying renal lesions associated with such nephrotoxic plants consists of selective structural injury in certain segments of the tubuli which leads to impaired tubular reabsorption. The excretion of low

molecular weight proteins is reported to be characteristic or disrupted tubular function. Affected glomerular filtration which is associated with the excretion of gross amounts of proteins of predominantly high molecular weight is brought about by only a few renal toxicants (De Bruin 1976).

Substances from plants which produce hepatotoxicity invariably also cause nephrotoxicity with the accompanying proteinuria. Therefore plants such as Lupines which are hepatotoxin also cause nephrotoxicity with accompanying proteinuria (Gardiner, 1965; Shupe et al., 1967).

1.5.2. Pigment Metabolism - Jaundice

Bilirubin, the pigment of bile, is derived from haemoglobin. It has been rather definitely established that the cells of the reticulo-endothelial system, especially those present in the bone marrow, spleen and liver (Kupffer cells) are concerned with the metabolism and formation of bile pigment. Bound to albumin, bilirubin is then transported from the reticulo-endothelial cell via the blood stream to the liver. In the hepatocyte, bilirubin is separated from albumin and conjugated with glucuronic acid and excreted in the bile as bilirubin-diglucuronide. The conjugated bilirubin in the intestine is reduced by

bacteria to urobilinogen is reabsorbed into the portal circulation and carried to the liver (enterohepatic circulation) where most of it is converted to a bilirubin-like compound and re-excreted into the bile.

Three of these pigments are of importance in the diagnosis of icterus (a) Bilirubin-diglucuronide or conjugated bilirubin or direct reacting bilirubin which gives a direct reaction to the Van den Bergh test.
(b) non-conjugated bilirubin also called in direct reacting bilirubin (indirect reaction to the Van den Bergh test), bilirubin and haemobilirubin, (c) urobilinogen.

Icterus is an important clinical and postmortem disorder in which biliburin reaches such a high concentration in the circulating blood that the tissues of the whole body are tinged yellow.

Icterus or jaundice may be divided into three types, mainly the hemolytic, toxic and obstructive icterus.
Hemolytic jaundice is the result of excessive destruction of RBC ordinarily in the circulating blood.
Granineous plants such as Trifolium subterraneum which accumulate copper is reported to cause jaundice in animal consuming it over prolonged periods during which the animal is asymptomatic (Hogan et al., 1968). It causes RBC destruction under conditions of stress when the copper is released. Ricinus

conmunis, the common castor plant also produces intravascular hemolysis resulting in icterus (Jenkins, 1963). Divers et al. 1982), and Tennant et al. (1981) have reported similar findings in horses who ingested Acer rubrum. Hemolytic icterus was also observed in cattle (Hutchinson, 1977), sheep (Van Kampen et al., 1970) and steers (Jain, 1986) which were fed feed containing the plant Allium cepa.

Toxic jaundice is caused by toxic substances acting upon the cells of the liver and producing hydropic degeneration, fatty change and necrosis. There are two ways in which destructive changes in the liver result in icterus. First, the hepatocytes may be damaged to such an extent that they cannot perform their excretory function. Haemo- bilirubin (unconjugated bilirubin) then remains in the blood just as hemolytic jaundice.

Secondly, the toxicant can cause swelling of the hepatocytes which cause occlusion of the bile canaliculi.
The bile is excreted from the cells but cannot pursue its course to the gall bladder and intestine. In this case it is bilirubin diglucuronide or posthepatic bilirubin which accumulates in the liver from where it is reabsorbed into the blood. Both processes are reported to go on simultaneously so that the bilirubin-glucuronide and unconjugated bilirubin are detected in blood. Hepatotoxic

plants are most commonly observed to produced toxic jaundice. For example plant of the genus Senecio, Crotalaria and Heliotropium europaeum which contain pyrrolizidine -alkaloids produce characteristic change of the enlargement of the parenchymal cells throughout the liver, a phenomenon which is termed megalocytosis (Bull, 1955). Shupe et al. (1967) also reports of similar observation with plants of the genus Lupinus.

Plants which cause toxic jaundice also produce swelling of the hepatocyte which results in the obstrction of the bile canaliculi or obstructive jaundice. Sharma et al. (1982) reports of marked increase in the conjugated form of bilirubin in guinea pig plasma during L. camara poisoning characteristic of obstructive jaundice.

It is recognized that discoloration of the sclerae and the skin and passage of bile pigment into the urine occur more commonly in obstructive and toxic jaundice than in hemolytic jaundice. This explained on the basis that in obstructive jaundice, the bilirubin is more readily diffusible (direct-reacting bilirubin) than in hemolytic jaundice (non direct-reacting bilirubin). For example it has been observed that in toxic or obstructive jaundice, small levels of bilirubin in the blood which persist for some days, diffuse through the capillaries and appear in

the tissues. Greater concentrations are usually necessary in hemolytic jaundice for the production of frank icterus (Kaneko, 1980).

1.5.3. The influence of Plant poisons on blood urea nitrogen

Urea is the chief end-product of protein metabolism and in formed normally largely if not entirely in the liver from amino acid metabolism. Ammonia derived from the amino acid by deamination, and Co_2, arising from oxidations (Kreb cycle) are carried by a derivative of glutamic acid (carbamylglutamic acid) and are transferred to ornithine. This undergoes amination (transfer of NH from aspartic acid) to form arginine, which is split by the enzyme arginase, into urea and a molecule of ornithine. The urea enters the systemic circulation and is excreted from the body mainly in the urine.

When liver function is severely impaired, as occurs in acute hepatic necrosis, there is rise in the blood amino acids and a fall in the urea concentration of blood and urine. Increase in blood urea nitrogen may be due to decrease in renal excretion due to organic disease of the kidneys with destruction of a considerable portion of functioning renal tissue. Glomerulonephritis is a common cause for abnormally high blood urea nitrogen concentration, evidence of renal functional impairment in acute and chronic

glomerulonephritis. Also conditions in which there is extensive destruction or inflammation of the kidneys, pyelonephritis, advanced nephrosclerosis, renal cortical necrosis, renal conditions accompanied by marked oliguria or anuria as in some poisoning. P. aquilinum was noted to produce increased BUN in calves (Singh et al. 1987) and rats (Rao, Joshi, Kumar, 1986), because of impairment of renal function. A similar observation with increased BUN was made by poisoning with the plant H. glomeratus in sheep (Shupe and James, 1969) which was attributed to reduced renal function as a result of organic changes in the renal parenchyma. Crotalaria retusa which contains pyrrolizidine alkaloids was found as a seed contaminant of grain sorghum at a rate of about 0.1% body weight in the diet fed at a piggery. The pigs were reported to show reduced weight gains and later severe illness with many mortalities. The dominant syndrome was severe nephroses with ureamia. The pigs showed elevated BUN between 95 and 275mg/100ml, (Hooper, 1977).

1.5.4. Serum Enzymes of Diagnostic importance

Each cell in an organ is known to have a specific function and contains enzymes unique to that function. With the disruption of the integrity of the cell, the enzymes escape into the surrounding fluid compartment

and into the serum or cerebral spinal fluid, where their activity can be measured as a useful index of that cell's integrity.

There are three readily assayable liver specific enzymes which are arginase ARG), sorbitol dehydrogenase (SDH) and alanine aminotransferase (ALT). Arginase and SDH are liver specific enzymes in many animals and are excellent markers of hepatocellular damage. Alanine amino-transferase is also reported to be specific and is assayed in place of ARG and SDH because its assay is simple and SDH is moreover not very stable (Kaneko, 1980).

When an enzyme lacks sufficient specificity for an organ, a second enzyme may be combined with the first to increase the diagnostic value. For example serum alkaline phosphatase activity increases with bone and liver disorders and to assist in identifying the source of the increase, alanine aminotransferase or gamma-glutamyltransferase (GGT) is assayed as part of the hepatic profile.

1.5.4.1. Creatinine Kinase

Creatinine kinase (EC 2.7.3.2) or creatinine phosphokinase is one of the most organ specific serum enzymes in clinical use. It catalyzes the reversible phosphorylation of creatinine by ATP to form creatinine phosphate and ADP. Creatinine phosphate the major storage form of high energy phosphate required by muscle for

contraction. Creatinine kinase is a dimer consisting of two peptides B (for the brain and M (for the muscle) which makes up the three isoenzymes CK_1, CK_2, and CK_3 or BB, MB and MM respectively. Many cells contain creatinine kinase but only the heart and skeletal muscle contain sufficient amounts of CK_2 and CK_3 to alter serum activity in organ specific disorders. The isoenzyme CK is found predominantly in the brain.

Creatinine kinase has a short half life in serum and it is relatively unstable when stored at room refrigerator or freezing temperature. However the activity of CK can be restored by incubating serum with sulfhydryl activators e.g. cysteine or glutathione. Elevations of creatinine kinase activities have been reported in myodegeneration due to ingestion of toxic plants in cattle (Henson et al., 1960. Elevations in creatinine kinase activities have been reported in polioencephalomalacia and focal symmetrical encephalomalacia of sheep and it has been suggested that creatinine kinase determinations may be of value in diseases of the central nervous system (Smith and Healy, 1968).

1.5.4.2. Alanine Aminotransferase

Alanine aminotransferase (ALT) (EC.2.6.1.2) and glutamic pyruvate transaminase (GPT) catalyzes the reversible transamination of L-alanine and & -oxoglutarate to pyruvate and glutamate. ALT is present in plasma and cells. In dogs and cats, there is sufficient ALT activity in the liver for it to be used as a liver

specific enzyme. Increase in plasma activity of GPT in these animals is associated with hepatocellular disorders. Acute hepatic diseases causing membrane damage or cell necrosis result in appreiable increase in plasma activity (Kaneko, 1980).

1.5.4.3 Aspartate Aminotransferase

Aspartate aminotransferase (AST)(EC.2.6.1.1) and glutamic oxaloacetate transaminase (GOT) catalyzes the transmination of L-aspartate and -oxoglutarate to oxaloacetate and glutamate. AST is present in mitochondria and cytosol of almost all cells and in plasma. The presence of GOT in so many tissues precludes the use of this enzyme as an organ specific enzyme but it is useful in conjunction with other enzymes as an index of hepatic or muscular cell damage (Kaneko and Cornelius, 1970).

1.5.4.4. Gamma-Glutamyltransferase

Gamma-glutamyltransferase (GGT)(EC.2.3.2.2.) is a carboxypeptidase which cleaves the C-terminal glutamyl group and transfers them to peptides and other suitable acceptors. Glycylglycine is the most suitable acceptor. The physiological function of Y-glutamyltransferase is unknown but it is speculated to be associated with glutathione metabolism. Cell cytosol and membranes are known to have Y-glutamyltransferase acticity. Most cells have GGT activity ranging from the kidney with the highest and the muscle the lowest.

Serum has GGT activity most of which is derived from the liver. Kidney GGT is detectable in serum and urine contains only kidney GGT (Bruin et al., 1978). Ford (1974) and Johnson (1976) observed that GGT may well be a more specific indicator of obstructive hepatic disorders than alkaline phosphatase.

1.5.4.5. Lactate Dehydrogenase

Lactate dehydrogenase (LDH) (EC.1.1.1.27) catalyzes reversible oxidation of L-lactate to pyruvate with the co-factor NAD. A large amount of LDH activity is present in all tissues. In the blood, there is as much as 150 fold greater LDH activity in the red blood cells than there is in plasma. Thus even minimal hemolysis alter the plasma LDH activity appreciably. Anticoagulants such as EDTA and oxalate, indirectly inhibit the enyzmes. There heparinized plasma or serum is the preferred sample.

LDH is a tetrapeptide made up of two types of peptides: (heart) and M (muscle).The two types of peptides in various combinations make up 5 isoenzymes LDH-1, LDH-2 LDH-3, LDH-4 and LDH-5 which are designated for peptide combinations HHHH, HHHM, HHMM, and MMMM respectively. Tissues contain various amounts of the LDH isoenzymes band serum isoenzyme profile are used to identify specific tissue damage by electrophoretic separation (Prase, 1969).

1.5.4.6 Sorbitol Dehydrogenase

The activity of Sorbitol dehydrogenase (EC.1.1.1.14) in plasma is very low.The major source of it is the hepatocyte. It catalyzes

the reversible oxidation of D- Sorbitol to D. fructose with the co-factor NAD. Sorbitol dehydrogenase is unstable and at room temperature appreciable amounts are lost in a few hours. Metal chelating anticoagulants such as EDTA and oxalate decrease the amount of sorbitol dehydrogenase activity. Therefore heparinize plasma may be used for its estimation but serum is preferred.SDH is liver specific and hepatic injury appears to be the only source of increased SDH activity.

1.5.4.7. Alkaline Phosphatase

Alkaline phosphatase (EC.3.1.3.1.) is an important enzyme for characterizing bone and hepatic disorders. Alkaline phosphatase is a non-specific enzyme which hydrolyses many types of phosphate esters and is present in multiple molecular forms i.e. isoenzymes. The endogenous substrate of ALP is unknown, but it catalyzes the dephosphorylation of ATP and is thought to be associated with energy requiring membrane pumps.

The term alkaline refers to the optimal alkaline PH of this class of phosphatases in vitro.The PH optimum for ATP is 10, a PH unlikely to occur in the body.

1.5.5. Serum Enzyme Tests

The level of serum enzymes which is measured by their biochemical activity changes as a result of processes involving the liver. Enzyme activity may be increased from disrupted hepatic parenchymal cells with necrosis or altered membrane permeability

e.g. alanine aminotransferase (SGPT) aspartate aminotransferase (SGOT), arginase, isocitrate dehydrogenase (SICD), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GD) ornithine carbamyltransferase (OCT), and lactate dehydrogenase (LDH).Increased levels of enzymes also result from their over production in cholestasis of obstructive icterus e.g. alkaline phosphatase. Elevations in serum activity provide little information regarding the reversibility, type of lesions or functional state of the liver. However the quantiative estimate of the extent of necrosis can be estimated.

Enzymes whose concentration is increased following hepatic necrosis are further divided into the liver specific enzymes because the liver contains their high concenration (SGPT) in dogs, cats and primates; SDH and GD in sheep and cattle; SDH in horses; arginase and OCT in all ureotelic animals and enzymes which are present in high concentration in other tissues including the liver (SGOT) LDH, and SICD).

1.5.5.1. Pathological Findings

The clinical-pathologic features of A. flavus poisoning have been demonstrated in experimentally poisoned swine (Gumbmann and Williams, 1969; Sisk, Carlton and Curtin, 1968). The serum levels of SGOT, OCT, ALP and SICD were markedly elevated. The enzymes

were reported to be concomitantly lost from the damaged liver. Himes and Cornelius (1973) attest to the usefulness of them

Pathology involving the skeletal or cardiac muscle and or the hepatic parenchyma allows for the leakage of large amounts of this enzyme into the blood. Since all major tissues contain high concenrations of SGOT, the finding of significant elevations in SGOT need not indicate hepatic necrosis unless diseases of other large organ systems can be ruled out. Elevations of SGOT activities have been observed in Senecio jacobaea intoxication in calves (Ford and Ritchie and Thorpe 1968) aflatoxicosis in swine (Cysewski et al., 1968) in feeding of Brassica oleracea to cattle (Grant et al.,) and on rams fed on the plant of the genus Astragalus (James and Binns, 1967).

In poisoning, there is more rapid disappearance of arginase which is a mitochondrial-bound enzyme as compared to the transaminases which are in the hyaloplasmic fraction to the cells. Harvey and Hoe (1971) and Kaneko (1980) suggest that if both serum arginase and transminase activities are continuously elevated, a progressive hepatic necrosis was most likely the cause. However if the serum arginase returns to normal and elevated transaminase activities are observed following significant elevations of both enzymes, prognosis is favorable as hepatic necrosis was likely subsiding. The estimation of serum arginase activity is a reliable

liver-specific enzyme test for active hepatic necrosis in all ureotelic species.

Harvey and Obeid (1974) reported that arginase was superior to alkaline phosphate and bilirubin levels for the diagnosis of hepatic disease in ruminants and camels in Sudan.

Sorbitol dehydrogenase is a serum enzyme which is been found to be liver-specific in large domestic animals. Sharma et al. (1982) observed a marked increase in the conjugated form of bilirubin in guinea pig plasma during L. camara toxicity characteristic of obstructive jaundice. Also activities of SGOT, lactate dehydrogenase and sorbitol dehydrogenase in plasma of guinea pig, were elevated. Elevated levels of sorbitol dehydrogenase was also observed in sheep with lantana poisoning (Sharma, et al 1981):

Boyd (1962) has shown that glutamate dehydrogenase is highly concentrated primarily in ovine and bovine liver and recommended its use in measuring necrosis in these species. Similarly GDH is present in high concentration in equine (Freedland et al., 1965). It has become the enzyme of choice in measuring hepatic necrosis in ruminants (Kaneko, 1980). Animals poisoned by the plants in the Lupine family showed elevated levels of SGOT in the early stages as are the serum lactate dehydrogenase and glutamate dehydrogenase (Shupe etal., 1968).

1.5.5.2. Urinary enzymes

Enzymes, ordinarly absent or negligible in the urine,are excreted significantly under pathological conditions involving renal cell damage. Enzymuria is a sensitive index of minor tubular lesion preceeding functional impairment as evidenced by renal function tests. Enzymes gain access to the urine either by impaired glomerula filtration or as a result of desquamation of kidney tubular cells.

Lactate dehydrogenase activity is reported to increase in the urine and serum of patients with urinary tract disease (Gelderman, 1965). Enzymuria is a more sensitive and early criterion of the nephrotoxic action of substances than are secretory dysfunction.

As certain enzymes are localized predominantly in specific segments of the renal tubuli, their urinary assay may delineate specific structural injury. Thus proximal tubular lesions are suggested from the rise of alkaline phosphatases while increases in lactate dehydrogenase or carbonic anhydrase is indicative of structural anomaly of the distal segments (De Bruin, 1976). Injury of the glomerula type is revealed by acid phosphatase assay.

1.5.6. Primary Renal Dysfunction

Acute failure

The kidney is particularly susceptible to noxious agents due to its high perfusion rate, the numerous enzymes and its role as an

excretory organs.Nephrotoxins commonly encountered in domestic animals include most plants poisons such as H. glomeratus (Buck et al., 1966 Shupe and James 1969), A.retroflexus (Marshall et al., 1967), aflatoxins (Cysewski et al., Sisk et al.,1968),

Nephrotoxins produce their effect by various mechanisms. Some remain in the renal lumen and are concentrated as water is reabsorbed.Others are taken up by tubular cells and concentrated in them. Such compounds are relatively inert and are toxic upon modification by host enzymes.Oxalates are for example precipitat-ed in the renal tubules during the process of elimination and fatal outcome may occur from renal insufficiency and ureamia. Such poisoning has been observed in H. glomeratus and A. retroflexus which contain high oxalate levels (Buck at al.,1966; Shupe and James, 1969).

There is no unanimity of opinion on the events that culminate in acute renal failure (Harrington and Cohen,1975; Levinsky, 1977). The first opinion assumes it is due to renal vasoconstriction. The second states that tubular blockage occurs as a consequence of intraluminal debris and interstitial oedema. The third surmises that glomerular filtrate forms but is totally reabsorbed (passive back flow) because of tubular necrosis.

Chronic failure.

Chronic renal failure is the consequence of slow insidous destruction of renal parenchyma. Due to the reserve capacity of

the kidney, clinical signs of disease may not develop for months or years following the initiation of disease. Morphological examination are of little value to identify causes of chronic renal failure because renal responses to injury are limited in variety and tend to assume a common character regardless of cause as chronicity develops. Unlike the abrupt sequence of events in acute anuric failure that reflects manifestations of sudden dysfunction,most changes take place gradually in chronic failure. The changes are initially undetectable both clinically and biochemically. It is only when over two- thirds of the normal parenchyma has been destroyed that clinical signs become apparent. For example the ultrastructual features of the hyaline lesions in glomeruli and renal arteries results deposition of amorphous and fibrillar material which obliterates lumens of glomerular capillaries following Crotalaria spectabilis intoxication in rats yet no clinical signs were obvious (Carstens and Allen, 1970).

1.5.7 Serum calcium and phosphorus

Extracellular calcium is present in free calcium ions, calcium ions that are ionically bound but ultra filtrable,and calcium bound to plasma proteins. The ionized calcium is diffusible; the complexed calcium is diffusible but not ionized; whereas the protein bound calcium is neither ionized nor diffusible.Although all these forms to calcium are in equilibrium

with one another, in the body fluids, only the ionized fraction is under direct control (Copp, 1969).

Most of the phosphorus of blood is present as organic ester of phosphorus within the red blood cells, these contain small amount of inorganic phosphorus (pi) at any given moment. Serum contains about 14-15mg oftotal phosphorus per deciliter but this 5-8mg are lipid phosphorus. A trace of the rest is ester phosphorus, the most significant portion of the remainder being Pi. The bone mineral is readily mobilized (regulatory mechanisms by parathyroid hormone, calcitonin and the active metabolites (of vitamin D) to maintain the level of serum calcium but less readily to maintain that of Pi so that a low serum Pi level is the first sign of deficiency of phosphorus. Pi level appears to be intimately tied to carbohydrate metabolism. During increase carbohydrate utilization, the level tends to decrease and during fasting and increase usually is observed.Measurement of Pi concentration (preferably heparinized plasma) provide the most readily determinable index of the phosphorus status of animals, (Newman, 1968; Little et al., 1971).

1.5.7.1. Toxic Plants affecting calcium and phosphorus metabolism

A series of so-called plant induced calcinosis in animals has been describeed. Cattle feeding on Solanum malcoxylon are known to develop a condition characterized by wasting stiffness, hypercalcaemia,,and hyperphosphataemia.The most typical lesions is

the calcification of the arteries, which is associated with a greatly increased calcium absorption from the diet (Samson et al., 1971). In describing this poisoning by S. malacoxylon, Krook et al. (1975) give a list of the different names by which the condition is called in various conuntries;entegueseco (Argentina) espichamento (Brazil) naalelia disease (Hawaii) Manchester wasting disease (Jamaica); enzootic calcinosis (Germany, Austria). A similar condition with hypercalcaemia and calcinosis has been reported caused by the shrub Cestrum diurnum in pigs (Kasali, 1977) and horses (Krook et al., 1975).

These plant poisonings show great similarities with vitamin D poisoning i.e.different degree of hypercalcaemia hyperphosphataemia, and calcifications in the circulatory systems, lungs, kidneys and flexor tendon. Investigations show that all these diseases most likely are caused by the presence of potent, active vitamin D-like substances in the plants and that these substances are responsible for the abnormalities of mineral metabolism in the animals (Krook et al., 1975' Shupe and James, 1969; Wasserman et al., 1977).

Hypocalcaemia has been reported in sheep poisoned by H. glomeratus (Littledike, James and Cook, 1976; Marshall et al., 1967) and other plants such as Sarcobatus vermiculatus, Oxalis cernua and A. retroflexus (Buck et al., 1966; Shupe and James, 1969> Littledike et al. (1976) report that the plasma concentration

of Ca and Ca activity decreased over several hours following H. glomeratus poisoning, to such low levels that tetany or coma occurred and death followed. Increases in plasma total inorganic phosphorus and magnesium concentration also occurred as the hypocalcaemia progressed.

1.6. GROSS AND HISTOLOGIC LESION IN TOXICOSIS

1.6.1. Death of cell and tissues

Poisonous substances which produce local injury (necrosis and inflammation) to the tissues have to come into contact with them. Obviously an appropriate degree of concentration is essential to the production of this effect. Some toxicants achieve their highest concentration at their site of toxic action. For example carbon monoxide which has a very high affinity for haemologbin, and paraquat which accumulate in the lung (Sharp et al., 1972). Other agents concentrate at sites other than the site of toxic action. Lead for example is stored in the bone while the symptoms of lead poisoning are due to lead in the soft tissues (Clarke and Clarke, 1975). The toxicant
while it is stored often does no harm to the organism.Storage depots therefore could be considered as protecting organs preventing high concentrations of the toxicants from being achieved at the site of toxic action.

Grossly, a dead tissue is regularly paler than a living one except where it is well filled with blood, which makes it

black. A dead tissues also has little strength, especially tensile strength. If one finds both living and dead tissues within the same microscopic sections, then the dead part represents necrosis.

Microscopically a necrotic tissue has the nucleus of its cells pyknotic or karyorrhetic.The cell nucleus may also show karyolysis or there may be no nucleaus at all. The cytoplasm is acidophilic because its reaction is more basic than during life and hence it takes the acid stain, which is usually red (eosin) or there may be cytoplasmolysis. With more advanced changes, there is loss of cell outline and differential staining.

Necrosis could be coagulative, caseous or liquefactive. There could also be the necrosis of fat and zenkers necrosis.

1.6.2. PLANTS PRODUCING DAMAGE TO THE GASTROINTESTINAL TRACT

1.6.2.1. Plants producing stomatiti

There are many specific diseases in which stomatitis is a product sign. The Merck Veterinary manual 1975) notes that plants of the genus Anemone which include A. nemorosa and A. pulsatilla; and family Amaryllidaceae produce stomatitis with reluctance of the animal to permit manual examination of the mouth cavity. Animals stand with their mouth open roll their tongues or chew with their heads turned sideways. Mycotic stomatitis produced by infections with Monillia species is also common (Blood andHenderson, 1974). A specific type of ulcerative stomatitis in dogs and cats caused by

Candida albicans and characterized by the appearance of ulcers and soft, white to gray slightly elevated patches onthe oral mucosa (Merck Manual 1979.

1.6.2.2. Plants producing gastritis

The feeding of damaged feeds,including mouldy and fermented hay and ensilage, commonly causes a moderate gastritis. Fungi can produce diffuse or ulcerative gastritis in new born animals, especially pigs.Mucormycosis and moniliasis are the two commonly recorded causes. In all species Mucor species and Aspergillus species frequently complicate gastric ulcers caused by other agents (Martins et al., 1957). Pharyngeal paralysis in the horse due to Aspergillus infection has been reported just as the infection of the gultural pouches.

Fish feeding on blue-green algae Lyngbya species are not themselves harmed,but become highly toxic to animals eating them. Cats have been poisoned showing among others anorexia and excessive salivation. The condition is known as Ciguatera poisoning (Clarke and Whitwell, 1968). The burrs of bardock (Archlium lappa) are known to give rise to granular stomatitis in dog. Small papules develop on the tongue which enlarge and develop necrotic centres which slough and become ulcerated (Thwienge, 1973).

1.6.2.3. Signs and lesions associated with other parts of the GIT

Disinclination to eat is one of the common response of most animals to plant intoxication. Agave lecheguilla gives

rise to a condition known as swell head chracterized by listlessness, and loss of appetite (Clarke and Clarke, 1975). James and Binnus (1966) reports of loss of appetite and weight in ewes experimentally fed with Allium validum. Watt and Breyer-Brandwijk (1962) mentioned that plants of the genus Aristolochus produced decreased appetite constipation,whereas in addition, plants of the genus Asclepias also produced weakness and diarrhoea.

Lobelia berlandieri has been one of the causes of stock poisoning in Mexico.Fed exerimentally to sheep at a level of 0.5 per cent body weight dialy, it caused inappetence and diarrhoea, followed by ulceration of the mouth, salivation and death. Scarisbrick (1954) gives evidence of the presence of an irritant glycoside on Beta vulgaris sub sp tops which induces indigestion and purgation in sheep. This he attributed to formation of large quantities of lactic acid in the rumen. Symptoms of poisoning produced by Sinapis nigra are those of acute gastroenteritis, colic, frothing around the mouth and nose, grunting and diarrhoea. Among the clinical symptoms that accompany Pteridium aquilinum poisoning is the enteric type which is seen most frequently in adult cattle, with the animal exhibiting depression,anorexia,enteritis with frequent blood clots in the faeces.

Clarke and Clarke (1975) reports of the presence of lycorine in members of the genera Amaryllis, Crinum, Haemanthus and Nerine, all of which cause poisoning in sheep and goats producing salivation, vomiting and diarrhoea in small doses. The usual symptoms associated with Nerium oleander poisoning are vomiting, convulsion, diarrhoea and colic. Lesions were those of severe catarrhal or haemorrhagic gastroenteritis.

Ricin is the toxic agent obtained from the plant Ricinus communis which is very toxic when administered by whatever route. All animals are susceptible to its effects. All animals show profuse watery diarrhoea but even in the most severely affected, there was no blood in the faeces (McCunn et al., 1945). Anderson (1948) has given account of poisoning in cattle with the same plant in which the main symptoms was bloody diarrhoea. Anderson mentions that with cattle, diarrhoea and bloody purgation have been held to be characterisitic. Geary (1950) noted vomiting and diarrhoea (not bloody) in pigs. The animals were weak and showed some incoordination and signs of abdominal pain. The skin of the ears, flanks and hams was cyanotic. In pullets, Geary (1950) noted dullness, dropping wings, ruffled feathers and greyish-coloured wattles and comb.

The symptoms of poisoning associated with Fagus sylvatica and oak (Quercus species) usually commences with acute abdoninal pain of such severity that the animal becomes unmanageable and delirious

(Llewellyn, 1962). Symptoms associated with poisoning by the leaves of Quercus species may not appear for some days after they have been eaten. Initially there is dullness. inappetence, cessation of rumination and constipation. Later there is persistent diarrhoea, with frequent passage of small amounts of dark coloured faeces which may contain blood. There is also severe urination (Towers, 1950).

Sinapis nigra of the family cruciferae causes poisoning, the symptoms of which are acute gastroenteritis, colic, frothing around the mouth and nose, grunting and diarrhoea. Postmortem examination reveals acute inflammation of the stomach, intestine and kidney. Clarke and Clarke (1975) mentions that black mustard seed has occasionally found its way into cattle cakes and has given rise to colic and respiratory distress. Linseed meal of S. nigra contaminated with swede and turnip seed has caused fatal haemorrhagic gastroenteritis in cattle.

With the cycads, there are two forms of poisoning noted in cattle. These are mainly an acute gastrointestinal disturbance generally terminating fatally in a few hours associated with extreme liver damage and congestion of other organs whereas the chronic condition is characterized by a progressive and irreversible paralysis generally of the hind legs.

Pteridium aquilinium poisoning produces enteric symptoms which is seen most frequently in adult cattle. These inlude

depression, anorexia, enteritis with frequent blood clots in the faeces. Numerous petechial haemorrages can be seen on the visible mucous membranes and there may be bleeding from the nostrils, intestinal and urogenital tracts especially at the later stages of the poisoning (Evans et al., 1954); Evans et al., 1972).

Poisoning by the plant Iris foetidissima of the family Iridaceae leads to acute diarrhoea often haemorrhagic with death resulting from exhaustion. Colchicum autumnale contains colchicine which exert mainly, purgative effect.It has colchicine which exert mainly, purgative effect. It has also the peculiar property of preventing mitosis from proceeding beyond the metaphase (by inhibiting spindle formation) and has been used experimentally for inducing polyploidy. In cattle the symptoms arising from C. autumnale poisoning include abdominal pain, violent purgation with the passage of foetid green or black faeces with general collapse. Death occurs from respiratory failure which may be delayed for several days according to the amount of the plant ingested. The only notable finding at autopsy is that of gastroenteritis (Goonerate, 1966).

Symptoms associated with poisoning by veratine and related alkaloids contained in hellebores such as Veratrum album are salivation, purgation,vomiting, diuresis; excitability followed by prostration, weak and irregular pulse,deep and slow respiration and death in convulsions or consequent upon paralysis.

Danke et al. (1965) noted that ruminants are generally not affected by Gossypium species (cotton plant) although young calves before the rumen is fully functional are susceptible while the compound is highly toxic if injected. Monogastric animals however, are readily poisoned, the gossypol having a cummulative effect. The horse is said to be relatively resistant. However, in pigs, even quite low levels incorporated in the ration of fattening pigs will cause inappetance, loss of weight and lowered food utilization, while larger quantities will give rise to weakness dyspnoea, emaciation and death. Poultry show loss of weight and anorexia with lowered hatchability and discoloured yolk (Morgan et al., 1988).

Aconitine is an alkaloid obtained from Aconitium napellus which acts as a gastrointestinal irritant. Symptoms of poisoning in cattle following ingestion of the plant Rhamnus frangula is diarrhoea, colic and moderate fever with death supervening in few hours.

The genus Solanum contains a poisonous fraction,solanine which is known to produce gastrointestinal signs, salivations, stomatitis, vomiting, tympanitis and diarrhoea. Lesions in the digestive tract are acute catarrhal or haemorrhagic gastritis and enteritis sometimes accompanied by ulcers which extend to or through the muscularis propria (Peterlik et al., 1976) Peterlik and Wasserman, 1978).

1.6.3. MORPHOLOGIC LESIONS IN THE LIVER DUE TO TOXIC AGENTS

These are also known as hepatotoxins. Hepatotoxins can be defined as a heterogenous group of naturally occurring and synthetic chemical agents that produce a variety of hepatic lesions. According to Casarret and Doull (1975) hepatotoxin have the following common characteristics. (a) the agents produce a distinctive lesion, (b) the severity of the lesions seems to be dose related, (c) while quantitative differences in potency can be found among individuals generally the same type of lesion can be produced in all individuals, (d) the lesions are reproducible in experimental animals, (e) they usually appear after a predictable, usually brief latent period.

1.6.3.1. Mechanism of liver injury

Generally substances which cause injury to the liver produce their effect through the following mechanisms:(a) they produce abnormal amounts of fat in the parenchymal cells i.e. fatty liver, (b) derailment of normal protein synthesis, (c) cholestatic reaction and (d) lipid perioxidation.

The lipid that accumlates is predominantly triglyceride. The general mechanism that accounts for triglyceride accumulation is described by Lombardi (1966) as when the rate of synthesis of hepatic triglyceride is normal but, the liver cell is unable to secrete the triglyceride into the plasma. Secondly the secretion of the hepatic triglyceride may be normal but the rate of synthesis

is increased. Thirdly there may be a situation where both an increase in rate of synthesis and a block in the secretion of synthesized triglyceride. Lastly a situation arises when the triglyceride synthesis takes place in a compartment of the cell other than the endoplasmic reticulum and thus, this pool is not accessible to the normal secretary pathway (Lombardi.,1966). It has also been shown that carbon tetrachloride,ethionine,phosphorus and puromycin interferee with the synthesis of the protein moiety of the lipoprotein complex.

Morphologic injuries associated with early hepatic injury include loss of cytoplasmic basophilic material, vacuolation in the cytoplasm, abnormal configuration of the endoplasmic reticulum and loss of ribosome particles from the membrane surfaces. These observations as associated with the inhibitory effects of the hepatotoxins on protein synthesis (Magee, 1966). For example ethionine inhibits amino acid incorporation in microsomes due to a deficiency of available ATP in the cell; by the formation of s-adenosylethionine by ethionine replacing methionine. This leads to a trapping of cellular adenine and a dimunition in the rate of ATP synthsis.

Dimethylnitrosamine affects protein synthesis by causing a loss of m-RNA from polyribosomes which may be due to methylation of RNA (Casarrett and Doull, 1975).

Also hepatotoxins may affect protein synthesis by influen-

cing single unit ribosomes and not on the polysomes.This is as observed with the effect of carbon tetrachloride (Farber., 1971).

Certain hepatotoxins are known to produce cholestatic reaction. Alpha-naphythylisothiocyanate (ANIT), Taurolithocholic acid and 2-ethyl-2-phenylbutyramide are known to produce such action. Goldfarb et al. (1962) produced bile stasis and hyperbilirubinaemia and sulforbromophythalein (BSP) retention in rats following ANT administration.

Cholestasis could be due to poor water solubility and precipitation of the substance in the biliary tract as is the case with taurolithocholate. Canalicular bile plugs as is observed after treatment with substances as norethsterone, methylestosterone, mestranol or norethandrolone also cause cholestasis.

Slater (1966) speculated a mechanism of action associated with the necrogenic action of some hepatotoxins such as carbon tetrachloride, based o the activation of the parent compound to a toxic metabolite (lipid peroxidation). He proposed the homolytic cleavage on the carbon-chloride bond in the endoplasmic reticulum and that this resulted in the production of free radicals which interact with neighbouring lipid-rich material causing alterations in structure and function.

1.6.3.2. Morphologic lesions in the liver due to toxic agents

Morphologically, chemical-induced injury can manifest itself in different ways. The acute effects can consist of an accumlation of lipids (fatty liver) and the appearance of degenerative processes leading to death of the cell (necrosis). The necrotic process can affect small groups of isolated parenchymal cell (focal necrosis); groups of cell located in zones (centrilobular, midzonal or periportal necrosis) or virtually all the cells within a hepatic lobule (massive necrosis).

Chemical-induced liver injury resulting from chronic exposure can produce marked alterations of the entire liver structure with degenerative and proliferative changes observed in the different forms of cirrhosis.

1.6.3.3. Classification of Chemical-induced liver injury

On the, basis of morphologic changes produced in the liver, Popper and Schaffner (1959) and Zimmerman 1968) differently proposed their own classifications.

Popper and Schaffner (1959) described five major group of reactions which could take place in the liver as a result of chemical injury. The first was called zonal hepatocellular alterations without inflammatory reaction example as produced by carbontetrachloride.The lesion is reproducible and dose-dependent.The second group is called intrahepatic cholestasis. The important histologic feature associated with this response are the

presence of bile stasis, the presence of bile plugs in the cannaliculi, dilatation of the canaliculi with subsequent loss of the microvilli and the occurence of focal necrosis. The lesion produced were not dose dependent nor reproducible e.g.lesions produced by anablic steroids and oral hypoglycaemics. The third category was called hepatic necrosis with inflammation. The prominent feature is progression to a massive necrosis characteristic of viral hepatitis. The forth group was called the unclassified group and consisted of hepatic injuries which does not fit those above. The fifth category consisted of those causing hepatic cancer such as aflatoxin.

On his part Zimmerman (1968) classified hepatotoxins according to the supposed mechanism of action, morphologic changes observed or according to circumstances of exposure.

On the basis of mechanism, all agents were divided into intrinsic hepatotoxins and those depending upon host idiosyncracy .The intrinsic hepatotoxins were further divided into direct agents which injure many tissues including the liver and indirect agents which affect a particular metablic pathway.

With agents which produced morphologic changes, Zimmerman (1969) divided them tinto cytotoxic, cholestatic and mixed agents. The final consideration concerned the circumstances of the exposure which were classified into "toxicologic" which consists primarily of chemical overdosage and the second iatrogenic which are made of

lesions produced during the course of therapeutic utilization of drugs.

1.6.3.4. Hepatitis

There is the view that hydropic degeneration, fatty change and necrosis are incipient stages of a process which soon becomes inflammatory. Hepatitis can be infectious or non-infectious (toxic hepatitis) (Smith,Jones and Hunt, 1974). The non-infectious or toxic hepatitis can be separated into acute toxic hepatitis, also known as acute yellow (or red) atrophy and chronic toxic hepatitis, for which the usual name is cirrhosis.

The acute toxic hepatitis is characterized by death of hepatic cells and changes which precede death mainly hydropic degeneration, fatty change and necrosis. Microscopially, the necrosis is coagulative in type followed by desintegration and disappearance of cells.

Regarding location, necrosis in the liver could assume any of the five forms.Diffuse necrosis occurs when necrosis spreads without recourse to lobular boundaries and is a more severe form. Focal necrosis, in which minute necrotic areas, or foci of sublobular size appear here and there which could be at any part of a lobule. A necrosis could be peripheral in which case only the peripheral zones of the lobules are regularly necrotic. This type of necrosis happens when strong toxic substances get to the liver lobule through the blood stream without any impairment of

circulation of the blood and oxygenation of cells. This is because circulation gets to the peripheral cells first. Midzonal necrosis occurs when necrotic changes involve the cells half-way between the periphery and the centre of the lobule. Centrilobular necrosis affects cell nearest to the cenral vein which suffer from blood borne toxins and from a stagnation of circulaton with consequent anoxia. This is common with acute hepatitis with primary disorder being responsible for both the production of toxic substances and impairment of circulation. Paracentral necrosis has necrotic areas which adjoins the central vein on one side but does not surround it, (Smith, Jones, and Hunt 1974.

Typically, but not invariable, acute toxic necrosis is charactertized by centrilobular necrosis with disappearance of most of the centrally located cells with blood filling their place. Peripheral to the lobule, the cells will show fatty change an hydropic degeneration, As the lesions progress, moderate infltration of lymphocytes into the periportal connective tissue (islands of Glisson) usually begins.

Grossly, a liver with acute toxic hepatitis is usually lighter in colour, even to the tan of severe fatty degeneration however it is also likely to be redder because of an increased content of blood.Some show accentuation of the lobular markings.

1.6.3.5. Chemical substances and Plant toxins producing Hepatitis

Some of the chemical poisons known to cause acute toxic

hepatitis include copper, arsenic and the arsenical drugs, phosphorus (if the patient survives more than three days) chronic mercury poisoning, chloroform (delayed poisoning developing tow or three days after anaesthesia) tannic acid (used in the treatment of burns), tetrachloroathane, trinitrotoluene and tetrachlorathylene. Gossypol produces hepatic lesions including swollen cells, foci of necrosis around the cenral veins, congestion of the portal triads,pyknosis, karyorrhexis and karyolysis of hepatocytes, perivascular oedema, bile retention and severe fatty change in fedder lambs (Morgan et al., 1988).

Ingestion of plant substances containing pyrrolizidine alkaloids has been shown to lead to profound change in the anatomy of the liver.Microscopically,there is characteriscally a combination of haemorrhages, necrosis and cirrhosis.Bull (1955) who studied a detailed histology of the liver of sheep poisoned with Heliotropium europaeum considers that in this species, the characteristic change is an enlargement of the parenchymal cells throughout the liver a phenomenon which he terms megalocytosis. According to the author, haemorrrhages, cwell necrosis, and fibrosis are commonly associated changes but are not specific. Hill et al. (1958) however observed that the pyrrolizidine alkaloid,causes an intimal overgrowth of connective tissue of the hepatic veins producing partial or complete occlusion; the ultimate result is generalized fibrosis of the liver.

Markson (1960) who followed the sequence of hepatic events in calves following Senecio jacoboea intoxication observed first slight parenchymal steatosis.A week later bile duct proliferation parenchymal cell changes diffuse fibrosis, and centrilobular endophlebitis developed concurrently. Smith et al. (1974) lists Senecio species as one of the stronger hepatotoxic substances.

Lupinosis is a chronic poisoning arising from congestion of the plant of the species genus Lupinus, and this is observed to cause progressive liver damage. The liver is bright yellow and friable at postmortem (Gardiner, 1966).

Manifestation of Lantana toxicity in sheep include enlargement and dilation of bile canaliculi and injury to microvilli in the liver (Pass et al., 1978) and intrahepatic cholestatis with decrease in canalicular membrane ATPase activity (Gopinath,1969).

Plants of the genus Phyllanthus e.g. P. abnormis and P. drumondii cause postmortem lesions which are examples of hepatotoxic and nephrotoxic poisoning.Changes of acute toxic hepatitis in these plants were observed to progress in chronic cases, to cirrhosis.

Toxicosis by Agave lechuguilla in sheep and goats showns marked fatty change of the liver which is principally responsible for its pale or yellow colour grossly.Centrilobular necrosis is of moderate degree and cloudy swelling is not conspicuous. Lymphocytic infiltrations in and around the islands of Glisson are

usually noticeable. There is considerable retention and inspissation of bile, which forms precipitated masses in some of the intrahepatic bile ducts.

With Heliotropium europaeum poisoning in sheep, the outcome is a slowly progressive toxic hepatitis with symptoms of illness appearing perhaps months after access to the plant has ceased. Jaundice was observed as a salient feature with the fatty change progressing to cirrhosis and a shrunken liver.

The principal lesion of A. flavus intoxication occur in the liver and may be classified as toxic hepatitis (Smith et al., 1974).Newberne and Butler (1969) reported that the most constant responses to aflatoxin B is proliferation of small bile ductules at the periphery of hepatic lobules. Changes in hepatocytes (vacuolization, fatty change, loss of parenchyma, pyknosis) leading to necrosis were usually localized in one part of the hepatic lobule.

1.6.4. Pathological lesions associated with the Kidney

Even though the kidney may constitute less than one per cent of the body weight, they normally receive 20 to 25 per cent of the resting cardiac output indeed the renal cortex has as much greater blood supply than many other organs (Casarrett and Doull, 1975). This extremely rich perfusion implies that large-amounts of any circulating drug or poison will quickly reach the kidney. Moreover renal toxicity is also influenced by ability of the organ

to extract substances from the blood and to accumulate them within the renal parenchyma or in the tubular lumen.

Another aspect of renal function also contributing to the frequency to toxic effects in the kidney is the fact that filtered substances may be concentrated in the tubular lumen as a result of salt and water reabsorption. This salt and water reabsorption process will also further increase the tubular fluid over plasma ratio of secreted solutes so that values as high as 500 are readily attained. In addition to high inratubular concentration of certain solutes significant interstitial accumulation may also result from counter current concentrating mechanisms in the renal medulla. This could result in higher levels of diffusible and potentially toxic molecules such as cyanide or flouride in the renal medulla than other tissues. Foreign compounds may thus become especially toxic to the kidney by virtue of their high intraluminal,intracellular or intersti- tial concentrations.

For example poisoning by Agave lechuguilla produces dilation of the kidney tubules.The distension is probably due to obstruction by large albuminous casts in the case of some nephrons but there is also evidence of hypertrophic dilation and also of generation of epithelium. Fatty change occurs in the ascending loops of Henle. Similar observations were made with Phyllanthus.

Equally, normal tubular functions as occurs with maintena-

nce of normal acid-base balance and correction of metabolic acidosis depends on secretion of hydrogen ions in both the proximal and distal portions of the kidney. This permits toxic interaction in the kidney, that cannot readily occur in other organs. A substance whose solubility changes in PH may precipitate in the acidified tubular fluid and block the normal flow of urine. Alternatively a toxic effect may be produced by a chemical species set free from a filtered precursor by the action of hydrogen ion. Such mechanism has been invoked to explain the toxicity of uranyl ions circulating in plasma as a relatively inert but acid, labile uranyl-bicarbonate - uranyl complex (Nomiyama and Foulkes, 1968).

In addtion, the kidney as in the case with any other metabolically active organ, is also sensitive to metabolic poisons.For example cyanide injected into the renal artery of dogs causes saliuresis by inhibiting normal sodium chloride reabsorption (Vander, 1963).

1.6.4.1. Toxic Tubular Nephrosis

In this condition various irritant substances act directly and without any previous hypersensitization to produce fatty change and necrosis of the delicate epithelial cells lining the tubules. There is also hyperaemia very limited infiltration by lymphocytes and proliferation of fibrous tissues.

The proximal convulated tubules with their large epithelial cells, suffer most as with the ingestion of the young leaves of

Quercus havardi (Llewellyn, 1962). The leaves of this plant is said to contain large amounts of tannic acid which is generally believed to be responsible for the toxic properties of Quercus havardi. Dollahite, Pigeon and Camp (1962) found that the lesions produced in the rabbit by Q. havarrdii are very similar to those produced by multiple doses of tannic acid. Lipidosis is observed to be most extensive in the proximal tubules or it may appear chiefly or exclusively in the epithelium of the ascending loops of Henle. The proximal convulated tubules may also show albuminous degeneration and calcification, usually in the form of granules no larger than on or two cells in the tubules (Battifora and Markowitz, 1969).

Most of the substances which cause acute toxic hepatitis also produce toxic nephrosis to some degree. Substances which can also produce tubular nephrosis also include oxalic acid and plants containing oxalates e.g. Amaranthus retroflexus, alphanaph thylthiourea and other chemical substances (Jaenike, 1966). Poisoning by A. retroflexus presents microscropic evidence of toxic tubular nephrosis with interstitial oedema in the renal cortex. There is presence of large amount of oedema surrounding the kidney between the renal capsule and perirenal peritoneum. Sometimes oedema fluid is tinged with blood but the affected kidneys are usually pale and normal in size. The renal capsule is not usually

affected although the oedema may extend into the renal parenchyma (Buck et al., 1966; Marshall, Buck and Bell, 1967).

Haemorrhages, usually petechial in size, located just beneath the capsule and visible through it, or in the intertubular connective tissues, are frequent in many types of poisoning including those from crotalarias and oak buds, (Boughton and Hardy, 1963). Extensive haemorrhages into the pelves, or peripherally with separation and distention of the capsule has been observed with poisoning by Melilotus alba.

1.6.5. Morphologic lesions associated with Central Nervous system toxicosis

The site of action most frequently involved in systemic toxicity is the central nervous system. Even with many compounds having a prominent effect elsewhere, the CNS particularly the brain, can be demonstrated to be affected using appropriate and sensitive methods. However, in general, the CNS is protected from toxicants by the blood brain barrier. The barrier is a functional concept based on observations that some substances that enter and affect many of the soft tissues of the body, such as the liver, kidney and muscle are excluded from the brain. Non Polar, lipid-soluble compounds usually penetrate the blood-brain barrier, while highly polar compounds tend to be excluded.

Even those toxic substances that can penetrate the brain tissue do not affect equally all of the cell types in the brain.

Different brain areas usually have different sensitivities to toxicants reflecting the unique biochemistry of the cells, the kind and amount of innervation of neurons as well as difference in degree of vascularization of vbrain areas. Neurotoxicants can be divided into those that cause damage subsequent to anoxia and those that cause damage because of affinity for specific structures.

1.6.5.1. Symptoms and lesions in the CNS due to plant poisons

Under certain conditions, the millet Paspalum scrobiculatum, widely grown in Africa has been known to cause poisoning in cattle with symptoms including tremors, clonic convulsions, coma, and death. The toxic agent is said to be a polycyclic hydrocarb-on which affects the CNS (Gupta and Bhide, 1967).

Since theobromine from Theobroma cacao is completely absorbed from the alimentary tract, and only slowly excreted, small doses are known to have a cummulative effect. Death from poisoning by T. cacao may thus be delayed until a critical level is attained.Black and Barron (1943) describe nervous excitabil- ity as a feature of theobromine poisoning in poultry. In calves given waste chocolate in the diet, Curtis and Griffiths (1972) noted excitement, sweating increased respiratory rate and rapid pulse, followed by convulsion and collapse.

In the chronic syndrome produced by Phalaris tuberosa called Phalaris staggers, there are degenerative changes in the mitochrondria of nerve cells and sometimes accompanied by

demyelination of the spinal cords (Gallagher et al., 1967).The acute syndrome associated with this perenial grass are neurological symptoms not unlike those seen in rye grass staggers while the chronic syndrome is characterized by head nodding, ataxia and weakness.

In horses, the symptoms first noticed associated with Pteridium aquilinum poisoning, is almost always some slight incoordination of movement inan animal in poor to fair condition. As the disease progresses, staggering becomes more pronounced, the feet and legs moving and being held in an unnatural manner. The animal stands with its feet well spread and with the back arched. Incoordination increases and severe muscular tremor appears. If no treatment is attempted, the animal goes down and may injure itself in an attempt to rise. Death is preceded by atonic spasms and the typical opisthotonus of a thiamine deficiency (Evans et al.,1954).

A paresis associated with spinal cord demyelination has been reported in pigs newly born of sows receiving very leafy green irrigated white clover (Trifolium rapens) cut and fed dialy to the limit of appetite. Farrowing was normal and the piglets normal as regards size and vigour, except that they were unable to stand and suck; they died in a few days apparently from starvation and dehydration. Mcclymont (1954) considers the diet to be the most likely aetiological factor and suggested among other things that the clover may contain a

neurotoxic factor whose concentration varied seasonally or that the demyelinating lesions could have been due to mild chronic cyanide intoxiccation.

Lathyrus latifolius and L. sylvestris contain the neurotoxic factor L-&, Y-diaminobutyric acid.It is suggested that lathyrogenic agents act by blocking certain carbonyl group normally present in collagen, and thus interfering with formation of cross linkages (Levene, 1962). The action may be retarded by reserpine or by calcium salts.

Spikes of Abrus precatorius coated with the crushedseeds have long been used for the malicious killing of cattle, the animal dying in 2-4 days after exhibiting salivation, stiffness, incoordination, muscular spasms and convulsions with extensive painful swelling round the site of the implant (Rahman and Mia, 1972). However, when the whole seed was given by mouth to cattle at had no effect, although they can kill fowls within a few days.

Rams that had eaten Delphinium hybridium thrown over the fence showed incoordination and violent tetanic spasms followed by recumbency , the spasms recurring at the animal were disturbed (Milne, 1966).

Symptoms of poisonig with Atropa belladonna (deadly nightshade) in pigs were nervous symptoms, dilated pupils,and very often animals were unable to stand with lesions as catarrhal inflammation in the stomach and intestine.A sydrome associated with the grazing

of Sorghum sudense (Sudan grass) characterized by urinary incontinence and incoordination of the hind-legs has been described (Knight 1968). There is ataxia and paresis of the bladder due to a focal axonal degeneration of the lumbar and sacral segments of the spinal cord (Adams et al., 1969).

Nerium oleander has been known for its extreme toxicity. Humans have been fatally poisoned not only by eating a few leaves, but even when oleander twigs were used as skewers in meat.Horses, cattle and sheep get poisoned when the leaves are mixed with hay. Tremors and tetanic stiffness give way to paralysis and death usually without convulsions (Liener, 1974).

The nervous disorder associated with consumption of plants of the genues Astragalus e.g. A pubentissimus and A. lentiginosus is observed to be slow in onset. In the horse, hyperexitability, fright and violent reactions to slight stimuli due to inability to see clearly were the signs observed. In the cow the same imparied vision and disordered judgement cause the animal to perform all movements of drinking while her mouth was still above the water (james, Van Kampen, and Hartley, 1970). The sensory and motor derangements increase uintil the animal is unable to get food for itself. A slowly increasing ataxia of the limb which results in ascending paralysis are the signs which top the nervous disorder which lead to the death of the animal. Microscop-ically Van Kampen and James (1969) observed vacuolation of the cytoplasm of cells of

various tissues which leads to necrosis in the neurons. Damage to neurons is most significant and is found in the central and peripheral nervous system including those in Meissner's and Auerbach' plexuses of the GIT. Later karyolysis, karyorrhexis or cytolysis leads to loss of neurons or mineralization of the necrotic remnants. Perivascular oedema was also observed throughout the central nervous system.

The basic sign associated with poisoning by A. decembens, A convallarius and A. hylophilas was nervous weakness and incordination involving the hind limbs in the cattle, sheep, rabbits and chicken which were experimentally poisoned (Williams, Van Kampen, and Norris,1969).Nervous incoordination results in crossing of the legs and weakness shown by knuckling over of the fetlock joints .

1.6.6. Plants producing teratogenic and developmental anomalies

Developmental anomalies ordinarily originate before birth i.e. in embryonic life when body structures are being formed. However, malformation are also possible in young growing animals. Errors in the developmental mechanisms which could produce malformations include the following: (a) arrest of development in a certain part of the embryo so that some structures dont develop or are small (hypoplasia).

(b) some embryonal or fetal structures fail to disappear when they are expected to.

(c) Some grooves, fissures or openings dont close properly.

(d) aberrant (ectopic or heterotropic) structures.

(e) duplications of structures.

Among the many causes of congenital anomalies are the intrauterine effects of poisons ingested by the mother. A distinctive congenital syndrome known as crooked calf disease has been observed in range cattle which is associated with the teratogenic effects of plants of the genus Lupinus. The cows had consumed the plant between the fortieth and seventieth day of pregnancy. A similar effect was reproduced in cows fed dried lupine plants especially Lupinus sericeus (Shupe, James and Binos, 1967). The affected calves were born with arthrogryposis, scoliosis, torticolis and cleft palate. Most of the calves were viable and could survive to become adults though some abortions were recorded among the pregnant cows.

Feeding to A. pubentissimus to pregnant sheep was reported to cause frequent occurrence of congenital anomalies in the offspring (James Keeler and Binns, 1969). The type of malformation was observed to be associated with the stage of pregnancy. If the plant was consumed by the ewes during the twenty-fifth to the forthyninth days of pregnancy, apiasia of the lower jaw was a dominant anomaly in the lambs. Between the 40th and 60th days of pregnancy often resulted in hypermobility of the hock and stifle joints whereas feeding between the 60th and 90th days of gestation

resulted in flexures of carpal joints in the lambs. Abortions were also recorded in some of the ewes. Shupe et al. (1968) in similar experiments observed loss of weight of the body and testes but no changes occurred in the libido or sperm counts of the rams fed with the plant.

Feeding of the fresh or dried plant Veratrum californicum by the ewe during her thirteenth or fifteenth days of pregancy is reported to cause a striking and grotosque congenital malformations in lambs, (Binns et al., 1964; Keeler and Binns, 1964). The deformed lambs were born alive singly or with a living or dead twin which may or not have been affected. The malformations include partial or complete cyclopia. A single-eye or two fused eyes usually occupy the same orbit. The upper jaw may be slightly distored with a cleft palate or almost totally agbsent. The nose is distorted in varying degrees and the lower jaw usually protrudes drastically. A large media cutaneous protuberance was often observed over the single eye. The cerebral hemispheres were often fused with the hydrocephalus involving the lateral ventricles (Clegg, 1971).

Feeding of Lathyrus odoratus to growing rats is reported to have produced skeletal deformities and changes in other mesodermal tissues (Levene, 1962). Periosteal new bone formation, kyphoscoliosis and dissecting aneurysms of the aorta was reported. Poisoning in sheep has been shown to result in

abortions, intrauterine death, contracted tendons and aplasia of the laower jaw in the offspring (Keeler et al., 1967).

1.6.7. Tumour forming Plant Agents

Tumour or neoplasm according to Smith, Jone and Hunt (1974) is a new growth of cells which (1) proliferate continuously without control (2) bear a considerable resemblance to the healthy cells from which they arose (3) have no orderly structural arrangment (4) serve no useful function (5) have no clearly understood cause.

In P. aquilinum poisoning, lesions in the urinary system involve the bladder, ureters or renal pelvis and appear to represent a chronic but violently hyperplastic and haemorrhagic inflammation which leads to frank neoplasia (Schacham, Philip and Cowden, 1970). The transitional epithelium undergoes localized proliferation with metaplasia to mucinous columnar or stratified squamous types. The hyperplastic epithlium may acquire neoplastic properties developing into a squamous cell or adenocarcinoma which is locally invasive and may matastasize to the regional lymph nodes and lungs. The capillaries of the inflammatory lesion may form haemangiomas in the stroma or project from the mucosal surface (Evans and Manson, 1965).

1.7. Aim and scope Scope of Study

In describing the importance of toxic plants in our environment both to livestock and humans. Hall (1977) and Clarke and Clarke (1975) emphasized that it is obviously impossible in

anything other than a work of encyclopedic proportions to mention all the species of plants which at sometime or the other, have been suspected of causing poisoning in livestock or man.

There can be little doubt that many instances of general malaise, inappetence and dullness in grazing animals which veterinarians are called in to deal with, and the cause of which are seldom diagnosed are due to consumption of subclinical doses of some harmful plants. Plant poisoning is essentially a local problem, occuring in localities where poisonous plants may form large proportion of the herbage available to grazing animals, (Achhireddy and Singh, 1984). Some of these toxic plants occur generally around the country, some occur only in restricted areas in perhaps only one country, some even well known quite deadly species in one region will be used as feed in another without any apparent effect, (Irvine, 1961; Hall, 1977). The reasons for these variations in toxicity can sometimes be accounted for, sometimes it is guessed at, and sometimes it is completely unknown.

Poisonous plants are often refused by animals (many have a repulsive smell or contain highly irritant juices e.g. Euphorbia and Asclepias species) and are eaten only when other herbage is scare. Whereas highly poisonous plants as D. cymosum are reported to be highly palatable (Van Dijk et. al., 1972). It is however, an unfortunate fact that many animals develop a tase for poisonous plants. Clarke and Clarke (1975) reported that there were many

instances on record of animals having to be forcibly restrained from returning to patches of poisonous plants after having recovered from poisoning by them.

Occasionally animals get poisoned when cutting from a garden are thoughtlessly thrown into a dry lot most especially where animals are accustomed to having hay placed before them.

In common with all plant life, the presence or absence of a poisonous plant is very much a matter of environment. Certain ecological conditions favour certain plant species. Variations in these conditions will cause variations in prevalence of plants and in the likelihood of these plants poisoning livestock. For example, plants of the genera Morinda, Astragalus, Naptunia, Oonopsis, Xylorrhiza and Stanleya have the ability to take up inorganic selenium and convert it to organic selenium, thus making it available to other plants and also as a source of selenium toxicosis (Avery et al., 1961; Harris et al., 1972; and Janses et al., 1970). Plants such as Amaranthus sp, Cucumis sativa, Brachiaria sp., Astragalus are nitrate accummulators (William and James, 1975; Andrade et al., 1971). Cynodon plectostachus, Eleusine indica, Vicia sativa, Phaseolus lunatus are some of the plants which accummulate cyanide (Herrington et al., 1977; Sidha, 1967; Aletor, 1983). Plants such Xanthscephalum sp. are reported not to be always poisonous. The differences in degree of toxicity is said to be related to variations in growing conditions (Shaver,

Camp and Dollahite, 1964). Such variations in plant toxicity, therefore calls for caution in trying to use information from other areas to evaluate plant usefulness in other localities.

The plants which have been chosen for the present study are commonly available in Nigeria and they can be found in areas where they are easily accessible to grazing livestock and to playing children. some of them belong to plant families which are reported to be poisonous in other countries.

Lantana camara is known to be notorious for its ability to escape from gardens where it is frequently grown as an ornamental and flourishes in grassland, (Achhireddy and Singh, 1984). It inhibits the growth of other vegetation (Sharma, 1984) and worst of all, it is said to be spreading very rapidly to areas hitherto free of Lantana. The plant is important for consideration because of the severe intoxication including jaundice and photosensitization it produces in livestock (Sharma et al., 1982; Sharma et al., 1987).

S. torvum belongs to the group of plants known as solanines. Most members of this family such as Datura spp. are known to be very poisonous. Animals have been seen to avoid S. torvum but in periods of drought when forage is scarce they serve as easy source of forage (Hall, 1977).

Luecaena leucocephala contains the toxic substance mimosime. The ingestion of large quantities of this legume has been reported

to cause hair loss in horses, dogs rabbits, pigs and may affect normal reproductive behaviour (Mullenax, 1963; Letts, 1963; and Seawright, 1963). Cases of suspected poisoning by L. leucocephala has been made (Akpokodje and Otesile, 1987). However the authors did not have experimental proof of their observation. Therefore it becomes necessary to ascertain such observation.

Tribulus terrestris is reported to cause photosensitization and animals eating the plant show inability to eat and drink, fever, oedema, especially of the limbs, blindness, jaundice, a purulent dermatitis and in advanced stages asphyxiation and death (Amjadi, Ahonrai and Baharse, 1977).

Eugenia uniflora is widely grown for its sourly sweet fruit (Irvine, 1961), while the plant is said to contain over 20 per cent of tannin. Levels of tannins of such in plants have been reported to be source of poisoning in animals (Barry and Forss, 1983). The leaves by personal observation have a very pungent smell. This could be indicative of its level of poisonous constituents.

Dichapetalum madagascasiense belong to a group of plant many members of which have been reported to be very toxic to livestock. Such other members include D. toxicarium (Irvine, 1961; Vickery and Vickery, 1971; Nwude, 1977; Watt and Breyer Brandwijk, 1962); D. cyamosum (Watt et al., 1962; Van Dijk et al., 1972). D. barteri (Nwudu et al., 1977). D. madagascasiense can widely be located in pastures, hedges and other places around yet little is known about

its poisonous potentialities. Therefore its inclusion in this study is significant.

Most of the reported cases of plant poisoning have been based on the clinical symptoms observed after the animals have consumed toxic quantities of the plant. Less effort has been devoted towards the mechanistic evaluation of such toxic substances. Such studies are often useful for the development of tests for prediction of risks, which facilitates the search for safer substances and for rational treatment of the manifestations of toxicity (Gilman et al., 1980).

This study was carried out therefore to evaluate the effects of the suspected poisonous plants, Solanum torvum Swartz, Tribulus terrestris Linn; Leucaena leucocephala Benth; Eugenia uniflora Linn; Lantana camara Linn and Dichapetalum madagascasiense Poir in rats and goats using the following parameters as indices of evaluation:

1. Haematology

This is an essential part of the studies since some of the most dreaded side effects of poisons are haematological e.g. agranulocytosis, thrombocytomenia, complete marrow aplasia or even red cell destruction. A complete blood count consisting of haemoglobin, white cell, red blood cell and differential count, packed cell volume and Winthrobe red cell indices evaluations was determined from each animal.

2. Biochemical estimations

These are important since biochemical changes are the earliest indicators of organic damage and moreover animals grazing on poisonous plants could be source of meat or other by products to man. Since the only way of observing such toxic effects while the animals are still alive autospsies are not anticipated, thus biochemical changes are useful. Such biochemical parameters include serum enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum electrolytes, total protein albumin and globulin fractions and blood urea nitrogen.

3. Gross and histopathological lesions arising from toxicity are the commonly encountered indices for evaluating toxicity.

4. Clinical signs associated with poisoning.

5. Chemical analysis of the toxic plants were attempted. Such analysis included etimation of inorganic substances as nitrate, nitrite, oxalate, cyanide, phytin, and metals as calcium, lead, molybdenum, manganese, iron, zinc and copper.

CHAPTER TWO

2. MATERIALS AND METHOD

2.1 Experimental Animals

The animals used in this study were :-

(a) Mice:

The mice were white albino mice,all male and weighing between 20-25 grams obtained from the Department of Veterinary Physiology and Pharmacology animal house. These were used for the pilot toxicity studies.

(b) Rats:

The rats used were of the Sprague Dawley strain weighing between 200 and 250 grams. They were all male rats and were maintained at the Department of Veterinary Physiology and Pharmacology and the University College Hospital, University of Ibadan. They were kept in rat cages and fed rat cubes (Ladokun and Sons Livestock Feeds. Nigeria Ltd) and allowed free access to clean fresh water in bottles.

(c) Goats:

The goats were all male West African dwarf goats about 3-5 years old purchased from a flock at Abadina, University of Ibadan. They were dewormed with Levamisole 15% (Phenix Pharm, Belgium) and observed for two weeks housed at the Veterinary Surgery and Reproduction animal house before their use.

2.2 The Poisonous Plants

The plants used in this study included:

i. Dichapetalum madagascasiense Poir - Plate 6

ii. Lantana camara Linn - Plate 5

iii. Eugenia uniflora Linn - Plate 4

iv. Solanum torvum Benth - Plate 1

v. Tribulus terrestris Linn - Plate 2

vi. Leucaena leucocephala Benth - Plate 3

2.2.1 Preparation of the extracts of the Poisonous plants

The leaves of the plants were always harvested fresh from their stands at different periods of the year. The drying operation was carried out under controlled conditions in good air draft. Some of the dried leaves were crushed into a coarse powdery form and weighed. The extraction procedure is as described by Harbone (1973).The powdered leaves were continuously extracted using absolute ethanol in a Soxhlet extractor until all the green pigment was in the extract. The extract obtained was clarified by filtration through celite on a water pump and was then concentrated in vacuo using a rotation evaporator which concentrates bulky solutions down to small volumes without bumping at low temperatures. The ethanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperatures of 40^{0}. This provided the semi solid materials which were administered to the rats

and mice in doses according to the LD50 of the particular plant.

2.2.2. Technique for Pelleting leave

Only the leaves of Dichapetalum madagascasiense were pelleted. The dried leaves were ground along with maize grain into coarse powdery form, in different proportions. Domestic starch was prepared and added to the mixture of maize and leaves or leaves alone in a proportions to hold them together i.e. binder. These were then pelleted using specially designed appliance and the pellet dried in he oven at a temperature of 40^{0}%.

2.3. Technique for administration of extracts and leave

(a) Mice and rats:

The mice used in this study were separated into 9 groups for each plant with a group consisting of 10 mice. A group was then administered with an assigned dose of the extract. The rats were divided into 4 groups with each cage containing the rats representing the 3 doses of the extract for each plant along with a control group.

The different groups of rats were administered with the different doses of each plant dissolved in propylene glycol daily by gavage (Po). The control group were given only 0.5ml of propylene glycol by the same route. These were repeated daily for 14 days for each plant extract. The groups of rats which were fed on the leaves of D. madagascasiense, had the leaves in pellets. The pellets consisted of the crushed leaves binder (starch) and

ground maize. The crushed leaves were mixed in proportion of only leaves, 75% and 50% with the maize. The control group were fed on rat cubes for 14 days.

(b) Experimental goats

The goats (four per plant) were fed with freshly harvested leaves of D. madagascasiense and L. camara daily in pens. The leaves were supplemented with freshly harvested grass and concentrates after consumption of the poisonous leaves. They were allowed fresh water ad-libitum. Aqueous extracts of L. camara (200-300 grams of the leaves were made latter and administered orally to the goats which were supposed to feed on L. camara. This was after it was observed that the goats did not actively feed on the leaves. This was by homogenizing the fresh leaves with small quantity of water. The homogenate was administered using a 20ml syringe in bits until the whole quantity was given to each goat.

2.4. Technique for obtaining blood and Serum samples

Blood was collected by Cardiac puncture from diethylether anaesthetized rats or the jugular vein in the goats, into clean bottles containing disodium ethylendiamine tetraacetic acid (NaEDTA) 1-2mg/ml of blood into bottles and allowed to clot. The sera was separated from the clot and centrifuged according to groups into clean bottles for the analysis.

2.5. Determination of inorganic constituents of Plants

2.5.1 Determination of Phytin content

Phytin content was determined by a method described by Wheeler and Ferrel(1971). The principle behind this procedure is that the phytate in the plant is extracted with trichloroacetic acid and precipitated as ferric salt.The iron content of the precipitate is determined colorimetrically and the phytate phosphate content calculated from the value.

Reagents

Barium phytate (Ba_4 Phy) standard was prepared by dissolving commercial phytin in 3% trichloroacetic acid (TCA) and filtered. Ferric phytate (Fe_4 Phy) was precipitated from the filtrate by adding concentrated $FeCl_3$ solution. The precipitate was washed with 3% TCA, slurred in water and converted to sodium phytate and Fe $(OH)_3$ by addition of NaOH. The $Fe(OH)_3$ was filtered out and the Naphytate solution adjusted to about PH 6 with HCl and Ba Phy precipitated by addition of BaCl. The Ba_4 Phy was redissolved in HCl and the double precipitate cycle repeated twice.

Procedure

About 30mg of the finely ground leaf material was placed in a 125ml flask and extracted with some of 3% trichloroacetic acid or 30 minutes with mechanical shaking. A suspension of this was

centrifuged and 10ml of the supernatant transferred to a 40ml conical centrifuge tube. A 4ml $FeCl_3$ solution (made to contain 2mg $FeCl_3$ per ml in 3% TCA) was added to the aliquot. The tube and its content were again heated in water bath for 45minutes. The solution was centrifuged for 10 to 15 min. and the clear supernatant decanted off. The precipitate was dispersed in 20 to 25ml 3% TCA and heated in boiling water bath for 10 mins. and centrifuged. The precipitate was then dispersed in few ml of water and 3ml of 1.5N NaOH added by mixing. The volume was increased with water to 30ml and heated again in water bath for 30 minutes. The solution was filtered hot. The precipitate was washed with about 60-70ml hot water and the precipitate from the paper dissolved with 40ml hot 3.2N NH_4No_2 into a 100ml volumetric flask. The filter paper was washed with several portions of hot water into the flask and the content cooled to room temperature diluting to volume with water. A 5ml aliquot of this was then diluted to approximately 70ml with water. A 20ml of 1.5M potassium thiosulphate was added and diluted to 100ml volume and the absorption of this read over a spectrophotometer at 480nm. There was a reagent blank prepared for each sample. The iron content was calculated from the ferric nitrate standard or alternatively from a standard curve prepared. The phytate phosphorus was calculated from the iron results assuming a 4.6 iron: phosphorus molecular ratio.

2.5.2. Determination of oxalate content of leaves

The oxalate content of the leaves was determined using the procedure of Baker (1952). This involves the extraction of the oxalate in the plant with HCl and its precipitation as calcium oxalate from the deproteinised extract and subsequent extraction with KMno4.

Reagent:

Dilute HCl (1+1)

NH_4 OH solution

Phosphoric-Tungstate reagent - dissolve 24g of sodium tungstate in water, add 40ml of syrupy phosphoric acid and dilute to 1 litre.

Calcium chloride buffer - dissolve 25gm of anhydrous $caCl_2$ in 500ml of 50% V/V glacial acetic acid and add this to a solution of 330g of sodium acetate in water and dilute to 500ml.

Wash Solution - A5% V/V solution of acetic acid kept over calcium oxalate at room temperature. This solution was shaken periodically and filtered before use.

Sulphuric acid - a 10% V/V solution

Potassium permanganate - an 0.02N solution prepared as required by diluting a 0.1N solution

Procedure:

For the determination of total oxalate, 60gm of chopped

green leaves was homogenized with 100ml of water and this transferred to a 600ml beaker. 2 volumes of diluted HCl was added to each 10 volumes of the mixture with 2 drops of capryl alcohol added and boiled for 15 minutes. After cooling, the mixture was transferred into a 500ml volumetric flask and diluted to mark with shaking and this left overnight.

The solution was then filtered through fitter paper after shaking. A 25ml of the filtrate was transferred into a tube with a stopper and 5ml of the phosphoric tungstate reagent added.By inversion,the mixture was mixed and set aside for 5 hours.This was then centrifuged for 10minutes at 3000rpm with the transfer of 20ml of clear solution to a 50ml centrifuge tube. Ammonium hydroxide was added drop wise from a burette until the solution was alkaline indicated by formation of slight precipitate of phosphotungstate. 5ml $CaCl_2$ reagent was added with stirring using glass rod and the tube left overnight in the refrigerator at 5^0C. This solution was centrifuged for 10 minutes. The supernatant was carefully removed and the precipitate washed with 20ml of wash solution stirring vigorously with a fine rod until the precipitate was broken up. This solution was centrifuged for 10minutes and the washings carefully removed. The precipitate was dissolved in 5ml of 10% H_2So_4 and this placed in water bath at 100^0C for 2 mins. The oxalic acid in this was titrated with 0.02N potassium permanganate. The oxalate content of the leaves was

estimated by assuming that 1ml of 0.02N KMno4 = 0.00090g of oxalate.

2.5.3. Determination of hydrocyanic acid in the leaves

The principle behind this method is that the bound hydrocyanic acid in form of the glucoside is liberated and along with the free HCN, converted to the red ferric thiocyanate which is measured colorimetrically.

Reagents:

The ferric reagent-this consists of 10% solution of ferric ammonium sulphate made up by dissolving 50gm of ferric ammonium sulphate in 250ml of distilled water. To this was added 250ml of concentrated nitric acid and the solution boiled to expel any oxide of nitrogen and the solution filtered.

Preparation of Sodium Polysulphide

A 20% solution of sodium hydroxide was saturated with sulphurretted hydrogen and an excess of finely powdered sulphur added.The mixture was shaken regularly for 10mins. and filtered. The filtrate was then diluted with distilled water until the color was like that of a 3.5% aqueous solution of potassium dichromate.

Preparation of solution of HON from leaves.

10gm specimen of leaves were stored in 200 ml of water containing 10ml of 5% solution of mecuric chloride in closed

containers for 5 days.The mecuric chloride reacts with liberated HCN from the glucoside formed during storage. Stannous chloride is added to this solution to liberated HCN before analysis.

Procedure:

This was carried out as described by Van der Walt (1944).

(i) Conversion of HCN to thiocyanic acid

Conversion of HCN to thiocyanic acid was carried out in stoppered vessel using 1.0ml of the sodium polysulphide solution per 50ml of the solution containing the hydrogen cyanide . The solution was then placed in water bath for 20 mins. for complete conversion of the HCN into thiocyanate.

(ii) Removal of the sulphur.

After conversion of the HCN into thiocyanic acid a slight excess of dilute HCN was added and the solution aerated for one hour to remove the sulphuretted hydrogen and render the precipitated sulphur filterable.This is important since if any sulphur passes the filter it may dissolve on subsequent evaporation of the filtrate in the presence of sodium hydroxide to form thiosulphate.

(iii) Concentration of filtrate

The was done by evaporation of filtrate after addition of slight excess of sodium hydroxide on a boiling water bath. The solution was then rendered acid with a slight excess of dilute

HNo_2. The acidified solution was then brought to 29ml with distilled water before 1ml of the ferric reagent was added.

(iv) Color intensity of ferric thiocyanate

1ml of the ferric reagent was used per 30ml of solution. Color intensity of the resulting ferric thiocyanate was measured using a spectrophotometer using a standard made of distilled water but treated the same way as above after the addition of the same quantity of NaOH at 530nm.

Varying quantities of thiocyanate, representing varying quantities of HCN were dissolved in 30ml of water containing 1ml of a 20% solution of NaOH and 7ml dilute HCl and used for the calibration curve.

2.5.4 Determination of nitrate content of the leaves

This is by reaction of the nitrate in the sample with phenoldisulfonic acid which gives a yellow color.It was performed as described by Snell Snell (1949).

Reagents:

Alumina cream

This was made of 125grams of alumina per litre of water to which was added slowly while stirring, 55ml of concentrated ammonium hydroxide.

Phenoldisulfonic acid reagent

It was prepared by dissolving 25 gram of colorless phenol in 150ml of concentrated sulfuric acid. To this was added 75ml of

fuming sulfuric acid containing 13% of free sulfur trioxide. This was stirred well and heated in a flask for 2 hours on a boiling water bath.

Procedure:

The dried ground sample 1mg was suspended in 10ml distilled water and heated on water bath for 30 minutes.The solution was then rendered slightly ammoniacal and decolorized with 3ml alumina cream. Half of this solution was then evaporated to dryness in a porcel-ain dish. From a pipette was added rapidly 2ml the phenoldisulfonic acid. The dish was rotated to ensure that the acid got into contact with all the residue to dissolve it. When this was cool 1:2 ammonium hydroxide was added slowly until the solution was slightly alkaline.The solution was then filtered and the absorbance read on a spectrophotometer with water as reference 520nm.

Series of Standards:

A solution into which was weighed 0.1mg of nitrate nitrogen was evaporated to dryness on a water bath.The residue was treated with 2ml of phenoldisulfonic acid reagent as above and dissolved in 15ml of water which was then diluted to 500ml. Each of this solution contains 0.01mg of nitrate nitrogen.For the calibration curve, 0.1, 0.3, 0.7,1.0, 3, 5, 10, 20, 30 and 40ml of the solution were taken and each diluted to 40ml and then with the addition of

1:2 ammonium hydroxide until faintly alkaline. These were each diluted to 50ml. The value of each standard in ppm is 0.02 times the number of ml of the standard solution used.

2.5.5. Determination of the Nitrite contents of the leaves

The methods of determination generally depend on diazotization and coupling reactions.

Reagents:

Alumina cream - prepared as with the determination of nitrates above.

Sulfanilic acid solution.

0.60 gram of the recrystallized sulfanilic acid was dissolved in 70ml of hot water.The solution was cooled with the additi-on of 20ml of concentrated hydrochloric acid and diluted to 100ml.and mixed thoroughly.&-naphthylamine hydrochloride reagent. This was prepared by adding 0.06 gram of the recrystallized material to water with 1ml of concentrated HCl and made up to 100ml.

Procedure:

This was done as described by Snell & Snell (1949). The 20mg dried ground sample was suspended in 250ml distilled water and heated on water bath for 30minutes. 200ml of the filtered solution was decolorized by placing in a 250ml glass-stoppered bottle with the addition of 3ml of alumina cream and mixed thoroughly leaving it for 15 minutes. 1ml of the sulfanilic acid solution was added to 25ml of the sample solution mixed and allowed to stand for

10minutes for diazotization at room temperature in the dark to prevent decomposition. 1ml of the &-naphthylamine hydrochloride reagent was then added to the diazotized solution and buffered to PH 2.0 - 2.5 with about 1ml of a 20 per cent solution of sodium acetate. This was diluted to 50ml and mixed well. After 10 minutes the intensity of the reddish purple color was measured at 520nm with water as reference. The concentration of nitrite was read off from a calibration curve prepared similar to that for the determination of nitrate.

2.5.6. DETERMINATION OF THE METALLIC CONSTITUENTS OF THE PLANT

Metallic contents of the leaves were determined by procedures as adopted at the International Institute for Tropical Agriculture using Model 403 Perkin Elmer Spectrophotometer. These are as described by Berman (1980).

About 0.5gm of the crushed dried leaves were wet ashed with 20ml of nitric acid and 1ml of sulfuric acid.Anti bumping beads were added and allowed to stand for about 5 hours. The various metals were then determined by using aliquots from these digestants.

2.5.6.1. Determination of the Copper content

An aliquot of the digestant as described above was placed in flask and heated over a flame with continuous addition of nitric acid until the digestant was clear.The digestant was then washed

into a 25ml volumetric flask and the volume made up to mark with distilled water.

5ml aliquot of this was taken and the PH adjusted to between 6.5 and 7 with 2.5N sodium hydroxide,1ml of a 2 per cent solution of sodium diethyldithiocarbamate (NDDC) 1ml of 1% Ethylene diaminetetraacetic acid (EDTA) and 3ml of Methylisobutyl ketone (MIBK) were then added to the solution. This was well shaken inorder to extract the chelate. This was allowed to equilibrate and the solvent layer removed and centrifuged. The samples above and the standards were hen compared at the 324.7nm resonance line for copper.

2.5.6.2. Determination of Iron content

An aliquot of the digestant as prepared above was transferred into a 50ml volumetric flask and the volume made up with distilled water.This was allowed to settle and 10ul of the supernatant of this was compared at the 248.3nm line in graphite furnace programmed to dry at 100 C for 40s, char at 800 c for 40s and atomize at 2400 C for 6s.

2.5.6.3. Determination of the zinc content

A 10ml aliquot of the digestant as prepared above,was transferred into a 25ml volumetric flask and this made up to volume with distilled water. The standard and the prepared samples were then compared as the 213.8nm line in an oxidizing flame using flow rates of 51 per min for acetylene and 71 min for air.

2.5.6.4. Determination of manganese content

A 10ml aliquot of the digestant prepared above was transferred into a 25ml volumetric flask and the volume made up with distilled water.

20ml of this dilution was transferred into a separatory funnel and the PH adjusted to 6-7.To this was added 1ml of 2% nDDC nd 3ml of MIBK and thoroughly mixed and set aside for 10 minutes. This was shaken to extract the chelate. Aliquots of the standard and the unknown in the solution were compared i the graphite furnace.

2.5.6.5. Determination of the lead content

A digestant of the leaves were prepared by the addition of 2ml of perchloric and 30ml of nitric acid to 1gram of the crushed dried leaves. An aliquot of the digestant was transferred into a 25ml volumetric flask and the volume made up to mark with distilled water.

5ml aliquot of this was transferred to a 60ml separatory funnel and the PH adjusted to 5.5.-6.5 with a 2.5N NaOH. To this was added 1ml of 2% NDDC and 2ml of MIBK mixed and left to stand for 10minutes. After equilibration, the solvent phase was removed and centrifuged. The standard and unknown in solution above were compared at the 282.3nm line.

2.5.6.6. Determination of molybdenum content

A digestant of the crushed leaves were prepared by adding perchloric acid to a quantity of the leaves.The PH of the solution was adjusted to PH 1 by the addition of more perchloric acid. The sample was then chelated and then extracted into methylisobutylketone.The level of molybdenum content of this extract was measured in an air acetylene flame at the 313.3nmk resonance line. A standard containing known concentration of molybdenum was similarly treated as above inorder to determine the concentration in the unknown sample.

2.5.7. Determination of Haematological Parameters

(a) Haemoglobin concentration

This was determined as described by Jain (1986) using the cyanmethaemoglobin method. After hemolysis of the red cells, the haemoglobin is converted to cyammethaemoglobin by the cyanide in the diluting solution (Drabkin; diluent).

Reagent:

Drabkin's diluent

The diluent was prepared by dissolving 1g sodium bicarbonate, 52mg potassium cyanide, and 198mg potassium ferricyanide in distilled water and this was made up to 1000ml.

Procedure:

A 0.02ml of the blood was added to 4ml Drabkin's diluent rising the pipette several times and the solution allowed to

stand for 10 minutes. The absorbance of the resultant solution was read in a spectrophotometer at 540nm with the Drabkin's diluent as reference.

Calculation:

Hb concentration (gm/dl) =

$$\frac{\text{Absorbance of test solution}}{\text{Absorbance of standard solution}} \times \frac{\text{Hb Concentration x dilution factor}}{\text{of standard solu.}}$$

where Hb concentration of std. = 0.0572gm/dl

Dilution factor = 201

(b) Packed Cell Volume (Pcv)

This was done by the conventional method of filling a capillary tube with blood. One end of the tube was sealed and the tube centrifuged in a microhaematocrit centrifuge for 25minutes. The Pcv in per cent was then read directly from a graphic reader. This is as described by Schalm et al (1975).

(c) Blood coagulation time

Blood coagulation time was determined by braking bits of plane non-heparinised capillary tubes containing blood at 10 seconds interval until a stringy clot was formed in between the two broken ends of the tube. Alternatively blood coagulation time was also determined by drawing one end of an office pin through a drop of blood on a clean slide until a stringy dot was drawn at the end of the pin. This is as described by Jain (1986).

(d) Differential Leucocyte Count

Reagent

Giemsa stain - this involved making a dilution of 1 in 10 of the original solution of the stain. This is by diluting 1ml of stain with 9ml dis. water

Procedure:

Identification of the different types of leukocytes was enhanced using color plates of Diggs Dorothy, and Ann Bell, Abbott Laboratories.

The differential leucocyte count was made by counting the different types of WBC mainly eosinophils, basophils, neutrophils, monocytes and lymphocytes viewed from each of thirty fields of oil immersion objective of a microscope on Giemsa stained slides.

(e) Total Erythrocyte Count

Reagents.

Hayem's solution - this consisted of mecuric chloride 0.5gm, sodium chloride, 1.0gm, sodium sulfate. 5gm, dissolved in 200ml of distilled water.

Procedure:

This was determined by the hemocytometer method as discribed by Jain (1986). Briefly a 0.5ml of blood containing anticoagulant (NaEDTA) was drawn into the blood pipette and by steady suction drawing the erythrocyte diluting fluid to the 101 mark. This gives a 1.200 dilution. With gentle shaking, the blood

was mixed and the solution used to fill the improved Neubauer hemocytometer ensuring no overflow. This was allowed to settle.

Using the objective lens of the microscope (x40) erythrocytes in 5 of the 25 smaller squares in the central area were counted.

Calculations.

Cell counted x 10 (0.1mm depth) x 5(1/5 of sqmm) x 200(1:200 dilution) = erythrocytes per cu mm (u/l) or

The sum of the cells in the five small squares multiplied by 10,000 = total erythrocyte per cu mm (u/l).

(f) Total Leucocyte count

Reagent:

Leucocyte diluting fluid.

This was made up of glacial acetic acid, 2ml gentian violet (1% aqueous 1ml; distilled water 100ml).

Procedure:

The technique described under the erythrocyte count was followed except for the diluting pipette which has the mark 11 above the bulbs. The blood drawn to the 0.5 mark was diluted to the 11 mark above the bulbs of the WBC pipette, using the WBC diluting fluid.

2 or 3 drops was discarded from the pipette before filling the counting chamber. After allowing 1 minute for the leukocytes to settle, the cells were counted under the x 16 objective lens of the microscope from the 4 large corner squares.

Calculations:

Cells counted x 20(1:20 dilution) x 10 (0.1mm depth) x 4 number of square millimeters counted = WBC per cm mm.or The sum of the cells counted x 50 = total leukocytes per cu mm.

(g) Erythrocyte indices

The erythrocyte-indices define the size and haemoglobin content of the erythrocytes from values obtained from the RBC count, haemoglobin concentration and hematocrit.

Calculation:

(i) Mean corpuscular volume (MCV)

This express average volume of the individual RBC

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

It is expressed in cubic microns (u3)

(ii) Mean corpuscular haemoglobin concentration (MCHC)

This expresses the concentration of haemoglobin in the average erythrocyte.

$$MCHC = \frac{\text{haemoglobin (gm \%)} \times 100}{PCV}$$

It is expressed in per cent

(iii) Mean corpuscular haemoglobin (MCH)

It is the amount of haemoglobin by weight in the average erythrocyte.

$$MCH = \frac{\text{haemoglobin (gm \%)} \times 10}{\text{RBC count (million/cu mm}}$$

It is expressed in micrograms (u gm)

2.7. Determination of serum biochemical parameters.

2.7.1. Measurement of blood urea nitrogen (BUN)

This was based on the Fearon reaction which is a direct interaction of urea with diacetylmonoxime Diacetyl Monoxine + urea Pink chromogen + hydroxylamine urea concentration is directly proportional to intensity of the color produced.

Reagents

BUN acid reagent: this contain ferric chloride in phosphoric and sulfuric acid.

BUN color reagent - Diacetyl monoxime, 0.18%(W/V) and thiosemicarbazide.

Urea nitrogen standard solution

Urea at a urea N level of 30 mg/dl with benzoic acid as preservative.

Procedure:

The Sigma diagnostic procedure based on the method of Crocker (1967) was adopted.

To each of three test tubes labelled blank, standard and test were added 3.0 ml of BUN acid reagent and 2.0ml BUN color reagent and these mixed by shaking. To the tube labelled standard was added 0.2ml of urea nitrogen standard solution; to tube

labelled test was added 0.2ml of serum sample. The tubes were shaken and simultaneously placed in boiling water bath for 10 minutes and these cooled in water for 5 minutes before reading the absorbance at 540nm with the blank as reference.

The BUN level in mg/dl was read from a prepared calibration curve.

2.7.2 Measurement of total protein

This is based o the biuret reaction copper + Serum Protein alkaline Ph ____> copper-protein complexes____>(purple)

The copper in biuret reagent react with peptide bands of serum proteins to form purple color.

Reagents

Biuret reagent: copper sulfate, 0.15 (W/V), sodium hydroxide 3 (W/V) with tartrate and iodide added

Protein standard solution:albumin(5g/dl) and globulin (3gm/dl).

Procedure:

The method of Gornall (1949) as described by the Sigma diagnostic was used.

To three different test tubes containing the following 0.1ml of water (reagent blank); 0.1ml protein (standard solution); and 0.1ml of serum sample, were added 5ml of Biuret reagent. These were individually mixed thoroughly and allowed to stand for 15 minutes at room temperature.

Reading of the absorbance were made on a spectrophotometer at 540 nm. The biuret reaction is said to be linear therefore a calibration curve was not prepared.

Calculations:

$$\text{Serum Total Protein (gdl)} = \frac{A\ TEST \times 8.}{A\ standard}$$

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

2.7.3. Serum bilirubin

This is the diazo reaction for bilirubin. According to this technique, bilirubin is coupled with diazotized sulfanilic acid (P-diazobezenesulfonic acid) to form azobilirubin.

Reagents

Caffeine reagent - accelerating reagent of Caffeine,25g/l,and sodium benzoate, 38g/l in sodium acetate solution .

Alkaline tartrate - sodium potassium tartrate 350g/l in sodium hydroxide solution.

HCl - 0.05mol/l (0.05N)

Diazo reagent - dry mixture of sulfanilic acid, .75 mol, and sodium nitrite 6.6 mol.

cysteine hydrochloride

Procedure:

The procedure used here is based on the work of Jendrassik and Grof (1938), Nosslin (1960) and Michealsson (1961) and reviewed by Sigma Diagnostics.

To labelled test tubes were added the following :

Component	Serum blank (ml)	Total Tube (ml)	Direct Tube(ml)
Serum	0.2	0.2	0.2
HCl	0.5		1.0
Caffeine reagent	1.0	1.0	-
Diazo Reagent solu.	-	0.5	0.5
This is mixed well			
Alkaline tartrate	1.5	1.5	1.5
This is mixed well.			

The absorbance of the total and direct bilirubin were read at 600nm with the blank as reference.

Serum Total bilirubin (mg/dl) = A x 13.2

Serum Direct bilirubin (mg/dl) = A x 13.2

Serum Indirect bilirubin (mg/dl) = Total - Direct.

2.7.4 Activity of Aspartate aminotransferase (Ast)

Also known as glutamic oxaloacetic transaminase (GOT) it catalyzes the transfer of & - amino group from aspartic acid to & - ketoglutaric acid (AKG)

Aspartic Acid + AKG GOT Oxaloacetic acid + Glutamic acid

Reagents

Prepared substrate - DL - Aspartate, 0.2mol/l and &-Ketoglutaric acid, 1.8 mmol/l in phosphate buffer PH 7.5

Color Reagent - 2,4- Dinitrophenylhydrazine (DNP) approximately 20mg/dl in acid solution.

Sodium hydroxide solution was prepared by dissolving 16 NaOH anhydrous in 1000ml water.

Procedure:

The procedure used is as described by Reitman and Frankel(1957)

1.0ml of the prepared substrate was pipetted into a test tube and placed in a water bath at 37^0. 0.2ml of the serum sample was added and left in the bath. About an hour later, 1.0ml of the color reagent was added and shaken leaving it at room temperature. 20minutes after this, 10ml of 0.4N NaOH was added and mixed by inversion.

5 minutes after this, the absorbance was read on a spectrophotometer at 340nm. The aspartate aminotransferase activity was determined in Sigma - Frankel (SF) units per ml from a calibrati-on curve prepared.

2.7.5. Activity of alanine aminotransferase (ALT)

Also known as glutamic pyruvic transaminase (GPT) and catalyzes the transfer of - amino groups from pyruvic acid to - ketoglutaric acid (AKG).

Alanine + - AKG GPT Pyruvic acid + Glutamic acid.

Reagents:

Alanine - &- KG substrate

DL - Alanine 0.2mol/L and &- Ketoglutaric acid

1.8 mmol/L in phosphate buffer, PH 7.5.

Sigma Color reagent:

2,4- Dinitrophenylhydrazine (DNP) 20mg/dl in acid solution

Sodium hydroxide solution 0.40N.This was prepared by dissolving anhydrous sodium hydroxide in 1000ml of water.

Procedure:

The procedure used in determining ALT (SGPT) is as described by Reitman and Frankel (1957).

Test tubes containing 1.0ml of the Alanine - KG substrate were placed in a water bath at 37 C to warm.To this were added 0.2 ml of the serum samples. About 30 minutes after adding the serum 1.0ml of the color reagent was added, shaking and leaving the tube at room temperature. 20 minutes after adding color reagent 10ml of 0.40 N NaOH solution was added and mixed by inversion.Absorbance of the solutions were then read at 340nm after 5 minutes.

The activity of ALT in the sera was read from a calibration, curve in Sigma - Frankel (SF) units/ml.The conventional units of transaminase (SF) who converted to u by multiplying the value by 0.48 (Kaneko 1980)

2.7.6. Activity of Alkaline Phosphatase (ALP)

Serum ALP activity can be measured using various phosphate esters as substrates. The principle is that ALP hydrolyses P-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate at alkaline PH. The nitrophenol formed shows as absorbance maximum at 405nm.

Reagents:

ALP reagent A contains diethanolamine buffer PH 9.8 (1.214 mol/L) and magnesium ions 0.607 mmol/L

ALP reagent +B consists of p-nitrophenyl phosphate 60.8 mmol/L

Procedure:

This is as described by Sigma Diagnostics.The ALP reagents A and B were maintained at 30 C in a water bath after reconstuition. To 2.7 ml of the ALP reagent A was added 0.05ml of the serum sample and mixed immediately by inversion. This was incubated for 1 minute at 30^0C. 0.25ml of the ALP reagent B was then added, mixing immediately and taking the initial absorbance at 405nm with water as reference.

The incubation was then continued at 30 C and the absorbance taken again exactly after 1, 2, and 3 minutes following the initial absorbance readings.

Calculations:

$$\text{ALP activity (U/L)} = \frac{A/mm \times TV \times 1000}{18.45 \times LP \times SV.}$$

where A per min = change in absorbance per minute at 405 nm

TV = total volume (ML)

SV = Sample volume (mL)

18.45 = millimolar absorptivity of p-nitro-phenol at 405 nm

LP = Light path (1-cm)

1000 = Conversion of units per ML to units per litre.

2.8. Histological Technique:

Gross and histologic evaluation of the tissues of the animals were done to determine toxicity of the plants to the tissues of the animals. The tissues analyzed include the brain, lungs, head, liver, spleen, pancreas, gastrointestinal tract, kidney and the skeletal muscles.

These tissues were observed grossly for any lesions after opening up the animal through a central incision on the abdomen. The tissues were examined for color, loss of strength, necrotic changes, size and hemorrhage.

Reagents:

10% solution of formalin in saline water

70% up to 100% ethanol

Xylene

Paraffin melted to 58 - 60^{0}C

Haematoxylin and eosin (H&E) dye

Procedure:

Relevant samples of the tissues were isolated and placed in containers with 10% buffered formalin solution for theirfixation. Next the tissues were dehydrated by passing through graded concentrations of ethanol (ranging from 70% absolute alcohol).

Clearing of the tissues were performed using xylene. This allows impregnation of the tissues with paraffin. The cleared tissues were then dropped in liquid paraffin at 58% -60 C for a period of 30 minutes to 6 hours. This makes the tissue more resistant to sectioning. Small blocks of paraffin containing the tissues were then sectioned using the blade of a microtome of thickness 3-8 um. These sections were placed in warm water before they were transferred to clean slides and stained with haematoxylin and counter stained with eosin.

Plate 1: Solanum torvum Benth growing along a cattle route from the abattoir in Bodija cattle market, Ibadan.

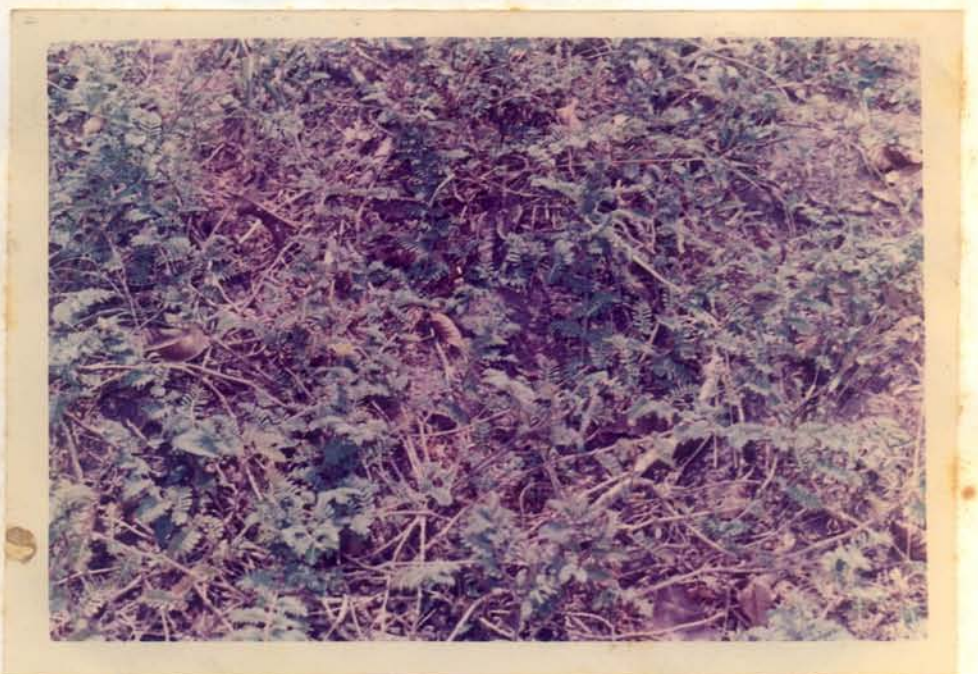


Plate 2: Tribulus terrestris Linn showing a cluster of growing and yellow flower heads.

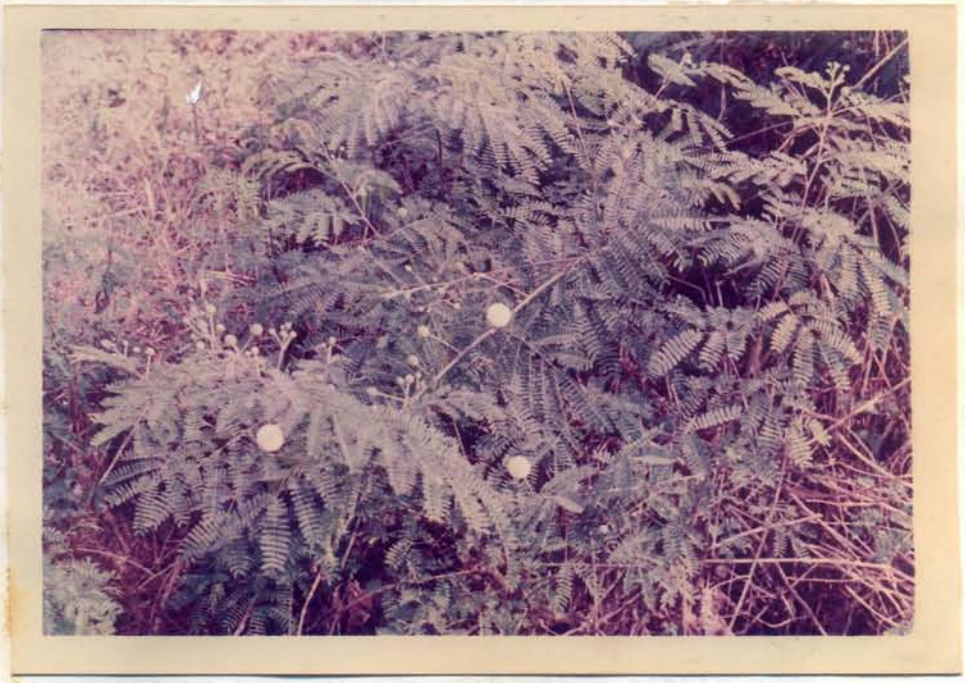


Plate 3: Leucaena leucocephala Benth with its flowers in white globose heads.

Plate 4: Eugenia uniflora Linn stands with other plants growing with it.

Plate 5: Lantana camara Linn growing in the grazing pasture at the Teaching and Research Farm, University of Ibadan.

Plate 6: Dichapetalum madagascasience Poir on a bush path.

CHAPTER THREE

EXPERIMENTS AND RESULTS

SECTION A

3.1. CHEMICAL ANALYSIS OF THE POISONOUS PLANTS

3.1.1. INTRODUCTION

Plants contain various chemical constituents. Such chemical constituents could either be organic or inorganic. Some of these chemicals are natural constituents of the plants whereas others are acquired from the soil types on which these plants grow (Clarke and Clarke 1975, Kingsbury, 1979).

The primary chemical compounds can be defined as those required for a plant's basic metabolism.Secondary compounds loosely are all others (Kingsbury, 1979).Most of these secondary compou- unds are reported to be toxic and are said to be originally for their defence against the pervasive pressures of herbivorous insects. That many of these compounds are also toxic to animals is accidental (Kingsbury 1978, Ewer and Hall, 1978).

According to Der Manderosian, Giler and Roia (1976) and Woodward (1985) the poisonous chemical constituents of plants can be classified into:

1. Glycosides-these contain sugar (pyran) and a non-sugar (aglycone) portion. There are four types of glycosides mainly:

(i) cyanogenic glycosides - many plants contain hydrocyanic acid either free or more usually in the form of cyanogenetic glycoside which is an organic compound containing a sugar and capable of yielding cyanide on hydrolysis.

(ii) saponin glycosides-these contain a sugar in combination with a steroid.

(iii) anthraquinone glycosides-these yield anthraquinone on hydrolysis with the aglycone or glycoside having a purgative effect.

(iv) cardiac glycoside - these include substances such as digitalis oleandrin, trophantin, etc.

2. Alkaloids - these are basically alkali substances which are nitro-genous. They are known to consists of various derivatives some of which are very toxic whereas others have medicinal importance and include pyrone e.g. caffeine; indole e.g. yohimbine; pyridine e.g. nicotine;tropine e.g. hyosine;quinoline e.g. quinine; phenanthrene
e.g. morphine; phenylalkylamine e.g. ephedrine and imidazole e.g. pilocarpine.

3. Proteins - they are phytotoxins such as ricin from Ricinus communis and abrin from Abrus precatorius (Wee, 1987).

4. Esters - such as oil of mustard (allyiosothicyanate) and oil of garlic (allylsulphate).

5. Resins - amorphous products of complex chemical structure.They occur as mixtures with volatile oils (oleoresins) gum (gum resins and sugars).

6. Oxalic acid - the only organic acid of plants toxic to animals under natural conditions. They occur in plants as soluble (Na+ and K+) or insoluble (Ca++) oxalates or acid oxalates (Littledike,James and Cook, 1976).

7. Nitrates and nitrites-nitrates are reported to be relatively non toxic.Their importance as cause of poisoning from plants is due to their conversion either in the foodstuff or within the alimentary tract into nitrites (Andrade, Peregrino and Aguiar 1971; Blood and Henderson, 1968).

8. The metallic constituents are reported to be derived from the environment or soil types on which these plants grow. They include copper, lead, magnesium manganese, zinc, selenium, arsenic, iron, cobalt phosphorus,etc.(Hall,1974).Such metals also form complexes with other substances inorder to be toxic e.g. phytin is an organic phosphate defined by most investigators as the mixed calcium and magnesium salt of inositol hexaphosphoric acid (Harland and Prooky, 1979).

3.2. EXPERIMENT 1

3.2.1. Determination of the inorganic constituents of the plants

Procedure:

The average of two estimations of the inorganic constituents of the leaves of D. madagascasiense, S.torvum, E.unifl- ora, T. terrestris, L. camara, L leucocephala were made using leaves from two different plant stands. The two determinations were made from the leaves of the plants harvested in October 1989 and April 1990.

The leaves were kept under shade at a place with good air draft after weighing them on a triple beam balance. The drying took almost 2-3 weeks depending on the moisture content of the leaves. The dried leaves were then crushed into a coarse form and weighed before subjecting them to analysis or extraction.

The percentage yield of the extract was determined

$$\text{by} = \frac{\text{Dry weight of extract} \times 100}{\text{Wet weight of starting material}}$$

The inorganic chemical constituents were determined by testing for the levels of nitrate, nitrite, oxalate, cyanide and phytin in the leaves using the methods as described on pages 164, 166, 167, 168, 169, 170,. 171, 172.

Results

The levels of inorganic and the metallic substances determined from the leaves of the poisonous plants L. leucocephala; L. camara; S. torvum; E.uniflora, D. madagascasiense, and T. terrestris are as presented in tables 1 and 2.

(a) Phytin content of the leaves of the poisonous plants. T. terrestris had the highest content of phytin of 20.18 percent. The level of phytin in S. torvum was 19.23 percent; level in L. leucocephala was 18.71 percent; level in E. uniflora was 18.66 percent; the level in D. madagascasiense was 17.07 percent whilst L. camara had the lowest content of phytin of 15.99 percent.

(b) Oxalate level in the leaves of the poisonous plants

D. madagascasiense showed the highest concentration of oxalate in its leaves of 0.24%.in decreasing order of oxalate concentration in the plants, the other plants showed the following: S. torvum, 0.18%, L. camara, 0.148%, T. terrestris, 0.14%, L. leucocephala, 0.126%, E. uniflora of 0.102%.

(c) Level of nitrate in the leaves of the poisonous plants.

As seen in table 1, the highest concentration of nitrate was found in the leaves of S.torvum.The level of nitrate in L. camara was 15.34 mg/g, the level in T. terrestris was 15.28 mg/g; the level in D. madagascasiense was 14.99 mg/g; level in E. uniflora was 14.66 mg/g and the level in L. leucocephala was 14.17 mg/g.

(d) Cyanide level in the leaves of the poisonous plants.

The highest concentration of cyanide in the leaves of the plants under study was found in D.madagascasiense of 15.06 ppm. Cyanide concentration in E. uniflora was 15.0 ppm.; the level in L.leucocephala was 14.2 ppm; level in T.terrestris was 14.4 ppm;level in S.torvum was 13.2 ppm;the level in L.camara was 12.7 ppm (Table 1).

(e) Nitrite level in the leaves of the poisonous plants.

T. terrestris had the highest level of nitrite content of 9.71 mg/g. The level of nitrite in L. camara was 9.5 mg/g; the level was 9.38 mg/g in E. uniflora; L.leucocephala had 9.2 mg/g; D.madagascasiense had 8.95 mg/g;S.torvum had 8.91 mg/g (Table 1).

Conclusions

The level of the antinutritional factor phytin analyzed from all the plants except L.camara was high enough to constitute a cause of toxicity by the plants. Similarly the level of nitrate and nitrites determined from all the plants were relatively high when compared with the minimum lethal dose of the pure compounds in animals.This implies consumption of these plants by livestock could produce some level of methaemoglobinaemia.

Furthermore all the plants analyzed contained hydrocyanic acid. The presence of HCN in these plants indicates they contain cyanogenetic glycosides or free HCN.

The level of oxalates determined from the leaves of the poisonous plants were very low.This implies that oxalate cannot be a source of poisoning to animals grazing on these plants.

3.3 EXPERIMENT 2

Procedure

Determination of the metallic constituents of the plants.

The leaves of the poisonous plants L. leucocephala; E. unifl ora, T terrestris, S.torvum, L. camara, D. madagascasiense were harvested weighed and treated as required for the different metals, iron manganese, molybdenum, copper, zinc and lead as outlined in materials and method in pages 173, 174, 175, 176 respectively.

3.3.1 Results

3.3.1.1. Molybdenum levels in the leaves of the poisonous plants

Table 2 shows that D. madagascasiense had the lowest level of molybdenum of 0.12 ppm. T. terrestris had 0.26 ppm; L. camara 0.25 ppm with L. leucocephala and S. torvum exhibiting equal levels of 0.14 ppm; E. uniflora had the lowest level of 0.12 ppm also.

3.3.2 Manganese level in the leaves of the poisonous plants

The level of manganese in the leaves of the plants from the plant with highest concentration to that with the lowest are D. madagascasiense, 1.11 ppm; T. terrestris, 0.060 ppm; L. leucocephala, 0.56 ppm; S. torvum, 0.052 ppm; E. uniflora, 0.51 ppm; L. camara, 0.10 ppm.

3.3.3 Iron level in the leaves of the poisonous plants

The order of the level of iron estimated in the leaves of the poisonous plants from the highest level to the lowest is L. leucocephala, 5.27 ppm; S. torvum, 4.93 ppm; E. uniflora, 4.87 ppm; T. terrestris, 4.78 ppm; D. madagascasiense, 2.54 ppm; L. camara, 1.55 ppm (Table 2).

3.3.4 Copper level in the leaves of the poisonous plants

The highest level of copper was observed in T.terrestris of 2.25 ppm. The level in L. leucocephala was 1.52 ppm; the level in D. madagascasiense was 1.87 ppm; the level was 1.28 ppm in S. torvum; 1.21 ppm in L. camara and 1.02 ppm in E. uniflora (Table 2).

3.3.5 Zinc level in the leaves of the poisonous plants

The highest level of zinc was found in D. madagasca-siense of 4.73 ppm. The level in E.uniflora was 2.39 ppm; the level in S. torvum was 2.35 ppm; the level in L. leucocephala was 1.57 ppm. The level was 0.49 ppm in L. camara and 0.33 ppm in T. terrestris.

3.3.6 Lead level in the poisonous plants

The highest level of lead of 0.21 ppm was observed in E. uniflora. The level in L. camara was 0.14 ppm, it was 0.12 pm in S. torvum; 0.1 ppm in L. leucocephala; 0.09 ppm in T. terrestris and 0.04 ppm in D. madagascasiense.

Table 1

Levels of inorganic anions in the poisonous plants.

PLANTS	LEVELS OF INORGANIC SUBSTANCES				
	Oxalate %	Nitrate mg/g	Nitrite mg/g	Phytin content %	Cyanide ppm
L. leucocephala	0.126	14.1	9.24	18.71	14.2
T. terrestris	0.140	15.28	9.71	20.18	14.4
S. torvum	0.180	16.10	8.91	19.23	13.2
S. madagascas.	0.24	14.99	8.95	17.07	15.06
L. camara	0.148	15.34	9.50	15.99	12.70
E. uniflora	0.102	14.66	9.38	18.66	15.0

Table 2.

Levels of metallic cations in the poisonous plants

PLANTS	LEVELS OF METALLIC IONS IN PARTS PER MILLION					
	Mo	Mn	Fe	Cu	Zn	Pb
L. leucocephala	0.14	0.56	5.27	1.52	1.57	0.10
T. terrestris	0.26	0.60	4.78	2.25	0.33	0.09
S. torvum	0.14	0.52	4.93	1.28	2.35	0.12
D. madagascas.	0.12	1.11	2.54	1.87	4.73	0.04
L. camara	0.25	0.10	1.55	1.21	0.49	0.14
E. uniflora	0.12	0.51	4.87	1.02	2.39	0.28

Each value represents an average of two determinations from leaves of two plants stands.

SECTION B

3.4 TOXICOLOGICAL EFFECTS OF THE POISONOUS PLANTS ON HEMATOLOGICAL PARAMETERS OF RATS AND GOATS

3.4.1 EXPERIMENT 1

Determination of the lethal dose 50(LD50) of the poisonous plants in mice.

INTRODUCTION

Most plants which have come to be known as poisonous, contain substances which depending on the rate of consumption or amount consumed, may not produce symptoms. Thus plants as Eleusine indica and Zea mays could be browsed by animals without the animal suffering any adverse effects (Kinghorn, 1979; Woodward, 1980).

However the same observation cannot be associated with plants such as Aconitum napellus or Colchicum autumnale which are reported to contain very toxic substances so that just a leaf in the meal of an animal could produce adverse reactions (Woodward, 1980).

It is customary in toxicological studies to define the lethal dose of poisonous substances and their effects on important functions of the animal. The basic test for the determination of the relative acute toxicity of a substance is by the observation of the median lethal dose (LD50) (Litchfield and Wilcoxon. 1949).

Procedure

The pilot toxicity studies on the poisonous plants under study were conducted in white albino mice weighing between 20-25 gm. For

each of the poisonous plants was assigned five groups of experimental mice with each group consisting of 10 male mice. Each group of the mice for each plant were then administered with dosages of the extracts of the plants dissolved in propylene glycol. A control group was simultaneously also administered with propylene glycol only. The administrations were orally using a canula. The different doses of the extracts from each plant ranged between 200 mg/kg to 1,200 mg/kg.

Immediately the dose of the extract of each plant was administered to a group they were kept in cages for observation for 120 hours. The number of mortalities observed per group were then recorded.

Results

The percentage of mortalities for each group under each plant was estimated and plotted on a graph of log- dose against % mortalities. The LD50 obtained for each of the plant extracts are as stated.

Table 3.

Acute LD50 values of the poisonous plants in mice.

Plant	LD50 (mg/kg)	
L. camara	1,460 -	figure 1
S. torvum	780	figure 2
E. uniflora	720	figure 3
T. terrestris	1,040	figure 4

L. leucocephala 1,400 figure 5

CONCLUSION

L. camara and L. leucocephala had the highest LD50 (oral) value among the other plants. T. terrestris produced intermediate LD50 values, whereas S. torvum and E. uniflora had the lowest LD50 values in mice. This means S. torvum and E. uniflora were more toxic acutely than the other plants evaluated in this study.

EXPERIMENT 2.

3.5. Effect of the Poisonous plants on the hematological parameters of rats and goats

Introduction

Toxic substances from poisonous plants affect the cellular elements of the blood by causing direct hemolysis of the circulating erythrocytes. Other agents such as from the plant Vicia faba produce hemolysis only in cells congenitally deficient in glucose-6-phosphate dehydrogenase (Casarrett and Doull, 1975). Some plants destroy the hematopoietic powers of the bone marrow or increase the number of circulating white blood cells by their mitotic effects (McPherson, 1979). Certain poisons owe their effects to or at least reveal their presence by numerous petechiae or ecchymoses, the result of injury to the endothelium of blood vessels (Smith et al., 1974).

Poisonous plants could also produce destructive lesions in the liver (Doerr et al., 1976). This activity may result in the failure of blood to clot due to the deficiency of essential blood coagulation factors such as prothrombin which are produced by the liver cells. Fibrinogen, factor VII, the Stuart Factor (factor x) and probably the Christmas factor (factor ix) are all synthesized by the liver. All these factors are important blood coagulatory factors (Jain, 1986). Some of the poisonous plants for example Dieffenbachia spp by the level of oxalate in their leaves cause the reduction in the level of blood calcium (Woodward, 1980). This results in the decreased ability of the blood to clot (Buck et. al., 1966; Marshall et. al., 1967). Fatal and uncontrollable hemorrhages in numerous bovine animals fed several weeks or longer on sweet clover hay have also been reported (Prier and Derse, 1962).

Some of the poisonous plants also cause the production of methemoglobin or cyanhaemoglobin. These complexes formation by haemoglobin of the RBC reduces the oxygen carrying capacity of the blood and thus results in toxicity to the affected animal consuming them (Andrade et. al., 1971; Miyazaki and Kanashima, 1975).

Certain of the plant lectins have been reported to have the capacity to release the developmental potential of specific resting or dormant cells in the circulating system and transform them to an active growth state (Nowell, 1960). Such plant lectins have been

termed mitrogens and the class of cells affected is primarily that of the immune responsive or lymphocyte (McPherson, 1979).

This study is an attempt to evaluate the toxic effects of the plants L. leucocephala, S. torvum, E. uniflora, T. terrestris, L. camara and D. madagascasiense on the hematological parameters of rats and goats. The parameters that were evaluated were the following:

(i) total erythrocyte count
(ii) haemoglobin concentration
(iii) packed cell volume
(iv) blood coagulation time
(v) total and differential leucocyte counts.

Procedure

Four groups each made up of ten rats were used for each plant. Three of the groups were administered with the three different doses of the extract of each group for a period of 14 days daily by the oral route. The fourth group of each set of groups for a plant were also administered with propylene glycol orally.

The doses of the extract for E. uniflora and S. torvum were 100, 150, and 200 mg/kg; for L. camara and L. leucocephala, they were 200, 400 and 600 mg/kg; they were 150, 200, and 400 mg/kg for T. terrestris. In case of D. madagascasiense the leaves were not extracted but pelleted (cf page 162) and fed to the experimental rats.

Freshly harvested leaves of D. madagascasiense were fed to a group of goats whereas the group of goats used for L. camara were administered with aqueous extracts of the leaves.

Blood obtained as explained in page 163 were analyzed for changes in hematological parameters.The determinations of total erythrocyte and leucocyte counts, packed cell volume, haemoglobin concentration, blood coagulation time and differential leucocyte counts are as described in pages respectively.

3.6. Results

3.6.1. Effect of the extract of Eugenia uniflora on the leucogram and erythron of the rat.

The effects can be observed in tables 4 & 5. The extract increased inconsistently the total red blood cell counts as well as the mean corpuscular volume as the doses increased. It produced increase in the PCV which is significant ($P<0.05$) with the 600 mg/kg dose. The extract also produced inconsistent increase in the haemoglobin concentration which shows significance at the 150 mg/kg dose level of the extract.

The extract did not cause significant changes in the blood coagulation time of the rats.

The extract of E. uniflora has not caused any serious changes in the total white cell count except that a significant increase was observed with the 150 mg/kg dose (Table 50). The eosinophil count showed a dose dependent increase.

FIG. 1. LOG-DOSE MORTALITY CURVE OF MICE ADMINISTERED WITH EXTRACT OF LANTANA CAMARA ORALLY EACH POINT HAS AN "n" OF 10. LD_{50} = 1460 mg/kg.

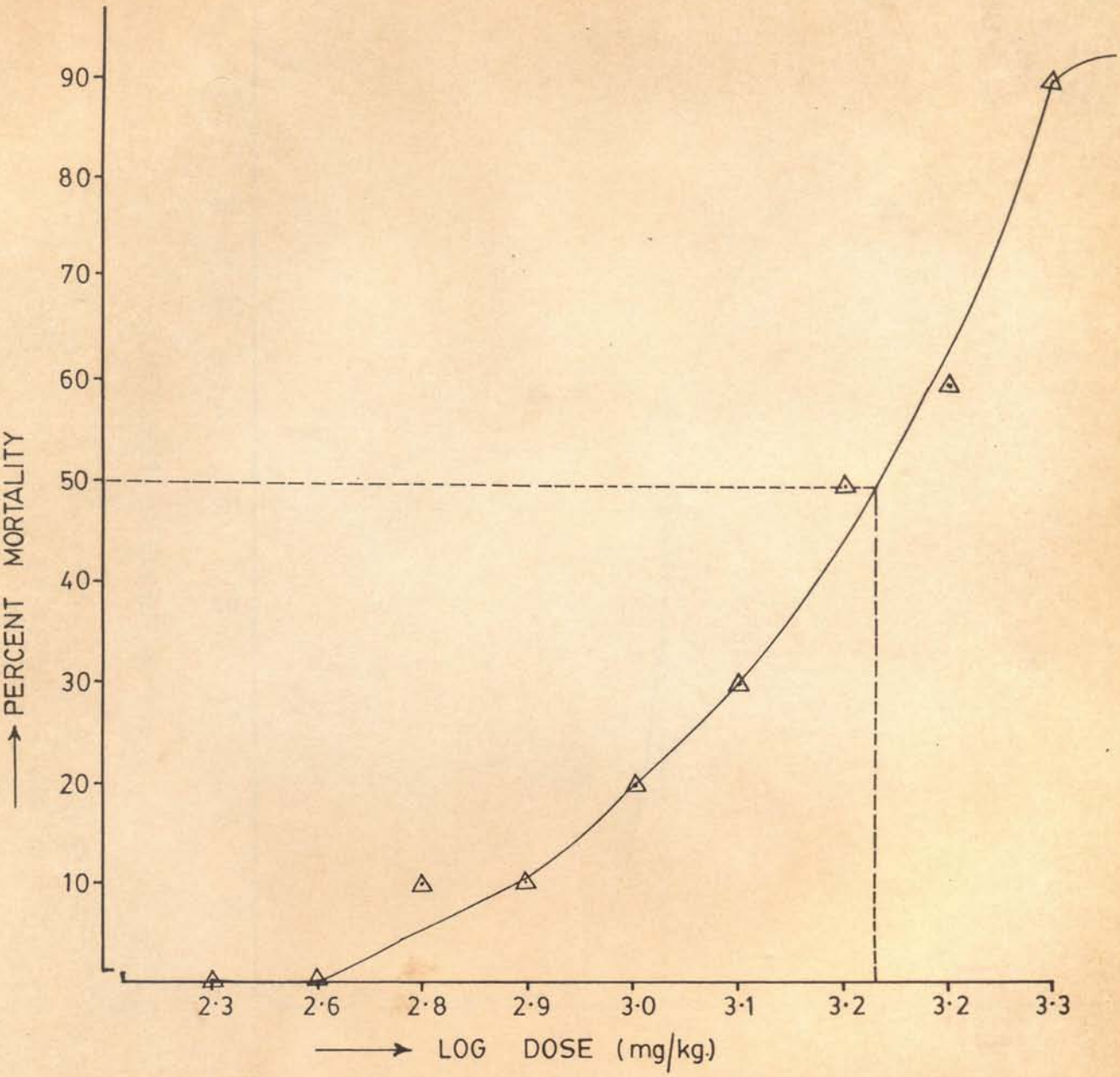


FIG. 2. LOG - DOSE RESPONSE CURVE OF MICE ADMINISTERED WITH EXTRACT OF SOLANUM TORVUM ORALLY EACH POINT HAS AN "n" OF 10. LD_{50} = 780 mg/kg

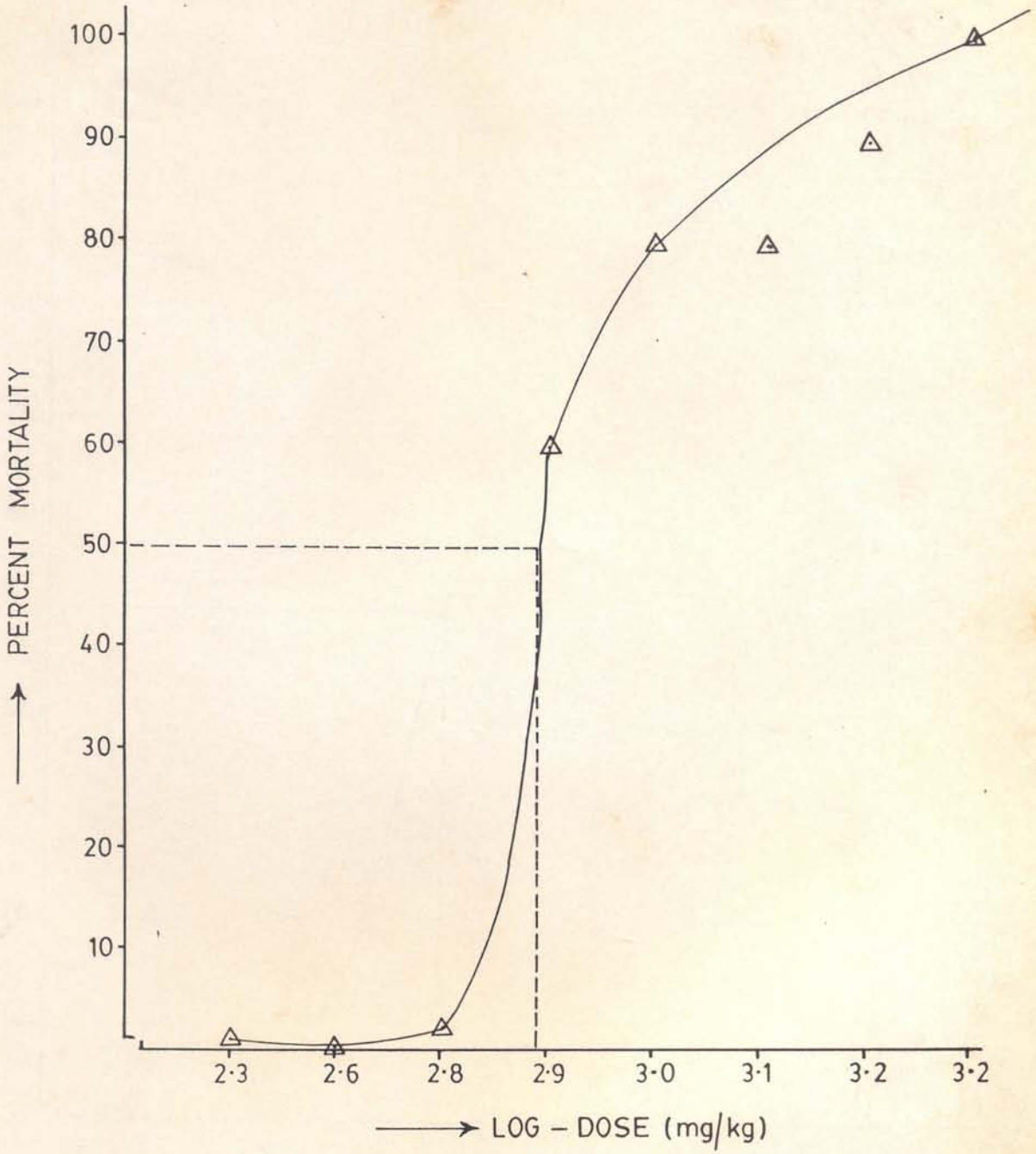


FIG. 3. LOG - DOSE RESPONSE CURVE OF MICE ADMINISTERED WITH EXTRACT OF EUGENIA UNIFLORA ORALLY EACH POINT HAS AN "n" OF 10. LD_{50} = 720 mg/kg

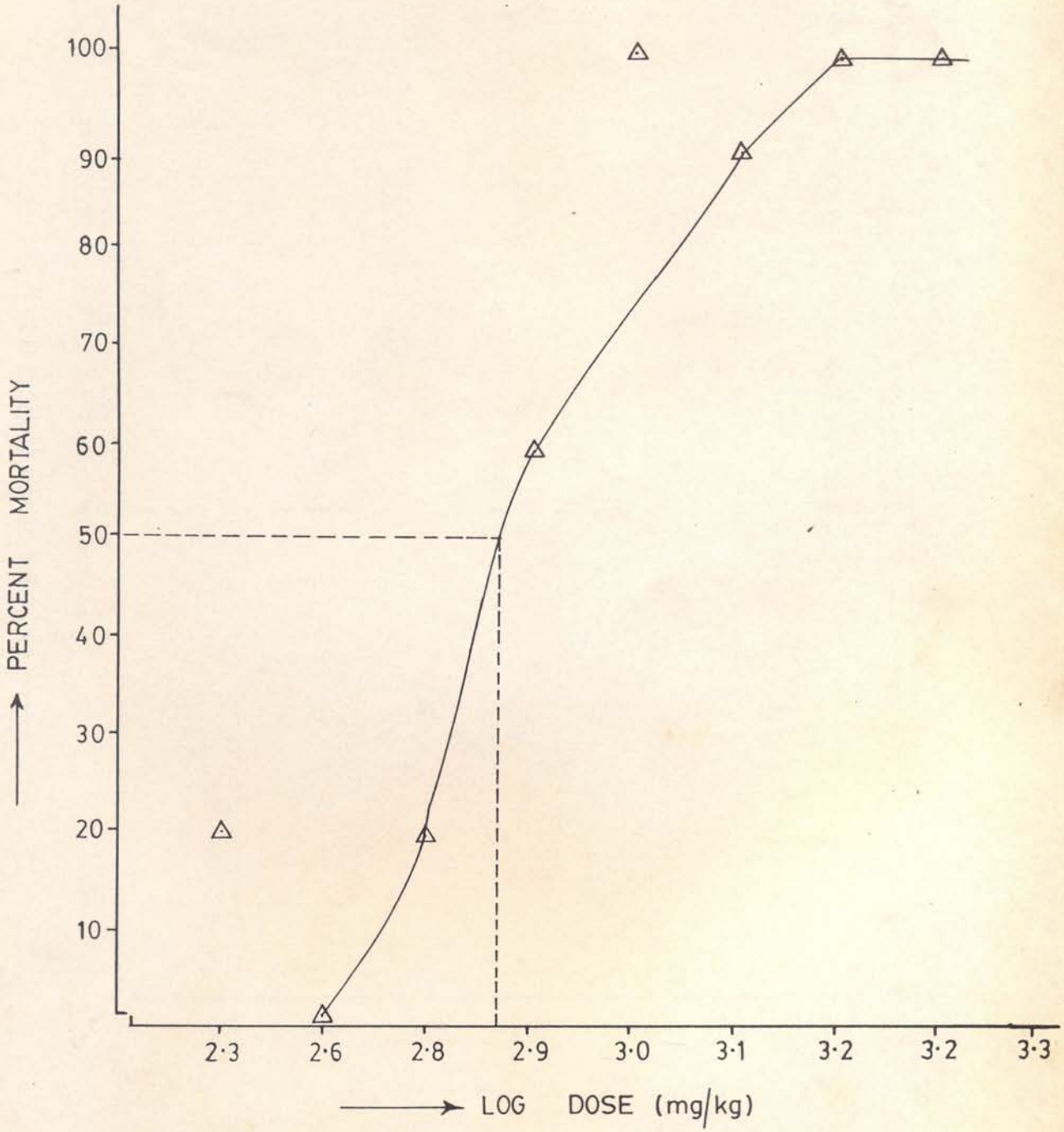


FIG. 4. LOG - DOSE MORTALITY CURVE OF TRIBULUS TERRESTRIS IN MICE. EACH POINT HAS AN "n" OF 10. LD_{50} = 1,040mg/kg

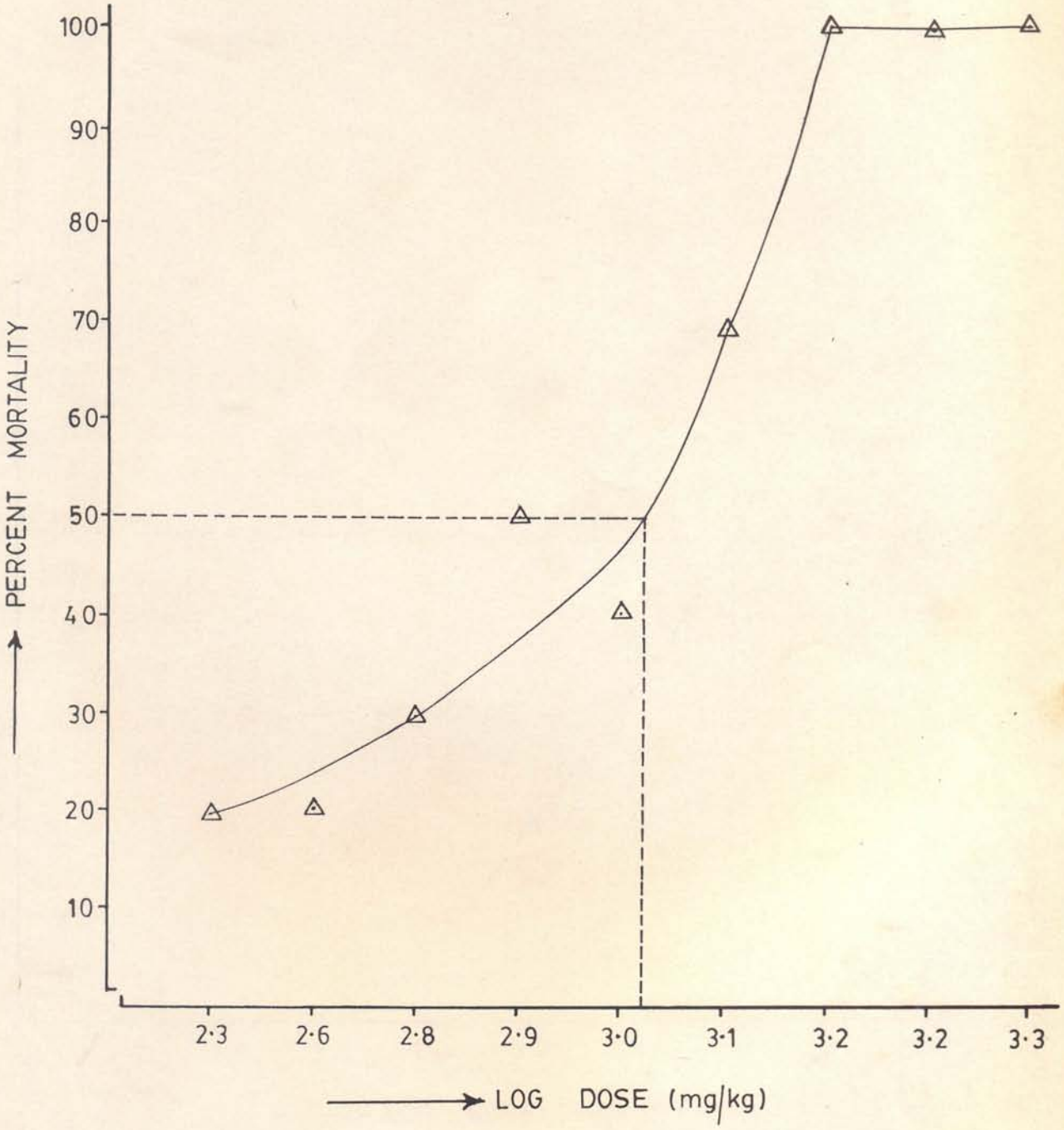
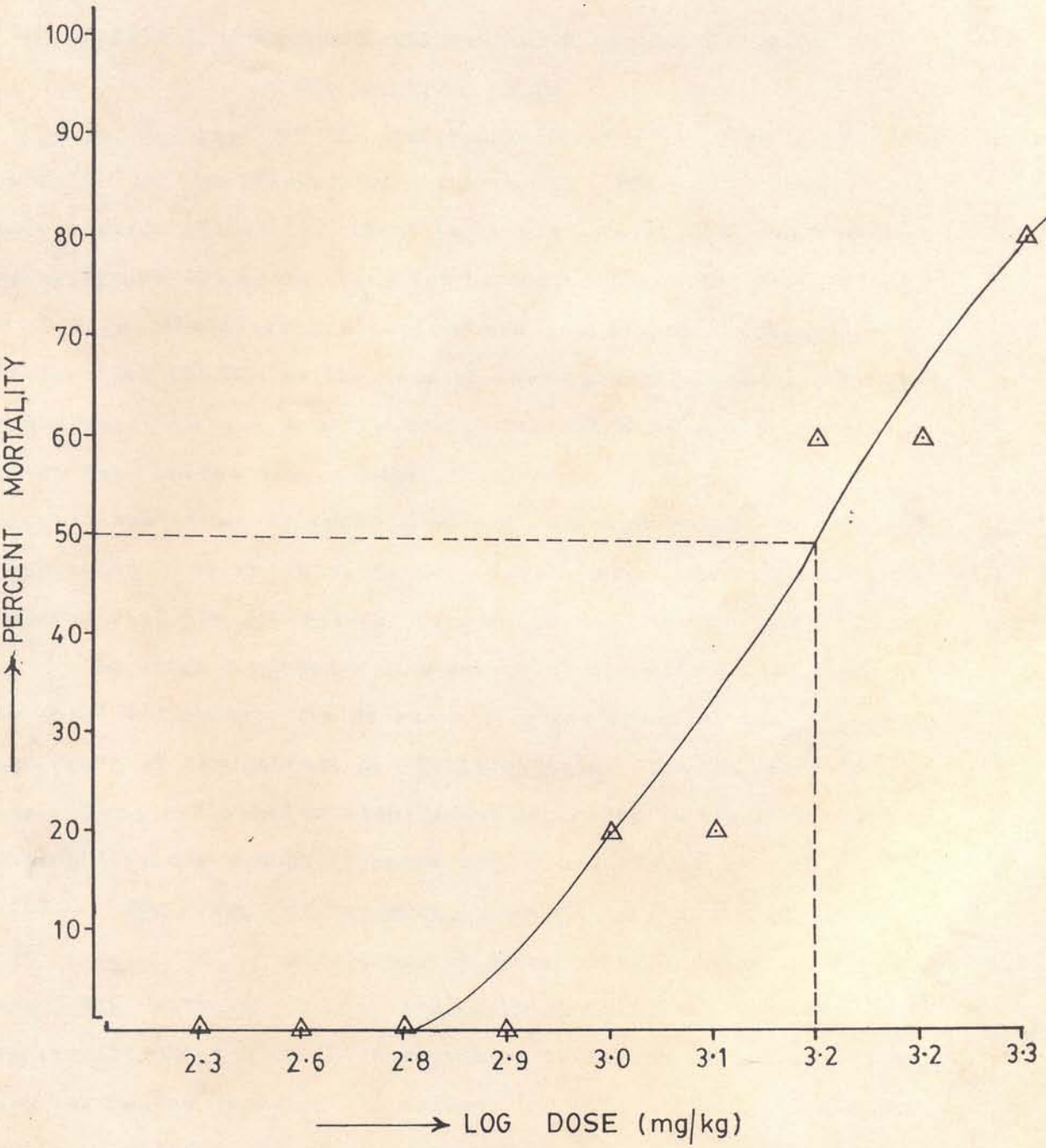


FIG. 5. LOSE-DOSE MORTALITY CURVE OF LEUCAENA LEUCOCEPHALA IN MICE. EACH POINT HAS AN "n" OF 10. LD_{50} = 1,400mg/kg.



3.6.2. Effect of Tribulus terrestris on the hematological parameters of the rat

The extract of T. terrestris caused a dose dependent decrease in the level of the total RBC and haemoglobin concentration (Table 6). These decreases were significant with the 200 mg/kg and 400 mg/kg doses for haemoglobin and RBC respectively (P 0.01). The extract also produced insignificant decreases in the level of the PCV as the dose of the extract increase. Changes in the total RBC count and PCV were reflected as slight increases in the mean corpuscular volume.

Though the extract produced increases in the blood coagulation time in rats, these changes were insignificant when compared with the control values (P<0.05).

The extract caused a dose dependent decrease in the level of the total WBC count. The decrease is significant at the 400 mg/kg dose level of the extract of T. terrestris. The decrease in the total WBC is reflected as significant decreases in the lymphocyte, neutrophil and eosinophil counts (P 0.05) (Table 7).

3.6.3. The effect of Leucaena leucocephala extract on the hematological parameters in rats

The extract of L. leucocephala did not produce any significant changes in the hemogram or leucogram of the rats even with increasing doses of the extract (P 0.05) (Tables 8 and 9). However the total WBC counts have shown increases as the dose of

the extract shown increases as the dose of the extract administered increased which is reflected as increase in the differential lymphocyte and monocyte counts (Table 9).

3.6.4. The effect of Solanum torvum on the hematological parameters in the rats

The extract of S. torvum produced a dose dependent increase in the level of the total RBC counts, PCV and haemoglobin concentration as the dose of the extract administered increased in the rats. The increase was observed to be significant with the doses administered, for the PCV; only the 150 mg/kg dose for haemoglobin concentration as well as only the 200 mg/kg dose level for the RBC.

The increases in the total RBC counts, PCV and haemoglobin concentration did not influence the values of the mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) or the mean corpuscular haemoglobin (MCH) (Table 10).

The extract did not produce any changes in the blood clotting time of the rats. The extract produced inconsistent decreases in total WBC counts as the dose of the extract of S. torvum administered increased. The decrease produced by the 100 mg/kg dose is significant (P 0.05) which was reflected as a decrease in the lymphocyte and neutrophil counts (Table 11).

3.6.5. The effect of Lantana camara on the hematological parameters of rats.

L. camara produced a dose dependent decrease in the total RBC and WBC counts (Tables 12 and 13). It also caused significant decreases in the haemoglobin concentration as the dose of the extract increased.

The decrease of haemoglobin concentration was reflected as significant decreases in the MCHC and MCH. The effect of the extract on the PCV was inconsistent.

The extract also produced slight increase in the blood coagulation time of the rats which was not significant ($P<0.01$).

The decrease caused by the extract on the total WBC count was reflected as decreases in the neutrophil and monocyte counts (Table 12).

3.6.6. The effect of Dichapetalum madagascasiense on hematological parameters in rats

The leaves of D. madagascasiense produced inconsistent increases in the PCV and inconsistent decrease of the total RBC. The leaves did not produce changes in the haemoglobin concentration and the Winthrobe indices or the rat blood coagulation time (Table 14).

The leaves of D. madagascasiense did not adversely affect the leucocyte differential or WBC counts in the rats (Table 15).

3.6.7. The effect of D. madagascasiense on hematological parameters in goats

After 14 days of feeding the goats on the leaves of D. madagascasiense in combination with maize, the hematological parameters examined did not show significant changes (Table 16).

After 21 days of feeding D. madagascasiense, the hematological parameters of the goats still did not show any serious alterations (Table 17).

3.6.8. The effect of Lantana camara on the hematological parameters in the goat.

After 14 days of feeding the goats with the aqueous leaf extract of L. camara, there were increases in the haemoglobin concentration, PCV, and decrease total RBC counts (Table 16). The total WBC count was increased with the corresponding increase in the lymphocyte and neutrophil counts (Table 16).

After 21 days of feeding L. camara, the haemoglobin concentration and PCV still remained elevated whilst the total RBC counts were decreased (Table 17).

The total WBC counts were also increased which were reflected as increased lymphocyte and neutrophil counts but decreased eosinophil, basophil and monocyte counts (Table 17).

3.7. CONCLUSIONS

The extract E. uniflora produced increases in the total RBC count or polycythaemia. It produced no adverse effects on the

leucocyte counts nor the blood coagulation of the rat. This suggest that E. uniflora does not have any destructive effects on the red blood cells nor any adverse effects on its production in the bone marrow.

Similarly L. leucocephala did not produce either decreases or increases in the blood parameters examined. It caused eucocytosis. This indicates that L. leucocephala has no adverse action on the hematological parameters of the rats.

T. terrestris reduced the level of circulating erythrocytes as well as the haemoglobin concentration. This implies that T. terrestris could produce anaemia. The increase produced by the plant on the blood coagulation time indicates the effect of the plant on blood coagulation factors. The plant also produced decrease in the leucocyte counts which may be indicative of the action of the plant on the hemopoietic system of the rats.

S. torvum produced polycythaemia with its increase of the circulating RBC and the PCV. Since these changes did not influence the Winthrobe indices, S. torvum may not have any adverse effect on the hematological parameters since it also did not affect the coagulation time of the rats.

The decreases in the hematological parameters, total RBC and PCV produced by L. camara implies that the plant can cause anaemia in rats. The leucopenia also produced by the plant shows its effect on the hemopoietic system of the rats. The

insignificant increase in the blood coagulation time, does not rule out the possibility of the plant producing serious coagulation defect if administered for much more prolonged periods.

A similar effect has been produced in goats by L. camara as was in rats. This may mean that the adverse effects of the plant may not be species specific but that it could produce harmful changes in blood factors of animals which may consume the plant.

On the other hand D. madagascasiense has not shown any serious abnormal changes in the hematological parameters of the rats and goats. This means that the plant does not contain compounds with adverse effects on blood factors.

TABLE 4.

Mean values (+ S.E.M.) of hematological parameters following exposure of rats to ethanolic extract of E. uniflora.

DOSE mg/kg	PCV %	Hb gm/ 100ml	RBC u/l	MCV u/3	MCHC %	MCH pg	COAG. TIME/ MIN.
0	45+2.7	14.1+2.3	6.8+1.9	66.2+4	31.3+1.3	20.7+1.4	1.95
100	58+2.0	18.6+3.5	8.2+0.4	70.2+16	32.1+0.7	22.6+3.1	2.80
150	57+2.5	19.3+2.2	9.7+2.6	58.8+02	33.9+1.2	19.9+0.86	2.0
200	67+1.0	17.7+1.3	7.7+1.6	86.8+3	26.4+0.6	22.9+2	2.0

n - for each dose is 10

TABLE 5

Mean values (+S.E.M.) of total and differential leucocyte counts of rats given extract of E. uniflora.

DOSE mg/kg	TOTAL WBC 10^3/ml	Lympho-cyte	Neutro-phils	Eosino-phils	Baso-phils	Mono-cytes
0	12.4+12.1	6200+662	4960+963	620+149	496+113	124+69
100	14.2+3.4	7526+992	5822+1041	483+134	483+134	0
150	26.7+7.2	14418+2627	1015+290	801+192	536+192	271+123
200	10.6+5.8	5276+561	4028+587	846+249	636+152	0

n - for each dose is 10

TABLE 6.

Mean values (+S.E.M.) of hematological parameters following exposure of rats to ethanolic extract of T. terrestris.

DOSE mg/kg	PCV %	Hb gm/100ml	RBC u/l	MCV u/3	MCHC %	MCH pg	COAGU TIM/MIN
0	44.4±32	15.4±0.65	9.5±0.86	48.7±4.9	35.7±2.4	16.8±1.0	2.0
150	44±2.9	13.9±0.81	8.4±0.83	54.2±3.3	32.7±0.36	17.3±16	2.14
200	42.4±36	12.6±1.1	8.5±0.66	50.9±4.3	30.3±2.4	14.7±12	2.25
400	28.2±60	14.3±0.61	7.6±0.61	57.9±5.9	35.0±2.9	14.4±13	2.30

n - for each dose is 10.

TABLE 7.

Mean values (+S.E.M.) of total and differential leucocyte counts of rats given extract of Tribulus terrestris.

DOSE mg/kg	TOTAL WBC	Lymph-ocyte	Neutro-phil	Eosino-phil	Baso-phil	Mono-cyte
	10^3/ml					
0	10.4±0.61	5387±689	4056±881	312±100	208±75	416±111
150	6.9±0.51	4623±294	1725±271	69±38	0	483±115
200	6.4±0.63	4416±103	1536±50	128±54	128±74	192±62
400	5.8±0.5	4524±146	1102±136	0	58±26	96±31

TABLE 8.

Mean values (+S.E.M.) of hematological parameters following exposure of rats to ethanolic extract of Leucaena leucocephala.

DOSE mg/kg	PCV %	Hb gm/100 ml	RBC u/L	MCV u/3	MCHC %	MCH pg	COAG. TIME/ MIN.
0	46.4±0.93	14,8±0.7	6.7_0.67	84.7±3.5	25.1.1	20.9±0.64	2.1
200	48.4±1.7	13.9±1.2	6.6±0.53	72.8±5.3	28.9±25	22.0±2.6	2.3
400	47.1±2.6	15.2±034	6.5±0.58	78.3±8.7	33.2±19	25.0±2.3	2.2
600	47.5±2.3	15.4±045	6.8±0.6	64.6±7.9	33.0±14	20.0±2	2.5

TABLE 9.

Mean values (+S. E.M.) of total and differential leukocytes counts of rats given extract of Leucaena leucocephala.

DOSE mg/ kg	TOTAL WBC 100^3/ml	Lymph- ocycte	Neutr- ophil	Eosin- phils	Baso- phil	Mono- cyte
0	12.9±0.25	6966±909	5418±8677	258±59	129±42	0
200	13.6±0.48	8092±667	2590±1153	672±114	420±127	560±20
400	14.0±0.34	7396±943	4488±990	952±290	408±98	277±85
600	15.0±0.39	9300±522	5100±656	0	0	300±68

TABLE 10

Mean values (+S.E.M.) of hematological parameters following exposure of rats to ethanolic extract of Solanum torvum

DOSE MG/ kg	pcv %	Hb gm/ 100ml	RBC 10/ ml	MCV u/3	MCHC %	MCH pg	COAGU. TIME/ MIN.
0	47.0±0.73	13.6±0.3	5.3±035	90.6±51	29.0±0.57	26.1±1.2	2.0
100	55.5±0.65	15.8±077	6.1±025	90±4.7	28.6±0.31	25.8±1.6	2.4
150	57.3±2.6	16.1±0.4	6.4±027	92.1±44	28.6±1.6	26.0±1.2	2.1
200	61.8±2.2	15.2±0.4	7.3±0.1	84.7±35	25.0±1.1	20.9±0641	.96

TABLE 11.

Mean values (+S.E.M) of total and differential leukocytes counts of rats given extracts of Solanum torvum.

DOSE mg/ kg	TOTAL WBC 10^3/ml	Lympho-cyte	Neutr-ophil	Eosin-ophil	Baso-phil	Mono-cyte
0	17.6±0.14	10376±1777	5810±913	404±106	56±37	49±35
100	10.2±3.2	6200±341	3325±445	581±90	112±34	0
150	18.2±1.1	11068±1430	6797±1174	322±105	92±59	3±4
200	15.6±0.28	10636±797	4555±776	343±93	125±59	8±7

TABLE 12.

Mean values (+ S.E.M.) of hematological parameters following exposure of rats of ethanolic extract of Lantana camara

DOSE mg/ kg	PCV %	Hb gm/100 ml	RBC u/L	MCV u/3	MCHC %	MCH pg	COAG. TIME/ MIN.
0	45.9±2.6	14.5±0.61	6.9±0.38	68.8±5.1	32.4±2	21.9±16	2.1
200	44.3±2.5	9.0±0.25	5.6±0.39	90.8±0.39	18.5±084	166±11	2.6
400	54.1±2.0	9.8±0.38	5.2±0.46	101.6±140	17.4±1.0	200±19	2.5
600	49.2±2.9	8.7±0.45	5.3±0.28	93.8±8.5	18.2±1.5	170±12	2.5

TABLE 13.

Mean values (+ S.E.M.) of total and differential leucocyte counts of rats given extract of Lantana camara.

DOSE mg/ kg	TOTAL WBC 10^3/ml	Lympho-cyte	Neutro-phil	Eosin-ophil	Baso-phil	Mono-cyte
0	18.4±0.93	10304±887	7176±720	368±102	184±84	368±132
200	133±0.82	8847±920	3485±726	606±136	266±61	0
400	13.9±0.96	9897±921	3475±825	139±45	264±72	139±45
600	16.3±0.62	10129±549	5226±534	489±117	326±91	163±52

TABLE 14.

Mean values (+S.E.M.) of hematological parameters following exposure of rats to ethanolic extract of D. madagascasiense.

TREAT-MENT	PCV	Hb gm/100 ml	RBC u/l	MCV u/3	MCHC %	MCH pg	COAGU. TIME/ MIN.
0	48.3±3.5	14.2±0.41	7.6±0.21	63.6±4.3	30.1±158	187±066	2.1
A	50.3±3.6	15.4±0.37	8.6±0.46	59.6±5.8	31.6±2.0	182±1.0	2.0
B	51.1±3.2	13.8±0.71	6.5±0.25	78.9±4.6	27.3±1.3	201±1.3	2.1
C	49.0±2.7	14.6±0.46	6.8±0.14	72.4±4.4	30.3±1.6	216±081	2.0

0 - rats fed rat cubes

A - rats fed pelleted leaves and maize in 50.50 ratio

B - rats fed pelleted leaves and maize in 75.25 ratio

C - rats fed pelleted leaves only.

TABLE 15

Mean values (+S.E.M.) of total and differential leucocyte counts of rats given extract of *Dichapetalum madagascasiense*

TREATMENT	TOTAL WBC 10^3/ml	Lymphocyte	Neutrophil	Eosinophil	Basophil	Monocyte
0	8.5±0.17	3655±391	3485±401	510±137	473±107	340±61
A	9.2±1.2	3772±618	4416±570	350±69	460±132	184±84
B	9.9±0.92	4514±755	4930±725	297±90	99±55	0
C	9.5±0.75	3686±561	5130±574	285±43	285±68	95±43

0 - rats fed rat cubes

A - rats fed pelleted leaves and maize in 50.50 ratio

B - rats fed pelleted leaves and maize in 75.25 ratio

C - rats fed pelleted leaves only.

TABLE 16.

Mean hematological values (+S.E.M.) of goats after feeding on D. madagascasiense or L. camara for 14 days

PARAMETERS	CONTROL	D.Madags.	CONTROL	L. camara
Hb gm/100ml	11.58±0.4	10.9±1.1	11.5±0.6	13.42±7.9
PCV %	23.5±0.78	23.5±2.1	23.5±0.93	25±3.3
RBC u/L	10.*±1.3	10.44±0.79	10.4±0.7	9.85±1.3
WBC 10^3/ml	14.75±0.49	15.3±1.3	134.2±1.0	14.6±1.6
Lymphocyte	7578±393	7956±11683	6061±649	6939±1087
Neutrophil	6785±253	6732±1026	5935.7±683	6714±688
Eosinophil	295±37	459±926	1194±180	583±107
Basophil	15+3	153+23	601+95	291+45
Monocyte	0	0	212+50	72.5+60

CONTROL A are the control values for goats fed on D. madagascasiense

CONTROL B are control values for goats fed on L. camara

TABLE 17.

Mean haemalological values (+S.E.M) of goats after feeding with D. madagascasiense or L. camara for 21 days

PARAMETER	CONTROL	D.madagascasiense	CONTROL	L. camara
Hb gm/100ml	11.58±0.4	10.8±0.93	11.5±8.3	15.5±1.3
PCV %	23.5 ±0.78	23 ±1.7	23.5±0.78	19.5±1.7
RBC u/L	10.8±1.3	11.0±0.68	10.4±0.7	8.9±0.43
WBC 10^3/100ml	14.75±0.49	15.0±0.80	13.2±1.0	16.1±1.3
Lymphocyte	7578±393	7724.5326	6061±649	9791±621
Neutrophil	6785±253	6673.5±854	5935±1683	7169±917
Eosinophil	295 ± 37	377.5±50	1194±180	895±56
Basophils	15±3	301±73	601±95	0
Monocytes	0	0	212±50	

CONTROL A are the control values for goats fed with D. madagascasiense

CONTROL B are the control values for goat fed with L. camara

SECTION C

3.8. EFFECTS OF THE POISONOUS PLANTS ON SERUM BIOCHEMICAL PARAMETERS OF RATS AND GOATS

EXPERIMENT 1

Introduction

The toxicity of any compound in the final analysis is the sum total of its interactions with cell constituents to produce chemical alterations and the cell response to these aberrations.

The untoward effects arising from poisons can usually be traced back to the functional incompetence of organs. Whenever such dysfunction happens in a tissue, it is traceable to biochemical derangements at the subcellular level. Therefore recourse could be made to appropriate function tests to elucidate subtle effects of this type even when the animal is still alive (De Bruin 1976). The biochemical abnormality revealed by such biochemical tests are often obvious long before the genesis of morphological damage and precedes the development of chronic degenerative disease (Kaneko, 1980).

Since the liver and the kidney are often the major targets of poisonous substances, organ function tests are directed at these two organs. One of the liver function tests is that for measuring hepatic transport including uptake conjugation and excretion of organic anions. These include measurement of unconjugated and conjugated bilirubin in serum. Bile pigments in mammals are waste

products. There are two forms of bilirubin which are distinguishable in serum of clinical icterus. Using the Van den Bergh test the two forms can be measured by their requirement (unconjugated bilirubin or indirect reaction) or non requirement (conjugated bilirubin or direct reaction) of alcohol to produce the diazo reaction color (Smith et. al., 1974).

Variation in concentration of certain enzymes as measured by their biochemical activity occurs primarily as a result of two processes involving the liver. These are mainly their elevation due to escape of enzymes from disrupted hepatic parenchymal cells with necrosis or altered membrane permeability (Kaneko, 1980).

A valuable adjunct to the clinical diagnosis of poisoning is the utilization of serum enzymes determinations. This involves the detection of enzymes in the serum or plasma which are normally confined to the tissues and whose activities or concentrations are normally low in the serum or plasma (Schmidt and Schmidt, 1967). Enzymes which are normally measured include alanine minotransferase (ALT) EC. 2.6.1.2., aspartate aminotransferase (AST) EC. 2.6.1.1., alkaline phosphatase (ALP E.C. 3.1.3.1.) Creatinine kinase E.C. 2.7.3.2 Sorbitol dehydrogenase (SD. EC. 1.1.1.14) Lactate dehydrogenase (LDH E.C.1.1.1.27) and Gamma-glutamyltransferase (GGT EC.2.3.2.2.).

Specific biochemical tests include protein metabolism test. Although none of the alteration in the serum proteins are entirely

specific for liver damage, the combination of an absolutely low albumin and or gamma globulin levels is quite typical (Kaneko, 1980).

In this study, measurements of changes in serum levels of the following biochemical agents have been used as indices of toxicity by the suspected poisonous plants S. torvum, Tribulus terrestris, Dichapetalum madagascasiense, Lantana camara, Eugenia uniflora and Leucaena leucocephala.

i. serum proteins (total), albumin and globulin

ii. blood urea nitrogen, direct and indirect bilirubin

iii. activity of some serum enzymes

iv. some serum electrolytes.

Procedure

The experimental rats were randomly separated into four groups consisting of ten rats per group. Three of these groups received the different doses of the extracts while the fourth group received propylene glycol only (cf page 162). The goats were given the leaves of D. madagascasiense and L. camara as described in page

At the end of the extract or plant leaves administration as described blood was obtained and treated as described in page 126. The following parameters were then determined by procedures as described in pages 181, 182, 183, 184, 185, 186, 187, 188.

i. serum total and direct reacting bilirubin.

ii. blood urea nitrogen

iii. serum total protein, albumin and globulin

iv. sodium, calcium, potassium, bicarbonate and chloride ions

v. The serum activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase.

3.9. Results

3.9.1. The effect of L. leucocephala on serum biochemical parameters in rats

The leaf extract of L. leucocephala produced a dose dependent decrease in the serum total protein which was reflected as decreased levels of serum albumin (Table 18). The decrease in the level of serum total protein is significant with the groups of rats which were given the 400 and 600 mg/kg doses of the extract ($P<0.05$). The globulin fraction did not show any significant change.

The extract also produced significant increases in the level of the serum urea nitrogen ($P<0.05$). This can be observed with the different groups of rats with the various doses of the extract (Table 18).

The leaf extract did not produce any changes in the serum electrolyte sodium and potassium (Table 18).

The extract produced increases in the activities of the serum enzymes alkaline phosphatase and aspartate aminotransferase though these increases were not statistically significant ($P<0.05$). However the 600 mg/kg dose of the extract produced significant changes in the serum activity of aspartate amino- transferase (Fig. 6 and Table 19).

3.9.2. The effect of T. terrestris on the serum biochemical parameters in rats

The extract of T. terrestris produced decreases in the serum total protein of rats. The decreases were significant at the 200 mg/kg and 400 mg/kg dose levels of the leaf extract (P 0.05) (Table 20). The serum protein decreases were reflected as decreases in the level of both albumin and globulin fractions.

The leaf extract produced dose dependent increase in the level of serum urea nitrogen which were significant at the 200 and 400 mg/kg dose levels ($P<0.05$). The extract produced insignificant changes in the level of the serum electrolyte sodium. It did not produce changes in the serum potassium values *Table 20).

The extract of T. terrestris caused dose dependent increases in the level of serum alanine aminotransferase and aspartate aminotransferase. These increases were significant at the dose levels of 200 and 400 mg/kg for SGPT and the 400 mg/kg for SGOT (Table 21 and Fig. 7). The 150 and 400 mg/kg dose levels

produced increases in ALP level with a decrease observed with their intermediate dose of 200 mg/kg (Fig. 7).

3.9.3 The effect of the extract of E. uniflora on the serum biochemical parameters in rats

The extract of E. uniflora produced dose dependent decreases in the level of serum protein. These decreases were reflected as dose dependent decreases in serum albumin level. The globulin fractions showed slight increases. The decreases in serum total protein and albumin levels were significant in rats which received the 150 and 200mg/kg doses of the leaf extract (Table 22).

The leaf extract of E. uniflora also produced increased levels of the serum electrolyte calcium and sodium. The increases were significant at the 150 and 200 mg/kg dose levels of the extract ($P<0.05$) (Table 22).

The extract did not produce significant changes in serum bicarbonate and chloride levels.

The extract produced dose dependent changes in the serum activities of SGPT and SGOT (Table 23). The increases were all significant for the doses of the extract used (P 0.05 and Fig. 8).

3.9.4 The effect of L. camara on the serum biochemical parameters in rats.

The leaf extract of L. camara decreased the serum total protein level significantly at the 400 and 600 mg/kg dose levels. This was accompanied by decreases in serum albumin level which is

significant only at the 600 mg/kg dose level ($P<0.05$). The level of the blood urea nitrogen also increases significantly with the 400 and 600 mg/kg dose levels of the leaf extract (Table 24).

There were no significant changes in the level of serum potassium and sodium though there appeared to be slight increases (Table 24).

There were no significant changes in the level of serum potassium and sodium though there appeared to be slight increases (Table 24).

All the dose levels of L. camara produced significant changes in the level of activities of the serum enzymes GPT, GOT and ALP (P 0.05) (Table 25 and Figure 4).

3.9.5 The effect of D. madagascasiense on the biochemical parameters of rats

D. madagascasiense caused increased levels of the serum total protein. This was followed by corresponding increases in the level of serum albumin and globulin fractions. The increase in the serum total protein level was significant when only the plant was pelleted for the animals. The plant also produced decreases in the blood urea nitrogen (Table 26). No changes were observed in the level of the serum electrolyte sodium and potassium (Table 26).

The leaves of D. madagascasiense when mixed in proportions with ground maize i.e.50:50 or 75:25 respectively or when fed alone to the rats in pelleted forms produced increases in the serum

activities of ALP, SGOT, and SGPT. These increases in case of the serum enzyme ALP were inconsistent because it was observed that when only the plant was fed to the rats without mixing with the ground maize, the level of ALP activity was even lower than obtained in the control group (Table 27).

3.9.6. Effect of S. torvum on the serum biochemical parameters of rats

The leaf extract of S. torvum did not produce any significant changes in the serum level of total protein, albumin, globulin, potassium or sodium (Table 28).

S. torvum produced dose dependent increases in the serum activity of the enzyme alkaline phosphatase. It did not increase the serum activity of aspartate aminotransferase. It was only the 200mg/kg dose that produced significant increase in the activity of the enzyme alanine aminotransferase (Table 29 and Figure 10).

3.9.7. The effect of D. madagascasiense on the serum biochemical parameters in goats

After 14 days of the goats consuming the leaves of D. madagascasiense, the serum total protein was slightly elevated with a corresponding increase in the serum albumin. The globulin fraction was unaffected. The conjugated and total bilirubin levels did not show changes (Table 22). The serum showed highly elevated levels of ALP and AST. The slight increase in the level of ALT was not statistically significant ($P<0.05$) (Table 30).

After 21 days of consuming the leaves, the total serum protein, albumin and globulin showed increases. The serum levels of conjugated and unconjugated bilirubin did not show changes (Table 31). The serum level of ALP was further elevated whereas the level of AST had slightly dropped though the level was still higher than the control values. The level of ALT had also slightly increased further (Table 31).

3.9.8 The effect of L. camara on the serum biochemical parameters in goats.

At the end of 14 days of the goats consuming the leaves of L. camara, the serum of the goats showed elevated serum total protein which was reflected as increased levels of serum globulin fraction. The total bilirubin and conjugated bilirubin were insignificantly reduced (Table 30). The goats showed highly elevated levels of the enzymes AST and ALP. The increase in the level of ALT was slight and not statistically significant (Table 30).

At the end of 21 days, the goats which were fed with the leaf extract of L. camara showed highly increased level of serum total protein as well as the albumin and globulin fractions. The serum levels of the total bilirubin and conjugated bilirubin were also significantly elevated (Table 31). The serum of the goats showed further increases in the serum activities of the enzymes AST, ALT and ALP (Table 31).

CONCLUSIONS

Leucaena leucocephala produced reduced levels of the serum total protein of rats which is reflected as decreases in the serum albumin level with a related increase in blood urea nitrogen.

This implies that the plant produced some toxic effect on the liver cells. This observation is further strengthened by the observed increases in the serum enzyme activities of alkaline phosphatase and alanine aminotransferase.

The extracts of T. terrestris, E. uniflora and L. camara are also hepatotoxic because they produced decreases in the serum total protein level of the rats which is reflected as decreases in the serum albumin. Furthermore they cause elevation of the blood urea nitrogen, the serum enzyme activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase.

The level of liver insufficiency caused by D. madagascasiense is not very serious since there were no dysproteinaemia produced but there were increases in the serum activities of ALT, AST and ALP.

The plant S. torvum is not hepatotoxic since it did not produce any adverse changes in the serum biochemical parameters utilized in evaluating liver function in this study.

TABLE 18.

Mean serum parameters (+S.E.) of rats following the oral administration of Leucaena leucocephala

DOSE mg/ kg	TOTAL PROTEIN g/dL	ALBU-MIN g/dl	GLOB-ULIN g/dl	BUN mg/ 100ml	K mmole/ l	Na mmole/l
0	6,2±0.1	4.1±0.36	2.0±0.46	9.7±1.4	6.1±0.38	160.1±2.1
200	4.8±0.87	2.1±0.52	2.7±0.58	123±1.9	5.8±0.68	148.8±0.51
400	4.1±0.53	1.8±0.27	2.3±0.27	144±043	6.6±0.9	158.1±0.88
600	3.3±1.0	1.4±0.45	1.9±0.51	165±1.3	5.4±0.42	153.8±0.83

n - for each dose is 10.

TABLE 19

Mean serum enzyme values (+S.E.) of rats following the oral administration of Leucaena leucocephala

DOSE	Activities of Serum enzymes		
	ALP	AST	ALT.
mg/kg	u/L	u/L	u/L
0	98.9±1.1	141.2±2.5	60±4.3
200	114.1±5.0	142.2±0.68	74.6±3.5
400	103.6±1.65	131.8±1.2	70.5±0.98
600	105.3±2.6	148.4±1.5	68.6±2.2

n - for each dose is 10.

TABLE 20

Mean serum parameter (+S.E) of rats following the oral administration of Tribulus terrestris

DOSE mg/kg	TOTAL PROTEIN g/dl	ALBUMIN g/dl	GLOBULIN g/dl	BUN mg/100 ml	K+ mmole/L	Na+ mmole/L
0	8.3±0.77	4.9±0.56	3.4±0.45	102,1±0.33	4.2±0.18	142.4±2.0
150	7.4±0.15	4.3±0.16	3.1±0.27	13.6±0.28	4.8±0.36	144.3±2.4
200	7.5±0.31	4.6±0.26	2.9±0.0.1	14.0±0.24	3.7±0.21	150.7±2.6
400	6.3±0.25	3.8±1.2	3.9±0.19	17.4±0.22	5.2±0.09	149.4±067

n - for each dose is 10.

TABLE 21

Mean serum enzyme values (+ S.E.) of rats following the administration of extract of Tribulus terrestris

DOSE	Activities of serum enzymes		
mg/kg	ALT	AST	ALP
	u/L	u/L	u/L
0	135±3.6	190.3±3.9	120.2±5.3
150	144.1±1.6	192.1±1.1	126.3±0.96
200	149.3±4.7	198.4±2.2	108.6±3.0
400	158.5±2.1	201.8±4.7	125.2±0.8

n - for each dose is 10.

TABLE 22

Mean serum parameters (+S.E.) of rats following oral administration of extracts of Eugenia uniflora

DOSE mg/ kg	TOTAL PROTEIN g/dl	ALB-UMIN g/dl	GLOB-ULIN g/dl	Ca mmole /L	K mmole /L	Na mmole /L	HCo2 oz mmole L	CL mmole/ L
0	5.6±0.6	2.6±0.8	3.0±0.4	7.9±0.9	43±0.3	148±13	192±06	85±1.1
100	4.7±3.4	1.6±1.9	3.1±1.9	8.5±2.7	52±1.0	141±15	201±09	86±0.7
150	4.1±1.9	0.6±1.4	3.5±1.2	9.1±0.6	63±1.0	145±02	216±20	84±1.8
200	3.4±0.7	0.2±0.9	3.2±1.6	9.6±0.7	74±0.4	149±13	189±13	85±0.9

n - for each dose is 10

TABLE 23.

Mean serum enzymes values (+S.E.) of rats following the administration of extract of Eugenia uniflora

DOSE mg/kg	Activities of Serum enzymes ALT u/L	GOT u/L
0	27.5±4.2	36.8±3.5
100	40.2±3.8	59.2±7.1
150	47.5±9.2	58.1±4.7
200	44.3±8.4	53.5±3.2

n - for each dose is 10.

TABLE 24.

Mean serum parameters (+S.E.) of rats following the oral administration of extract of Lantana camara

DOSE mg/ kg	TOTAL PROTEIN g/dl	ALBUMIN g/dl	GLOBU- g/dl	BUN mg/100 ml	K+ mmole/ /L	Na+ mmole/ L
0	9.4±1.1	6.9±0.68	2.5±0.52	10.2±1.6	4.8±0.46	151±2.6
200	8.6±0.13	6.5±2.21	2.1±0.19	136±0.46	5.5±0.33	150.2±2.1
400	8.1±0.9	6.3±0.99	1.8±0.54	15.2±1.5	5.9±1.2	161.3±2.1
600	6.6±2.4	4.4±0.68	2.2±0.4	18.2±1.9	6.8±0.73	169.4±3.9

n - for each dose is 10.

TABLE 25.

Mean serum enzyme valued (+S.E.) of rats following the administration of extract of Lantana camara

DOSE mg/kg	Activities of Serum enzymes		
	ALP u/L	AST u/L	ALT u/L
0	78.3±1.3	158.0±3.1	105.6±0.8
200	108.3±3.7	162.4±2.2	120.7±1.1
400	126.4±5.2	167.3±6.1	134.1±0.3
600	141.6±0.6	178.1±11.6	130.3±4.3

n = for each dose is 10.

TABLE 26

Mean serum parameters (+S.E.) of rats fed with *Dichapetalum madagascasiense*

TREATMENT.	TOTAL PROTEIN g/dl	ALBUMIN g/dl	GLOBULIN g/dl	BUN mg/dl	Na+ mmole/L	K mmole/L
A	6.7±0.3	3.4±0.2	3.3±0.2	16.8±0.17	140.8±0.6	4.3±0.13
B	7.5±0.39	40±0.32	3.5±016	16.1±0.6	136.3±6.2	3.8±0.57
C	7.8±0.17	39±0.09	4.0±014	14.2±1.3	143±6.2	4.4±0.32
D	8.1±0.33	4.2±0.2	3.9±021	15.6±0.84	141.3±16	4.6±0.19

A - control fed rat cubes. B - fed leaves in ratio of 50:50 with carbohydrate. C - fed leaves in proportion of 60:40 with carbohydrate; D - fed only pelleted leaves

TABLE 27.

Mean serum enzyme values (+ S.E) of rats fed with *Dichapetalum madagascasiense*

TREATMENT	Activities of Serum enzymes		
	ALT	AST	ALP
	u/L	u/L	u/L
A	72±6.3	139.3±13.9	103±7.8
B	164±7.9	160.8±8.6	128.8±18.2
C	174.7±6.0	176.7±3.8	148.7±6.2
D	178.3±5.7	189.0±3.1	88.3±16.3

n - for each dose is 10.

TABLE 28.

Mean serum parameters (+S.E.) of rats following administration of extract of Solanum torvum

DOSE mg/kg	TOTAL PROTEIN g/dl	ALBU-MIN g/dl	GLOBU-LIN g/dl	K+ mmole/ L	Na+ mmole/ L
0	7.1+0.21	2.4+0.19	4.7+0.14	5.0+0.1	149.7+1.0
100	7.0+0.15	2.0+0.2	5.0+0.21	4.4+0.14	148.6+1.3
150	7.4+0.13	2.1+0.1	5.4+0.014	4.7+0.21	144+0.88
200	7.0+0.03	2.5+0.07	4.6+0.06	4.5+0.04	142.2+0.84

n - for each dose is 10.

TABLE 29

Mean Serum enzyme actives (+S.E.) of rats following administration of Solanum torvum

DOSE	Activities of Serum enzymes		
mg/kg	ALP	AST	ALT
	u/L	u/L	u/L
0	116.0±8.4	194.3±2.1	139.7±3.3
100	141.3±6.2	191.7±1.2	123.7±4.5
150	64.5 ±5.2	165.5±11.6	90.5±2.0
200	52 ±1.8	183±3.9	94.5±0.52

n - for each dose is 10.

TABLE 30

Mean Serum values (+ S.E) of goats after feeding with *D. madagascasiense* or *L. camara* for 14 days

PARAMETER	CONTROL	*D. madagas.*	CONTROL	*L.camara*
AST U/L	83±9.8	273.5	79.5±5.4	130.512.3
ALT u/L	17±1.2	18.5±1.6	16.0±1.6	19.5±2.6
ALP u/L	262±12.7	463.6±18.9	522.5±38.8	14875±802
Total Protein g/dl	4.7±0.69	5.0±0.84	4.7±0.7	6.2±0.83
Albumin g/dl	2.1±0.39	2.3±0.46	2.45±0.39	2.4±0.37
Globulin g/dl	2.7±0.43	2.6±0.2	2.2±0.42	3.8±0.8
Total Bilirubin mg/dl	0.75±0.19	0.7±0.6	0.85±0.23	0.7±0.2
Conjugated bilirubin mg/dl	0.35±0.17	0.35±0.12	0.35±0.1	0.3±0.1

CONTROL A indicates the control values for goats fed the leaves of *D. madagascasiense*

CONTROL B are the control values for goats administered with extracts of *Lantana camara*

TABLE 31

Mean Serum values (+S.E.) of goats after feeding *D. madagascasiense* or *L. camara* for 21 days

PARAMETER	CONTROL A	*D.madagas.*	CONTROL	*L.camara*
AST U/L	83±9.8	262.5±5.0	79.5±5.4	185.5±3.5
ALT u/L	17.0±1.2	22±1.0	16.0±1.6	33.5 ±2.5
ALP u/L	262±12.7	1003±3.7	522.5±38.8	1493±351
Total Protein g/dl	4.7±0.69	5.7±0.1	4.7 ± 0.7	7.7±0.6
Albumin g/dl	2.1±0.39	2.55±0.7	2.45±0.34	3.7±0.55
globulin g/dl	2.7±0.43	3.2±0.35	2.2±0.42	4.05±0.05
Total Bilirubin mg/dl	0.75±0.19	0.75±0.15	0.85±0.23	1.35±0.15
Conjugated bilirubin mg/dl	0.35±0.17	0.3±0.1	0.35±0.1	0.6±0.1

CONTROL A indicates the control values for goats fed leaves of *D. madagascasiense*.

CONTROL B are the control value for goats administered with extracts of *Lantana camara*.

FIG. 6.

EFFECT OF LEUCAENA LEUCACEPHALA ON SERUM ENZYME ACTIVITIES IN RATS. "n" IS 10 FOR EACH POINT.

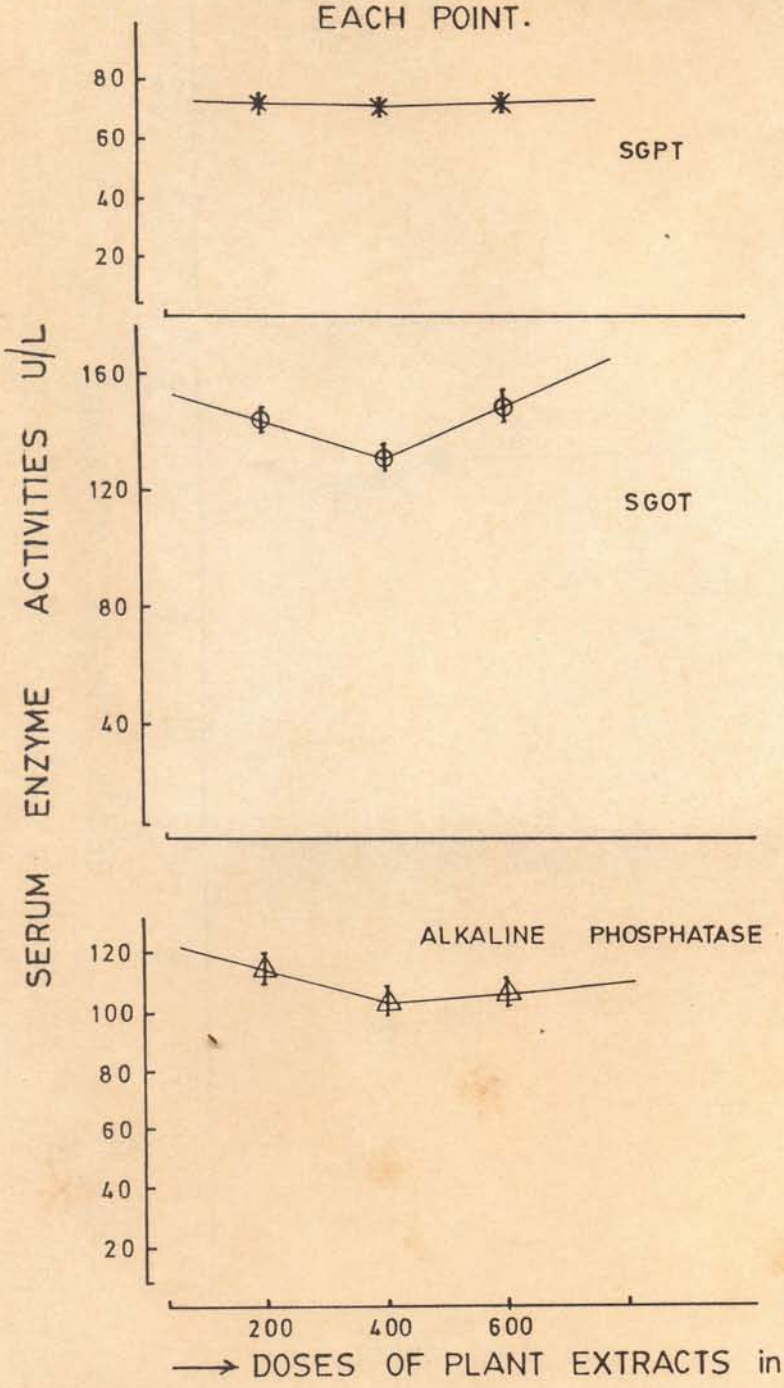


FIG. 7.

EFFECT OF TRIBULUS TERRESTRIS ON SERUM ENZYMES IN RATS "n" IS 10 FOR EACH POINT.

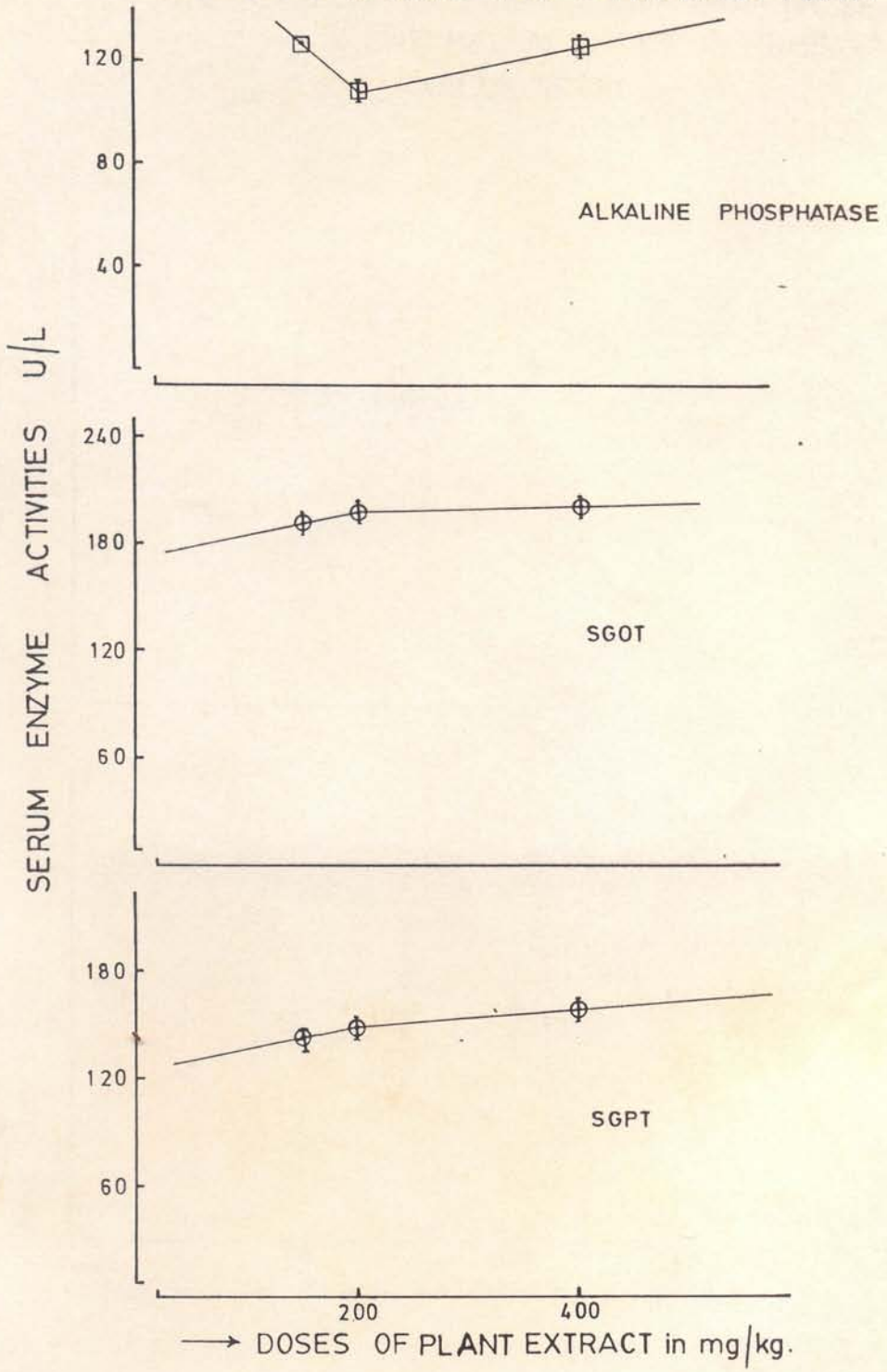


FIG. 8.

THE EFFECTS OF EUGENIA UNIFLORA ON SERUM ENZYME ACTIVITIES IN RATS "n" VALUE IS 10.

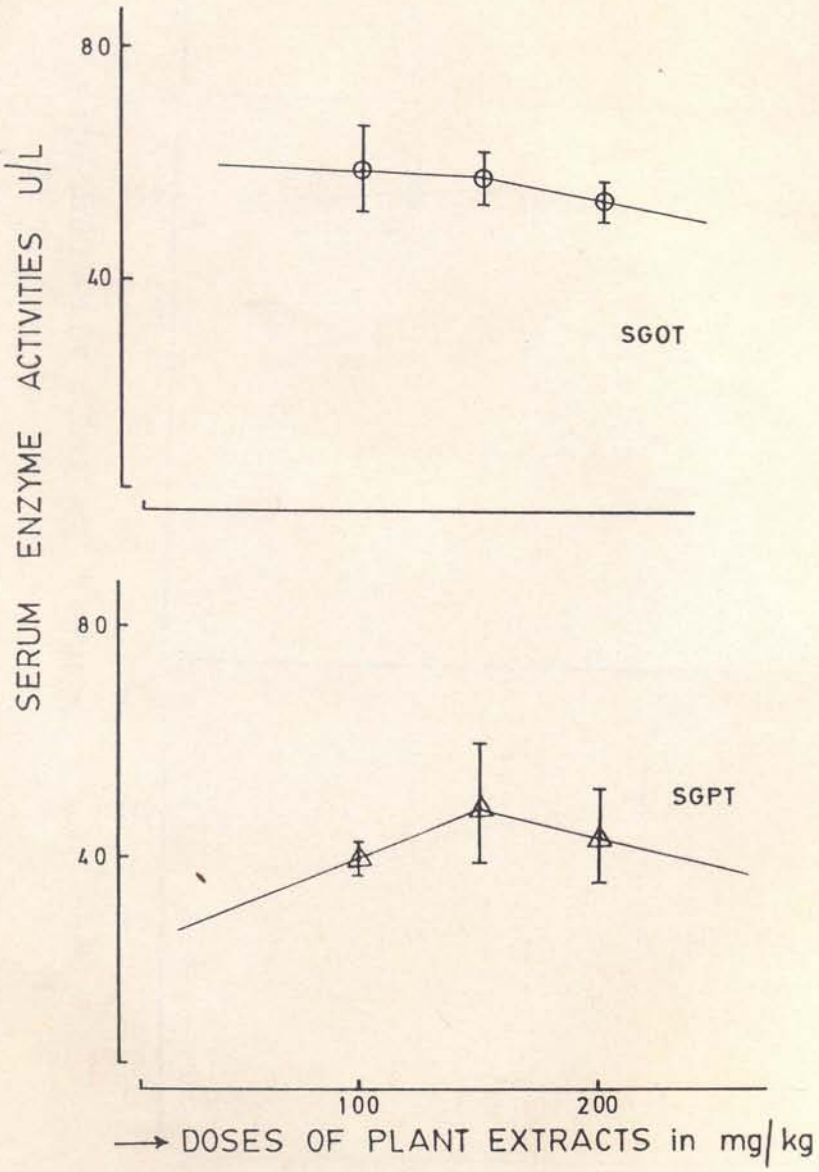


FIG. 9.

EFFECT OF LANTANA CAMARA ON SERUM ENZYMES ACTIVITIES IN RATS "n" VALUE IS 10

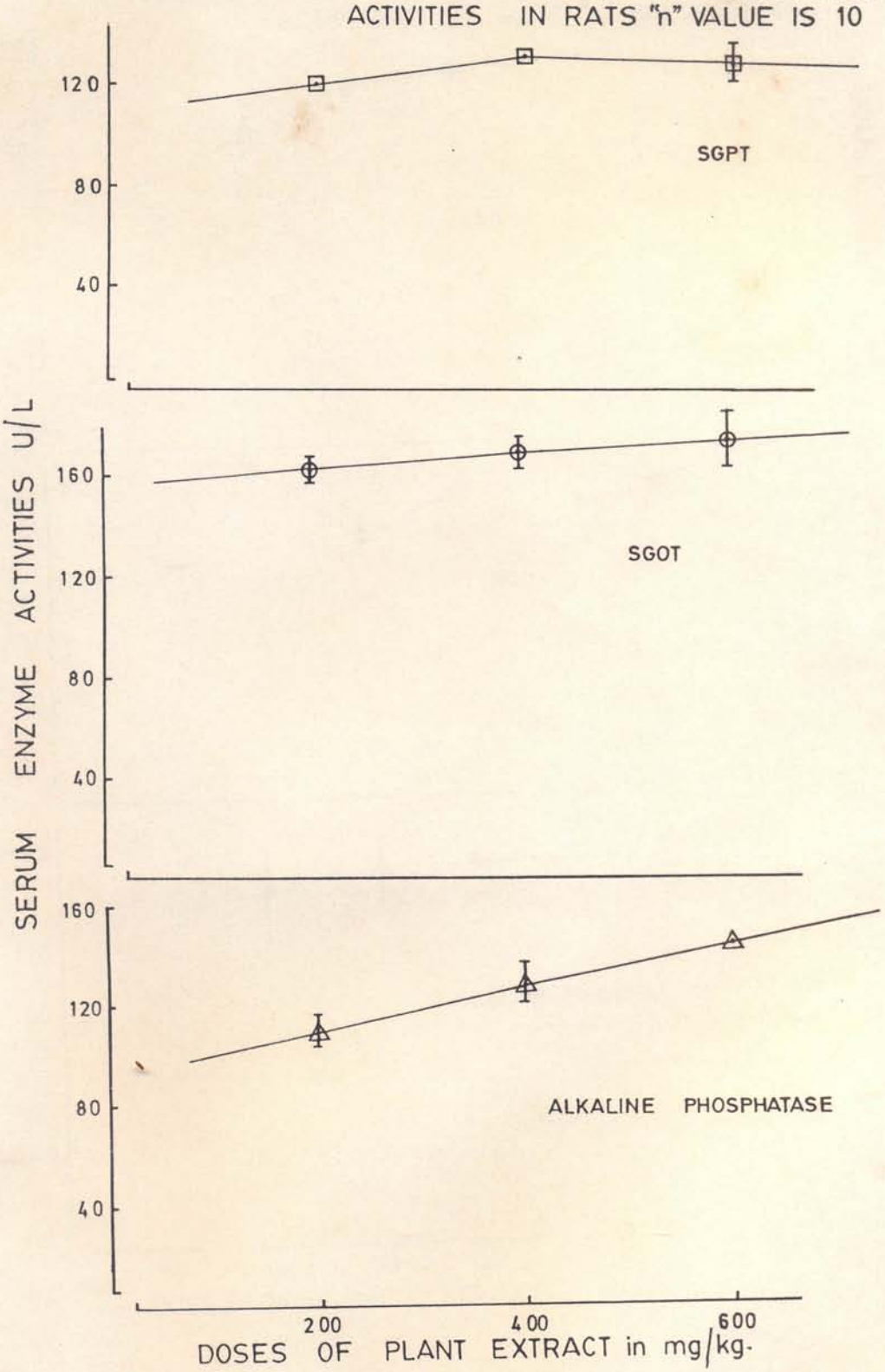
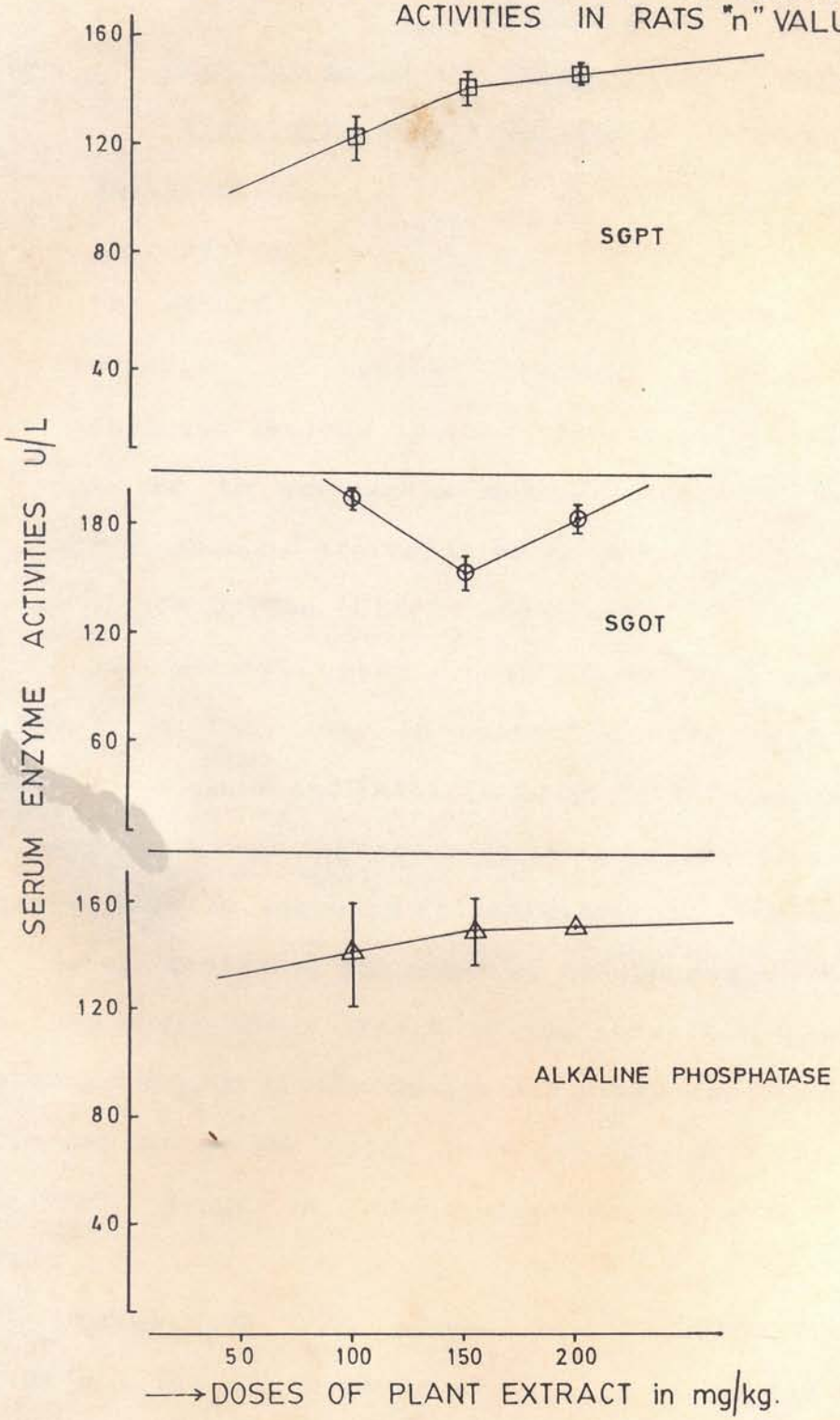


FIG. 10.

EFFECT OF SOLANUM TORVUM ON SERUM ENZYME ACTIVITIES IN RATS "n" VALUE IS 10.



SECTION D

3.10 CLINICAL SIGNS AND PATHOLOGICAL CHANGES IN RATS ASSOCIATED WITH INGESTION OF POISONOUS PLANTS

EXPERIMENT 1

Introduction

The effect of many poisonous substances often manifest by the production of typical clinical symptoms and gross and histopathologic lesions in the tissues. The lesions of poisoning are reported to be rarely characteristic. Nevertheless, the findings at autopsy are reported to provide definite clues to the nature of the poison (Clarke and Clarke, 1975).

The poisons, when in sufficient concentration kill the tissue which they come in contact or if the action is milder, injure the tissues and initiate an acute inflammatory reaction. If the poison has been ingested, it is the alimentary mucous membranes which suffer the necrosis or inflammation. The most powerful ones destroy the lining of the mouth or oesophagus as they are swallowed and then carry their effect to the stomach. Others pass through the stomach with little damage and cause superficial necrosis and inflammation in the upper or lower intestine presumably because they remain longer in contact with the injured part (Smith et. al., 1974).

Furthermore, some poisons are known not to have immediate action but are absorbed to produce their action on the delicate

epithelial cells of such parenchymatous organs as the liver, or kidney which they reach by the blood stream.

The skin and visible mucous membranes may have a characteristic discoloration. Jaundice has been reported as a frequent sign of hepatic damage by plant poisons (Jenkins, 1963; Hutchinson, 1977, Tennant et. al., 1981, Dwers et. al., 1982). A cherry red or pink color is seen in cyanide and carbon monoxide poisoning. Methaemoglobinaemia due to nitrates, nitrites or chlorates may impart a brown coloration (Andrade et. al., 1971).

In toxicology therefore, the most important evidence of toxicity even to the untrained person will be the observation of clinical symptoms and gross pathologic lesions in the tissues of the affected animals. The level of tissue damage can then be further evaluated with the examination of histologic studies.

This study was therefore carried out to evaluate the organotropic effects of the poisonous plants, S. torvum, E. uniflora, T. terrestris, D. madagascasiense, L. camara and L. leucocephala in rats and the re-examination of the effect of L. camara and D. madagascasiense in goats.

Procedure

The extracts of the different plants were administered in doses to groups of rats daily for 14 days. Each group consisted of 10 rats. After administration the rats were left in their cages and observed for periods of up to three hours for any obvious

clinical signs until the next dosage was administered. The goats consuming the leaves were similarly observed in their pens.

The extract administration was stopped on the 14th day and on the 15th day, the rats were sacrificed by a blow to the head and exsanguination. The experimental goats were fed the leaves of D. madagascasiense and L. camara for 21 days and were slaughtered on the 22nd day.

Gross and histologic evaluation of the tissues of the animals were performed to determine the toxicity of the plants to the tissues of the animals. The tissues examined include the brain, lungs, heart, liver, spleen, pancreas, gastrointestinal tract, kidneys and the skeletal muscles.

For histopathology, tissue blocks were fixed in 10% buffered formalin, processed routinely and stained with hematoxylin and eosin (page 188).

3.10.1 Lesions due to L. camara in goats

Grossly the lesions were those of a pale, swollen, friable liver and ecchymotic hemorrhages in the kidney.

At histology the liver from this group of animals showed mild fatty degeneration. The intestinal lesion was characterized by marked oedema, moderate glandular degeneration and marked lymphocytic infiltrates into the laminar propria. These infiltrates were accompanied by few macrophages and plasma cell. There was also goblet cell hyperplasia. In the spleen, there was moderate depletion of lymphocytes from the germinal centers and in addition there was very mild macrophages and plasma cell hyperplasia.

3.10.2. Lesions due to S. torvum in rats

Although gross lesions were not observed, the lungs at histology showed vasculitis with moderate perivascular lymphocytic and macrophage infiltration.

3.10.3 Lesions due to L. terrestris in rats

At necropsy, petechial hemorrhages were observed in the kidney and liver. Histology showed a moderately congested heart while the kidney lesions were characteri by moderate messangial proliferation, thickened glomerular basement membrane and presence of protein casts in renal tubules (Plate 7).

3.10.4 Lesions due to L. leucocephala in rats

Grossly the kidney was markedly congested and the liver pale. Microscopic examination of tissues confirmed renal congestion, while the liver lesion was that of marked perivascular lymphocytic cuffs around central veins and portal vessels (Plate 9). Although lesions were not observed grossly in the heart, histology revealed degenerative and necrotic changes characterized by cellular swelling and clumping of nuclear chromatin. These changes were accompanied by moderate lymphocytic infiltrate and fibroblast proliferation.

3.10.5. Lesions due to E. uniflora in rats

The only gross and histologic lesions observed in rats dosed with this plant were those of congestion in the kidney and moderate fatty change in the liver.

3.10.6. Lesions due to D. madagascasiense in rats

Gross lesions were not observed at necropsy but histological examination of tissues revealed mild pulmonary oedema associated with perivascular lymphocytic cuffs in the lungs. In the liver, there was mild fatty change and most of the bile ductules were distended with bile. The intestinal lesion was that of marked macrophage, few lymphocytic and eosinophilic infiltrates into the laminar propria. In addition, there was goblet cell hyperplasia.

3.10.7. L. camara in rats

At necropsy, only animals that received 600 mg/kg doses of L. camara showed lesions which were characterized by ecchymotic hemorrhages in the heart, kidney and liver.

Histology of these tissues revealed marked diffuse fatty degeneration in the liver which also showed moderate periportal lymphocytic infiltrates (Plate 10).

3.10.7. L. camara in rats

At necropsy, only animals that received 600 mg/kg doses of L. camara showed lesions which were characterized by ecchymotic hemorrhages in the heart, kidney and liver.

Histology of these tissues revealed marked diffuse fatty degeneration in the liver which also showed moderate periportal lymphocytic infiltrates (Plate 10).

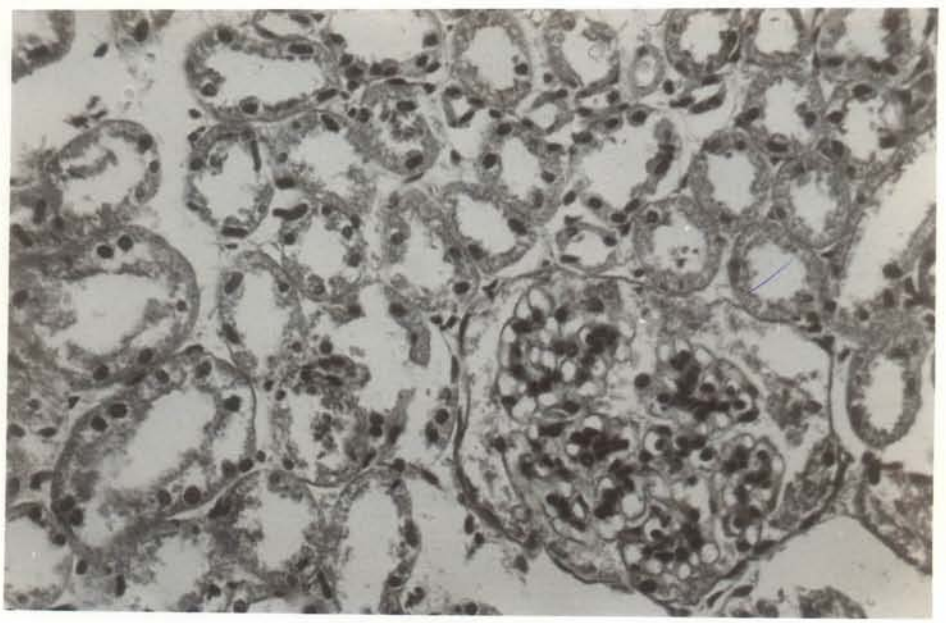


Plate 7:

Renal glomerulus of a rat dosed with Tribulus terrestris showing moderate messangial proliferation and proteinaceus material in glomerular space (H & E X 750)

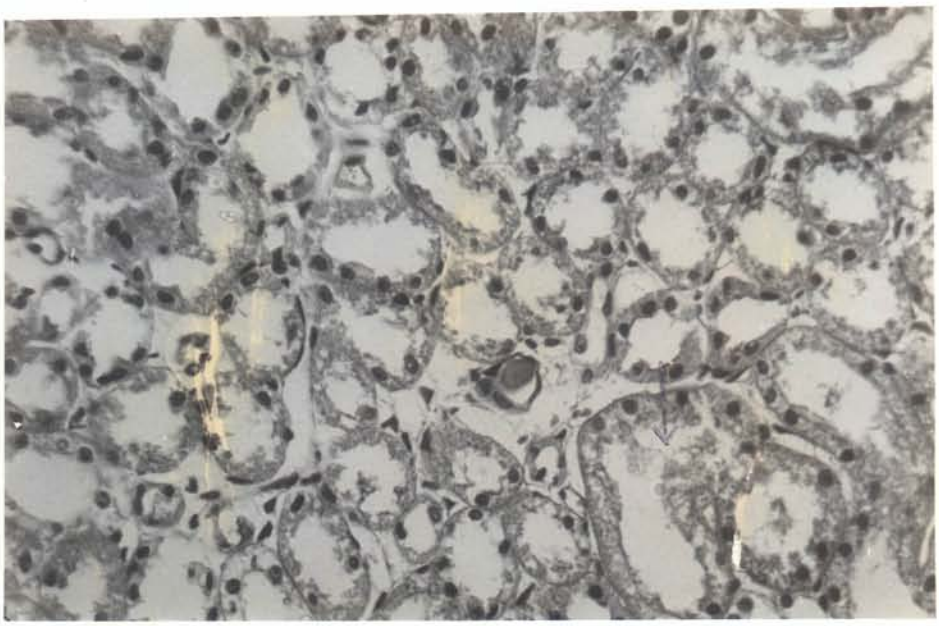


Plate 8:

Sectioning the same kidney as Plate 7
with protein cast (arrow) in tubule
(H & E X 750)

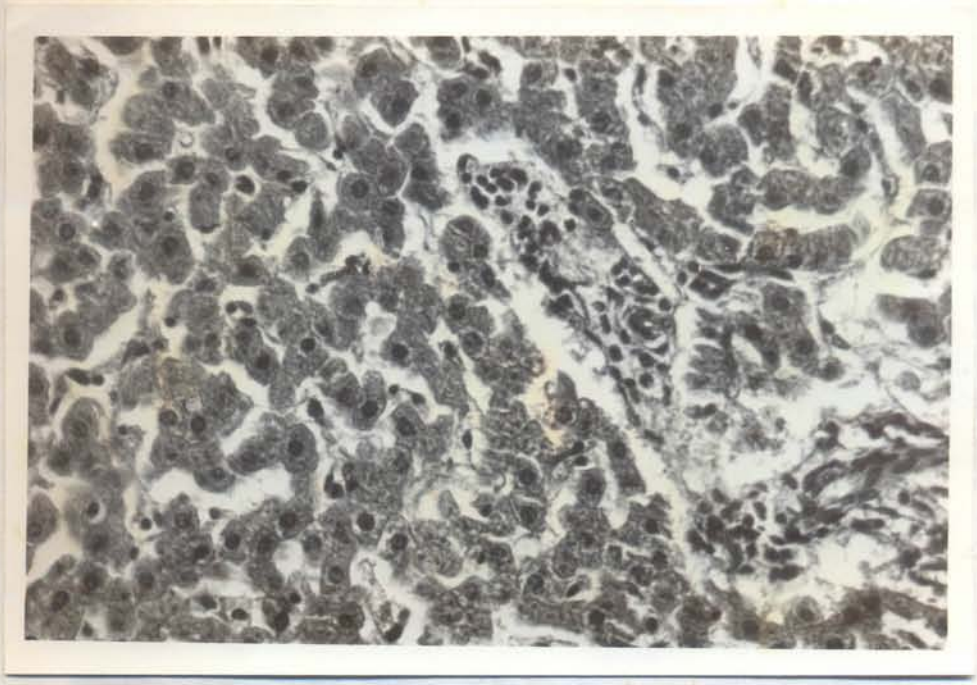


Plate 9: Section of the liver of a rat dosed with Leucaena Leucocephala showing lymphocytic cuffs around the portal vessel (H & E X 750).

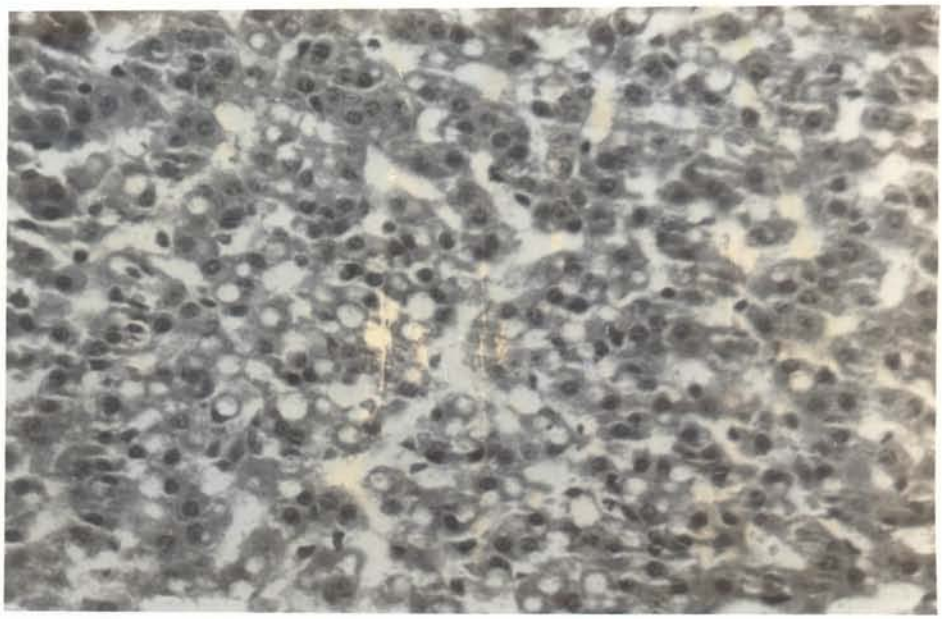


Plate 10:

Histological appearance of the liver of a rat dosed with Lantana camara showing moderate fatty degeneration (H & E X 750).

CHAPTER FOUR

DISCUSSIONS

It is a striking fact that majority of the plants are,at least to some extent toxic. Caution is necessary even in the consumption of many crop plants (Ewer and Hall, 1978). In describing the occurrence of toxic plants in our environment, both to human and livestock, it has been observed that it is obviously impossible in anything other than a work of encyclopedic proportions to mention all the species of plants which sometime or the other have been suspected to cause poisoning in man and livestock (Clarke and Clarke, 1975; Hall, 1977 and Kinghorn, 1979).

However because Nigeria has a poor or non existent reporting system to document the incidence and occurrence of poisoning by plants, coupled with the system of livestock management, incidence of phytotoxicology assumes less significance. Taking into consideration the transhumans nature of livestock management in Nigeria, it is estimated that about 10 per cent of all grazing livestock are seriously affected by poisonous plants each year (Nwude, 1975). The nature of the losses from such encounters is variable. The effect can be either direct, causing death, debilitation, reduced weight gain or management problems in grazing animal or indirect causing abortions or congenital deformities in the unborn offsprings of that animal (Keeler, 1973).

4.1 TOXIC CHEMICAL CONSTITUENTS OF POISONOUS PLANTS

Poisonous plants produce their toxic effects by virtue of their secondary chemical constituents such as gums terpenes, glycosides, alkaloids, tannins and phenolic compounds (Ewer and Hall, 1978; Kingsbury, 1979). Cocoyam is not only disagreeable to eat when raw because of the piercing action of raphides, but is also slightly toxic when cooked because of the calcium oxalate of which the raphides consist of. Cassava tubers always contain poisonous cyanogenic glycosides in the peel; bitter varieties contain them in the flesh also (Osuntokun, 1973; Tewe and Pesu, 1982). Wild forms of the yam Dioscorea dumetorum contain a poisonous alkaloid dioscorine and poisoning may result if they are eaten by mistake for cultivated less toxic forms of the same species.

Poisonous plants may not always be poisonous depending on certain factors. For example plants such as Amaranthus retroflexus are able to acquire dangerous levels of their toxic factor i.e. nitrates only when they are grown on nitrate soils or when the pasture is fertilized by artificial manures containing sodium, potassium or ammonium nitrate (Clarke and Clarke, 1975) i.e. environmental factors affect poisoning.

Furthermore handling also affects the toxic constituents of plants. For example a plant like Melilotus alba is reported to

produce toxicity only when spoiled (Frasser and Nelson, 1959; Prier and Derse, 1962).

4.1.1. Nitrate and nitrite content of the Poisonous plants

The principal hazard to livestock from nitrates or nitrites arises from the fact that certain plants grown on soils containing excess of the salt may take up sufficient to render them toxic to animals eating them. Outbreak of nitrite poisoning have been reported to have occurred in sheep grazing normally safe plant, after a prolonged drought. In such circumstances some normally safe plants with a capacity for fast growth are said to become dangerous (Blood and Henderson, 1976).

In this study Tribulus terrestris, Lantana camara, Leucaena leucocephala, Solanum torvum, Dichapetalum madagascasiense, and Eugenia uniflora were all observed to contain nitrate and nitrites (Table 1). The highest concentration of nitrate was analyzed from S. torvum with an amount of 16.10 mg/g. The lowest concentration was determined in L. leucocephala with an amount of 14.1 ug/g. Plants which accumulate more than 1.5 per cent by dry matter of nitrate are reported to be potentially toxic (Blood and Henderson, 1974). Similarly, Clarke and Clarke (1975) reported that the minimum lethal dose of nitrate in form of potassium nitrate was 4.5 per cent. This implies that the level of nitrate analyzed from the six toxic plants are relatively low to contribute to acute toxicity which may be produced by such plants. However in

the event of an animal being exposed to pasture containing mainly a variety of one of these plant, it is possible that the level of the nitrate in these plants could build up as the quantity of the plant consumed increases Poisoning by nitrate from these plants could also be feasible in a situation of prolonged drought as nitrate and nitrite level are reported to increase under this circumstances (Blood and Henderson, 1976, The Merck Veterinary Manual, 1979).

If the minimum lethal dose of nitrate in form of potassium nitrate is 4.5 per cent (Clarke and Clarke, 1975) then an animal exposed to a pasture infested only with S. torvum will have to consume as much as 279.5 grams of the leaves inorder to suffer nitrate poisoning. The animal has to consume as much as 293.4 grams of L. camara leaves to show symptoms of nitrate intoxication; consume 294.5 grams of T. terrestris leaves; consume 300.2 grams of the leaves of D. madagascasiense; consume 319.2 grams of the leaves of L. leucocephala inorder for such animals to accumulate enough substance to suffer from nitrate intoxication.

Nitrate per se are reported to be relatively non toxic. They become important as toxic factors only when they are converted either in the plant or within the alimentary canal into nitrite (Andrade et. al., 1971; Merck Veterinary Manual, 1979). Nitrates are reduced by the microflora in the alimentary tract especially the rumen, to nitrite, hydroxylamine and finally to ammonia. The

rate at which this reduction takes place is an important factor affecting toxicity (Clarke and Clarke, 1975). It is the nitrite which is then absorbed into the blood circulation to convert haemoglobin into methemoglobin which then disturbs the oxygen carrying capacity of the blood. If the level of methemoglobin conversion is considerable, then the animal suffers tissue anoxia. Symptoms are reported to be observed when about 20 per cent of the haemoglobin is converted into methemoglobin. This becomes increasingly more adverse as the proportion increases and death is reported to supervene when the conversion reaches 80 per cent (Harris and Rhodes, 1969; London, Henderson and Cross, 1967). Poisoning by these poisonous plants resulting from their nitrate content will therefore also depend on the rate of nitrate conversion into nitrite or production of ammonia by the microflora.

Environmental factors are also known to influence the amount of nitrate concentrated by plants. Low temperatures, limited sunlight and poor mineral sources are known to contribute to increased nitrate level (Merck Veterinary Manual, 1979). Harris and Rhodes (1969) reported that on dull days or at night, the nitrate level is higher than it is in the sunlight as conversion to amino acids does not take place. Since the sunshine is no limiting factor around here, the level of nitrate analyzed in these poisonous plant could have been affected

Similarly, in this study, the highest level of nitrite was determined in T. terrestris with an amount of 9.71 mg/gm. The lowest concentration was analyzed in S. torvum with an amount of 8.91 mg/g (Table 1). It is reported that pigs are more susceptible to nitrite poisoning than are cattle and sheep (Crawford, Kennedy and Davison, 1966). The minimum lethal dose that was reported for the pigs was in the order of 70 to 75 mg/kg in form of sodium nitrite. With the determinations obtained for the various plants in this study, it means the following quantities of the plant material has to be in the animals food, for nitrite to contribute to the animals poisoning. T. terrestris 7.7 kg of the leaves; L. camara 7.9 kg; E. uniflora 8 kg of the leaves; L. leucocephala 8.1 kg of the leaves; D. madagascasiense 8.4 kg of the leaves; S. torvum 8.4 kg of the leaf material.

Sinclair and Jones (1964) further alluded that it is difficult to make a categorical statement as regards the toxic dose of nitrate since according to them this depends on the rate at which substance is consumed. A single dose of nitrate of level 220 mg/kg was reported to be lethal to sheep whereas the same dose consumed over a long period (about 24 hours) showed no ill effect. Thus poisoning by these poisonous plants will have to depend on how long they were exposed to them. Under this circumstances, none of the plants under study can produce nitrate/nitrite toxicity with transhumans cattle management.

Furthermore, toxic levels of nitrate or nitrite are reported to be found in common pasture species such as Brachiaria species during rapid growth (Andrade et. al., 1971; Blood and Henderson, 1976). The leaves used in these studies were harvested from already matured stands, therefore the observation that rapidly growing plants have ability to accumulate nitrate may not be a factor in consideration.

Fasting has been reported to increase the susceptibility of animals to poisoning (Clarke and Clarke, 1975). This is an important observation since livestock in Nigeria are exposed to reduced availability of pasture during the dry seasons when there are no rains and they have to trek long distances in search of pasture. Unfortunately, plants are reported to be able to concentrate the level of their nitrate and nitrite content during the dry season (Blood and Henderson, 1976). It is not impossible that the level of nitrate and nitrite in L. camara, S. torvum, D. madagascasiense, E. uniflora, L. leucocephala and T. terrestris could be speculated to be able to increase during such dry season, for the level of these inorganic substances to build up to a level contribute to their toxicity. In such a situation chronic exposure then becomes a very important factor to be considered in determining toxicity by these plants. This is because as the animal continuously consumes the plants, the level of nitrate and

nitrite accumulates with their subsequent conversion of haemoglobin into methemoglobin to precipitate symptoms of poisoning.

4.1.2. Oxalate content of the poisonous plants

The poisoning of animals by pure oxalate is reported to be very rare. Cases of poisoning by oxalate are reported to be encountered due to the ingestion of large amounts of plant containing oxalates (Clarke and Clarke, 1975). In these studies the highest concentration of oxalate was determined in D. madagascasiense with an amount of 0.24 per cent whilst the lowest concentration was found in E. uniflora with an amount of 0.102 per cent. The oxalate content of plants are known to be mainly in form of sodium and potassium oxalate (Shupe and James, 1969). These salts of oxalate are reported to be soluble whereas the calcium oxalate is not soluble and is therefore not assimilated during digestion. Mckenzie et. al. (1981) reported that potassium oxalate at the level of 2.6 and 4.3 per cent, when added to a diet of oaten and lucerne chaff and fed to horses produced typical symptoms of oxalate toxicity. On the other hand, Stewart and McCallum (1944) gave two doses of 454 gm of oxalic acid as sodium oxalate or ammonium oxalate within 24 hours or 200 gram of ammonium oxalate daily for 5 days in order to kill the animals.

Utilizing the observations of Mckenzie et al.(1981), it is estimated that the following amounts of the poisonous plants have to be consumed by a horse inorder for it to have enough oxalate to

produce toxicity. L. leucocephala 3.4 kg of the leaves; T. terrestris, 3.07 kg of leaves; S. torvum 2.39 kg of the leaves, D. madagascasiense 1.79 kg of the leaves; L. camara, 2.91 kg of the leaves; E. uniflora 4.22 kg of the leaves.

The oxalate content of plants is reported to be highest at the leafy stage of growth where plants which have been associated with oxalate toxicity have been observed to contain high levels of the substance. Many of such plants are reported not to contain less than 20 per cent total oxalate (Blaney et al., 1981). It can then be inferred that the level of oxalate in the plants under study are too low to be associated with toxic effects they may produce.

Nutritional status, the ratio of calcium to oxalate in the diet, as well as the level of oxalate ingested are reported to be very important factors in the production of oxalate toxicity (Blaney et al., 1981). This observation is important in that a malnourished and hungry animal which becomes exposed to D. madagascasiense, from which was analyzed the highest level of oxalate in this study, most especially at the leafy stage when oxalate absorption is known to be highest, may suffer toxicity associated with oxalate. This is with the assumption that D. madagascasiense possesses the ability to accumulate oxalate at the leafy stage.

4.1.3. Phytin content of the poisonous plants

A large part of the phosphorus in plant is reported to be present as phytin which is the calcium magnesium salt of phytic acid. Harland and Prosky (1979) reported that as the intake of the dietary fibre from fruits, nuts, vegetables, and whole grains increases, then the intake of phytic acid also increase.

These studies have shown that T. terrestris leaves contained the highest concentration of phytin of 20.18 per cent, whilst the lowest level was obtained in L. camara of 15.99 per cent (Table 1). Edman and Forbes (1977) reported that at dietary levels of one per cent or greater, phytic acid can interfere with mineral availability. It is reported that phytin decreases the bioavailability to animals and humans of minerals such as zinc and iron. Morris and Ellis (1981) observed that the dietary requirement of zinc for growth was generally higher if seed protein was taken as source of protein. This is because seeds have rather high level of phytate than animal protein.

The phytate contents of all the leaves of the poisonous plants determined in these studies were rather very high. This is an indication that phytin in these plants could contribute to the toxic effects associated with these plants. Consequently a prolonged exposure of livestock to the leaves of these plants will affect zinc and iron metabolism in these animals. Such toxic effects are expected to be most obvious in chronic exposure to the

plant considering the restorative ability of the animal in terms of the substances that will be lost from the body.

4.1.4. Cyanide content of the poisonous plants

Many species of plants contain hydrocyanic acid either free or more usually in the form of cyanogenetic glycoside, an organic compound containing a sugar and capable of yielding cyanide on hydrolysis (Fernando, 1987). The glycoside itself is reported not to be poisonous but becomes so when the hydrocyanic acid content is released by an enzyme. The release of such enzymes is associated with the plant damage or decay (Herrington, Elliot and Brown, 1971; Fernando, 1987). In the poisonous plant assayed in this study the highest concentration of total cyanide was analyzed in D. madagascasiense. This concentration was 15.06 ppm whilst the lowest level of cyanide was determined in L. camara with a concentration of 12.7 ppm. The observation of hydrocyanic acid from these plants may be an indication that among other chemical substances they contain, they also possess cyanogenetic glycoside. According to Clarke and Clarke (1975) the minimum lethal dose of free hydrocyanic acid and of potassium cyanide given per os is about 2.0 to 2.3 mg/kg HCN. Taking into consideration the level of cyanide determined from these poisonous plants under study (Table 1) it becomes obvious that the level of cyanide in them are considerably high enough to participate in producing toxicity that may be associated with these plants. Infact it is

reported that materials containing over 20 mg HCN per 100g is potentially dangerous to stock. However, most authors working on cyanide poisoning allude that it is not possible to state with any certainty the toxic dose of cyanide in form of the cyanogenetic glycoside. This is because the level of cyanide varies according to the conditions obtaining in the plant and that of the animal at the time of consuming the plant (Van der Walt 1944; Chen et. al., 1934; Clarke and Clarke, 1975).

Cyanide concentration are highest in the young actively growing plants. Van der Walt (1944) mentioned that poisoning in ruminants depends upon the quantity of the plant ingested, the previous diet of the animal, the pH of the stomach contents, the percentage of the total hydrocyanic acid present in the free state in the plant, the concentration of cyanide liberating enzyme present in the plant, and the total hydrocyanic acid content of the plant.

In view of the foregoing variables, in determining the toxicity of cyanide by cyanogenetic plants, it becomes difficult to attribute the toxic effects that could be produced by S. torvum, E. uniflora, D. madagascasiense, L. camara, T. terrestris, or L. leucocephala to their HCN content. Infact it is reported that it is only those animals which rapidly eat the
plants that die. It is also observed that ruminant are more susceptible to poisoning by cyanogenetic plants than are horses,

and pigs, since the enzymes concerned in the release of hydrocyanic acid are destroyed by gastric hydrochloric acid. Moreover, hydrogen cyanide is detoxicated by conversion into thiocyanate and is excreted in the urine over a period of several days. Owing to the rapid detoxication of cyanide, it is reported that it is possible for animals to ingest amounts of cyanide only slightly less than the lethal dose over extended periods without harm (Hazzard et al., 1982). In this situation these plants under study could be consumed over prolonged periods with their cyanide content not constituting danger.

4.1.5. Metallic ion concentration in the poisonous plants

As shown in table 2, the metallic ions which were assayed for their contribution or otherwise in the toxic nature of S. torvum, D. madagascasiense, L, camara, E. uniflora, T. terrestris, L. leucocephala were molybdenum,manganese, iron, copper, zinc and lead.

4.1.5.1. Level of molybdenum in the poisonous plants

Plants have been reported to concentrate excessive amounts of molybdenum from the soil on which they grow. Normal herbage are classed as those which contain 1 to 3 ppm (Clarke and Clarke, 1975). Analysis of the leaves of the plants used in this study showed that T. terrestris contains the highest concentration of molybdenum with an amount of 0.26 ppm. The lowest concentration was found in E. uniflora and D. madagascasiense. The

presence of a small excess of molybdenum of up to 2 to 25 ppm in young growing herbage has been reported to cause deficient bone metabolism in herbivores consequent upon the effects of molybdenum on the utilization of copper and of phosphorus (Hall, 1977). If the lowest level of molybdenum cited above as causing symptoms of molybdenosis i.e. 2 ppm is used as a measure to determine whether the toxic plants studied could have enough molybdenum to produce toxicosis, then about 14.3 kg of L. leucocephala will have to be consumed; 7.7 kg of T. terrestris; 14.3 kg of S. torvum; 16.7 kg of D. madagascasiense; 8 kg of L. camara and 16.7 kg of E. uniflora would have to be consumed. These observations are mainly presumptive since Cunningham and Hogan (1959) had reported that high molybdenum levels are not toxic to sheep or cattle unless the diet contains enough inorganic sulphate. When the intake of sulphate is low, the excretion of molybdenum in the urine is negligible consequently the level of molybdenum in the blood rises. The implication of this is that the level of sulphate in the plants understudy, have to be manipulated inorder to cause a toxicity.

4.1.5.2. The level of manganese in poisonous plants

The highest concentration of manganese in plants is reported to be found in the roots as opposed to its occurrence in the leaves of the plant (Berman, 1980). The level of manganese in diet which has been observed to interfere with haemoglobin formation is 125 ppm whilst supplementation of the diet with 1250 to 2000 ppm was

reported to have depressed growth (Martone, Hartman, and Clawson, 1959).

The level of manganese that was estimated in the leaves of the toxic plants was low to be considered to be of any consequence in the toxic effects produced by the plants. The highest concentration of manganese was determined in T. terrestris. Considering the observation of Matrone et al. (1959) it means the plants in this study cannot produce manganese toxicity. The level of manganese in T. terrestris was 0.60 ppm (Table 2).

4.1.5.3. The level of copper in poisonous plants

Poisoning of animals by copper of plant source could take either the acute form in which case the animal consumes herbage with high levels of copper or chronic situations in which successive non-toxic doses of copper from the plant have a cumulative effect. Cases of animal poisoned by copper in plants have been associated with situations where there was no growth of herbage so that animals were forced to consume the grass which was heavily contaminated with copper. Poisoning of sheep which grazed areas previously sprayed with salts of copper has been attributed to the ability of certain plants to accumulate copper (Muth 1952, Gracey and Todd, 1960).

The toxic dose of the sulphate of copper by mouth is reported to be of the order of 25 to 50 mg/kg in lambs, 130 mg/kg in sheep and in the cow about 200 mg/kg. If these concentrations are

reckoned with then the level of copper determined from S. torvum, T. terrestris, E. uniflora, L. camara, madagascasiense, L. leucocephala are relatively low. This shows that these plants are not able to accumulate copper which may pose danger for copper toxicity. The implication of these observations is that copper is of no consequence in what ever type of toxic symptoms these plants may produce.

Damage to the liver caused by some plants e.g. Heliotropium europoeum can lead to abnormal accumulation of copper and death resulting from the hemolytic crisis of chronic copper poisoning (Clarke and Clarke, 1975). Gracey and Todd (1960) were able to find 200 ppm of copper in some grasses sprayed with copper sulphate. Tait et. al. (1971) estimates that a level of 27 ppm of copper in the diet is fatal to lambs.

4.1.5.4. The level of zinc in poisonous plants

The metallic zinc is reported to contaminate pasture which are found in areas where zinc is produced from its ores. However, it was observed that symptoms could not be produced in domestic animals by feeding them on pasture contaminated with zinc chloride (Clarke and Clarke, 1975). The average daily intake of the cow and of the sheep and goats used in the experiments were 527 and 53 grammes respectively. The amount of zinc estimated in the poisonous plants under this study were relatively low. The highest concentration of zinc of 4.73 ppm was determined in D.

madagascasiense. Thus in general, it appears relative large proportions of the leaves of such plants will have to be consumed inorder to produce zinc toxicity.

4.1.5.4. The level of Lead in the leaves of the poisonous plants

Lead is reported to be the most common cause of poisoning in animals especially in dogs and cattle (Todd, 1962). Contamination of pasture near open cast or disused lead mines have been reported to be the cause for serious losses in farm livestock (Harbourne et. al., 1968). The herbage in such areas are said to contain up to 580 ppm of lead on a dry matter basis.

The highest concentration of lead was estimated in E. uniflora with an amount of 0.21 ppm. This represents quite low concentration so that lead toxicity cannot be connected with the symptoms that may be associated with these poisonous plants. Plants which are located near busy streets have been known to concentrate up to 500 ppm of lead due to their contamination by the exhaust fumes of cars (Scott, 1963). The stands from which the leaves of D. madagascasiense, S. torvum and L. leucocephala were collected were all along busy streets. This means that the level of lead analyzed from their leaves could have been acquired from the exhaust fumes. Therefore depending on how busy the street is with cars and the length of growth, the level of lead could increase in such plants.

4.2. Effect of the poisonous plants on biochemical parameters

This observation of biochemical changes is very relevant since some toxic substances take long periods to exhibit any symptoms or produce any organic damage.

During the process of degeneration leading to necrosis, several biochemical alteration occur. The changes in capacity of oxidative phosphorylation is one of the earliest manifestation measured by adenosine triphosphate (ATP) level. (Beutler, 1968). Such an assault results in decreased intracellular pH and lack of energy, for energy dependent cation pump working against normal electrochemical gradients. Failure of the later and loss of integrity of cell membrane results in an influx of sodium chloride, calcium and water culminating in cellular swelling and leakage of intracellular ions (especially potassium) proteins and enzymes (Schmidt and Schmidt, 1967; Smith, Jones and Hunt, 1974; Morgan et al., 1988). These leaking enzymes provide an important diagnostic aid for the recognition of dead or dying tissues in living patients (Kaneko, 1980; Cornelius et. al., 1965). Measurements only allows for recognition of necrosis, and by characterization of these enzymes by electrophoretic separation, exactly localization of necrosis to specific tissue is said to be possible. This is the rational for the use of serum biochemical changes as one of the parameters for evaluating toxicity of the poisonous plants in this

study taking into consideration that the plants were fed subacutely.

4.2.1 The effect of the poisonous plants on serum proteins

In this study, E. uniflora, L. camara, T.terrestris and L. leucocephala have all produced dose dependent decreases of the total protein level. These decreases in the total protein are accompanied by decreases also in the level of serum albumin. The globulin levels also increased in the serum of the rats treated with T. terrestris and only slightly i.e insignificantly with rats treated with L. camara (Tables 20 & 24). Globulin levels for animals treated with E. uniflora and L. leucocephala did not show changes (Tables 22 & 18).

Changes in the serum protein are quite significant for these four plants. With E. uniflora, the 100 mg/kg dose produced 16.1% decrease; the 150 mg/kg 27% decrease; the 200 mg/kg dose 40.3% decrease. With L. leucocephala, the 200 mg/kg dose produced 23.6% decrease; the 400 mg/kg dose a 34.9% decrease; the 600 mg/kg dose 47.8% decrease. In case of T. terrestris, the 150 mg/kg dose produced 10.8% decrease; the 200 mg/kg dose 10% decrease; and the 400 mg/kg a 24.1% decrease. With L. camara poisoning the 200 mg/kg dose produced 8.5%; the 400 mg/kg a 13.8% decrease and the 600 mg/kg dose a 29.8% decrease in the serum total protein.

Factors responsible for decreased level of serum protein include toxic liver deficiency (Cantarow & Trumpert, 1956; Kaneko, 1980). This result from defective protein manufacture in the liver. The response either arise from the direct effects of hepatotoxicants on the Kupfer cells or are secondary to hepatocellular insult. Albumin is said to notably decrease though hypoalbuminaemia may also follow upon excessive loss of protein into the urine in case of severe kidney damage.

The decreases in the serum protein therefore observed with the rats treated with L.camara, L.leucocephala,T.terrestris and E. uniflora indicate that these plants produced hepatocellular toxic changes and or nephrosis. Decreases in total serum protein levels were also reported in layer strain after 16, 28 and 56 days of feeding the plant Brassica oleracea (Pearson et. al., 1983) or B. campestris (Fenwick and Curtis,1980).Such observation were associated with hepatic damage.

The decrease in serum total protein in the rats treated with the poisonous plants L. leucocephala, L. camara. T. terrestris and E. uniflora were associated with decreases in the serum albumin levels. Hypoalbuminaemia is reported to be most consistently demonstrable in acute and subacute hepatic necrosis (Kaneko, 1980). The liver is the organ chiefly if not entirely responsible for the formation of the plasma albumin. It would therefore be anticipated that hepatic function impairment might result in decrease in the

plasma albumin concentration. Since the globulin levels in the rats treated with these poisonous plants did not show significant changes it can be inferred that the decrease in serum total protein in these animals is a result in their reduced serum albumin levels. Decreases in serum total protein and hypoalbuminaemia have also been reported in poisoning by A. flavus in pigs (Cysewski et. al., 1968).

In the goats which were poisoned with.L. camara, the observation made with the serum protein levels was quite different (Tables 30 and 31) L. camara increased the serum total protein level as the days of consumption of the plant was increased. The goats which consumed the plant D. madagascasiense also showed elevated serum total protein levels. The increases in the total protein observed in the goats were associated with decreased albumin content but with increased globulin levels. It has been observed that in some liver damages, the level of total protein is increased as a result of increased serum globulin level to offset the hypoalbuminaemia that occurs (Cantarow and Trumpert, 1956; Kaneko, 1980). In some cases, it is even reported that the total protein concentration may not reflect either the nature or the extent of an existing abnormality and may indeed even fail to indicate presence. This is as a result of changes in the albumin,, globulin fractions in opposite directions to offset each other (De Bruin, 1976).

The decrease in serum protein level observed in the rats treated with L. camara in this study is not in consonance with the observations of Sharma et. al. (1981) who reported increases in the serum total protein in guinea pigs which were poisoned with L. camara though this observation is in agreement with the observation of L. camara treated goats (Seawright, 1964). However, the observation of increase in serum total protein level in the goats which were fed with L. camara agrees with the work of Sharma, Vaid and Dawra (1984) who observed serum protein increases in goats fed with L. camara.

Observations made with L. leucocephala toxicity are associated with hyperactivity and incoordination (Falvey, 1976; and Jones et al., 1984) emaciation and drooling of saliva (Jones and Hegarty, 1984) enlarged thyroid glands and death of offspring (Jones, 1979) loss of hair, swelling of face and severe bilateral oedematous palpebral conjunctivitis (Akpokodje and Otesile, 1987). All these authors did not include biochemical observations in their studies.

4.2.2 Effect of the poisonous plants on blood urea nitrogen

Increase in blood urea nitrogen in poisoning situation, has been attributed to decrease renal excretion associated with organic disease of the kidneys with destruction of a considerable portion of functional renal tissue. Glomerulonephritis is a common cause for abnormally high blood urea nitrogen which is an evidence of renal functional impairment in acute and chronic glomerulonephritis. Renal conditions accompanied by marked

oliguria or anuria, lower nephron nephrosis are associated with increase BUN (De Bruin, 1976).

As urea is normally formed in the liver, decrease blood urea nitrogen are observed in conditions associated with acute hepatic insufficiency (Cantarow & Trumpert, 1956).In this study L.leucocephala, T. terrestris and L. camara were observed to produce dose dependent increase in the BUN in rats. This is an indication that the urea nitrogen formed in rats poisoned by these plants was not being adequately excreted as a result of renal insufficiency.

This observation agrees with the observation that L. camara produces elevated levels of BUN in guinea pigs (Sharma et. al., 1981). Plants which are poisonous to animals and cause nephrotoxicity usually raise blood urea nitrogen. For example H. glomeratus is nephrotoxic and produces increased levels of the BUN (Shupe and Jane, 1969). Plants as A. retroflexus which produce perirenal tissue oedema with renal tubular degeneration and necrosis is reported to produce elevated BUN in pigs (Marshall et al., 1967). The elevated levels of BUN associated with L. leucocephala, L. camara, E. uniflora and T. terrestris may therefore be an indication of their nephrotoxic effect.

4.2.3 Effect of the poisonous plants on serum electrolytes

Increased levels of serum calcium and potassium was observed in the rats treated with E. uniflora. Significant increases were

also observed of potassium from the serum of L. camara treated rats.

Potassium is known to be the most abundant cation in the intracellular fluid of both plants and animals. For this reason this element is present in high concentration in almost all normal animals. Changes in serum potassium observed with E. uniflora may be associated with the tissue damage these plant caused thus allowing outflow of the elements.

An increase in serum calcium concentration is found during hyperparathyroidism and may also be induced via the administration or intake of excessive amounts of vitamin D or vitamin D like substances A series of so called plant induced calcinoses in animals has been described: enteque seco (Argentine espichaments (Brazil), naalelia disease (Hawaii, Manchester wasting disease (Jamaica); enzootic calcinosis (Germany, Austria) Cestrum diurnum poisoning (Florida) Krook et al., 1975, Wassermann et al., 1977; Kasali (1977). However the cause of the hypercalcaemia observed with rats treated with E. uniflora cannot be explained since there has not been an account of vitamin D-like substance isolated from this plant. Investigations however indicate that all the diseases associated with the plant induced calcinosis are caused by the presence of potent, active vitamin D like substance in the plants and that these substances are responsible for the abnormalities of

mineral metabolism in the animals poisoned by them (Wassermann et. al., 1977).

4.2.4 Effect of the poisonous plants on serum enzyme activities

Variation in the concentration of certain enzymes as measured by their biochemical activity, occurs primarily as a result of elevation due to the escape of the enzymes from disrupted parenchymal cells with necrosis or altered membrane permeability. In this study, S. torvum decreased the serum activities of the enzymes alkaline phosphatase (ALP) and alanine aminotransferase (SGPT); L. leucocephala caused inconsistent increase in ALP, ALT (SGPT) and aspartate aminotransferase AST (SGPT); E. uniflora caused increases in SGPT but inconsistent increase in SGOT; T. terrestris in SGPT and SGOT; L. camara produced increases in SGOT, SGPT and ALP; whiles D.madagascasiense caused increases in SGOT and SGPT (Figures 6-12).

Although elevations in serum activity profile provides little information regarding the type of lesion or functional state of an organ (Kaneko, 1980) it can be used to gain a quantitative estimate of the extent of necrosis. Enzymes which increases in concentration in the blood following hepatic necrosis are divided into two groups; enzymes which are liver specific in that high concentrations are present primarily in hepatic tissue such as SGPT and enzymes which are high in concentration in other tissues in

addition to the liver such as SGOT, lactate dehydrogenase and serum isocitric dehydrogenase.

Moreover, the activities of the liver specific enzymes are the most sensitive and reliable test available for detecting mild to severe hepatic necrosis.

4.2.4.1. Effect of plants on serum activities of aspartate aminotransferase (SGOT or AST)

All the plants used in this study except L. leucocephala and S. torvum produced elevated levels of SGOT which were significant ($P < 0.05$) compared with untreated controls in the rats. However the elevations produced by T. terrestris, L. camara and D. madagascasiense were the only ones which were dose dependent (Tables 21, 25 and 27). Elevations in the activity of SGOT can be associated with alterations in cell necrosis of many tissues. For example pathology involving the skeletal or cardiac muscle and or the hepatic parenchyma allows for the leakage of large amounts of this enzyme into the blood. The elevations in SGOT levels produced by the plants under study is therefore an indication of tissue necrosis (Kaneko, 1980).

Similar elevations in SGOT activities have also been observed in some plant poisoning such as with members of Senecio (ragworts) (Ford and Ritchie, 1968); in calves; aflatoxicosis in swine (Cysewski et. al., 1968). Lantana toxicity in guinea pig

(Sharma et. al.,1981). All these observations were associated with tissue damages such as liver damage as well as nephrotoxicity. D. madagascasiense, L. camara, E. uniflora, and T. terrestris should have produced tissue damage to have caused elevated levels of SGOT in the sera of the rats and/or goats which were fed with these plants.

4.2.4.2. Effect of the poisonous plants on alkaline phosphatase

L. leucocephala and L. camara produced elevations in the level of alkaline phosphatase (ALP). L. camara produced dose dependent changes in ALP activities (Table 25).

ALP is reported to be present in a large number of cells but only in a few is the activity sufficient to be of clinical importance. In general ALP activity is associated with the microvilli of secretory and absorptive cells such as epithelium of bile duct canaliculus, intestinal tract, renal tubular epithelium and placenta. It is also found in liver cells and in associated with osteoblastic activity in the bone (Pickrell et. al., 1974; Hoffman et. al., 1977; Hoffman and Dorner, 1977; and Saini and Saini, 1978). When obstruction of duct system occurs at any level in the liver, there is increase in hepatic ALP activity in serum (Hoffman et. al., 1977). Liver ALP activity is also reported to increase in hepatic fibrosis produced by poisonous substances.

Increases in the serum activities of ALP associated with the plants L. leucocephala and L. camara can therefore be adduced to be

most likely associated with microvilli of absorptive or secretary cells in the rats. In similar studies, Sharma et. al. (1981) reported elevated levels of ALP in guinea pig poisoned with L. camara. In their clinico - pathologic features associated with A. flavus poisoning in pigs other authors reported the observation of elevated levels of ALP among other serum-enzymes and concluded that the increased levels of the enzymes is related to the hepatic damage caused by the plant (Gumbmann and Williams 1969; Cysewski et. al., 1968; and Sisk et al. 1968). The principal lesions occur in the liver and they conclude it can be classified as toxic hepatitis. Similarly increases in the serum alkaline phosphatase were observed in the goats poisoned with D. madagascasiense and L. camara.

4.2.4.3. Effect of the poisonous plants on serum alanine aminotransferase

All the plants used except S. torvum in this study produced increases in the serum alanine aminotransferase activity (SGPT) in the rats. Such increases were dose dependent with only D.madagascasiense and T. terrestris, L. camara, and D.madagascasiense also produced elevated levels of SGPT in the goats.

SGPT is present in plasma and cells. Acute hepatic diseases causing membrane damage or cell necrosis result in appreciable increase in plasma activity of the enzyme. Since SGPT is one of the specific assayable liver enzymes, the elevated levels of SGPT

observed in the rats and goats poisoned by these plants used is an indication of the hepatic damage produced by the plants.

4.3 Effect of the poisonous plants on hematological parameters

Extraneous poisons often reveal their toxic effects on the blood circulating system by numerous petechiae or ecchymoses, the result of injury to the endothelium of capillaries some hemolyze the circulating erythrocytes; a few destroy the hematopoietic powers of the bone marrow or the spleen whereas other produce their effect by blocking vital enzyme systems, usually without any identifiable lesion (Smith, Jones and Hunt 1974, Beutler 1969).

Poisonous plants may affect the formed elements of the blood by their actions on the production in the bone marrow; by increasing their rate of peripheral destruction or hemolysis, or by influencing their distribution in various body portions. Anaemia can result from a lack of production of the various erythropoietic factors, inactivation of the factors (as by antibodies) or a failure of the marrow to respond to erythropoietin. (Swenson 1975) if the damage to bone marrow is severe enough, a decrease may be observed in the numbers of the major groups of formed elements (pancytopenia) (Harris and Kellermeyer 1970, Jain 1986).

4.3.1. The effect of the poisonous plants on erythron

In this study, T. terrestris, and L. camara were the only plants which produced a dose dependent decrease in the total RBC

counts, packed cell volume and haemoglobin concentration (Tables 6 and 12).

The same decreases in PCV and RBC were observed in the goats that were fed L. camara. D.madagascasiense did not affect the RBC count in goats. This means that L. camara and T. terrestris have adverse effect on the blood circulating system and can cause anaemia in animals that browse on them. The observation of anaemia in this study supports the work of seawright (1964) but is contrary to the work of Sharma, Makkar, Dawra and Negi (1982) who observed increases in the total RBC counts and packed cell volume. Sharma et al (1982) worked with guinea pigs whereas this work was in rats and goats (Seawright (1964), worked with sheep)and it may also lend credence to variations in terms of toxicity of the same plant at different areas or it may reflect some species variation in susceptibility to the effect of Lantana poisoning. However it is interesting to note that Sharma, Makkar, Pal and Negi (1981) in an earlier work observed that L.camara affects erythrocyte fragility. This means that L.camara consists of chemical substances which could cause hemolysis of blood cells. It is therefore acceptable to infer that the anaemia produced by Lantana in this study is hemolytic though hemorrhages could also contribute to it.Similar works (Seawright 1904, Sharma et al 1982) mention of observation of hemorrhages in Lantana poisoning.

The variation in the response of the RBC in animals to Lantana poisoning may also be related to environmental differences in poison accumulation in plants. Environmental conditions as earlier on reviewed plays significant role in the degree of toxicity produced by plants.

The observation of anaemia with T. terrestris supports the earlier work of Tonder, Basson and Va Rensburg (1972) and Amjadi, Ahonrai, Baharce (1977) who observed anaemia in clinical poisoning of T. terrestris in animals. Amjadi et al (1977) make mention of their observation of serious poisoning of a herd of cattle who fed on the plant and the experimental reproduction of the anaemia among other adverse clinical signs in sheep.

The clinical implications of the production of anaemia by those poisonous plants is quite obvious most especially in the system of livestock management in this country. With the trans humans in livestock management, the animals are constantly exposed to different plants which they are not used to and therefore are unable to differentiate between the poisonous ones and non poisonous. For example, in this study the locally acquired goats which were used in the experiment refused to eat the freshly harvested L. camara leaves mixed or unmixed with their normal pasture. They smelled the plants and moved away from it or extracted the normal pasture among the included Lantana. The Lantana leaves had to be aqueous extracted and administered to

produce the poisoning. The goats however readily ate the other freshly harvested plant. The plant is eaten by animals elsewhere (Amjadi et al 1977, Sharma et al 1982). The anaemia produced by T. terrestris and L. camara was microcytic and hypochromic due to their production of decreased mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (Tables 6 and 12) Literature examined have not mentioned the type of anaemia produced by T. terrestris.

Anaemia produced by poisonous plants generally can arise if for any reason, the rate of red cell destruction in the peripheral blood exceeds the normal rate of production. Such plant poison could have a direct hemolytic effect as occurs with plants containing saponin e.g. Agrostemma githago, Stellaria media, or vicin e.g. in plants as R. communnis (Geary 1950, and Bierer and Rhodes 1960) . Others infact may produce hemolysis in cell congenitally deficient in glucose - 6 - phosphate dehydrogenase e.g. Vicia fava (Bowman and Walker 1961) whereas hemorrhagic anaemia is reported in plants like P. aquilinum (Singh, Joshi, and Kumar 1987), Rao, Joshi and Kumar 1988).

E. uniflora and S. torvum in this study all increased the level of circulating erythrocytes (i.e. polycythemia). The mechanism by which these plants have produced their polycythemia is difficult to predict since non of them produced the typical

symptoms of diarrhea and vomiting observed in plants which produced such symptoms. For example among the clinical symptoms associated with Solanum tuberosum was a gastric type, which was characterized by salivation, vomiting, diarrhea and other sign of gastrointestinal irritation (Clarke and Clarke 1975). These are all symptoms which could produce fluid loss and this decrease blood volume. According to Smith et al (1974) in great majority of cases, an excessive number of erythrocytes noted in the blood cell count is purely relative; the total number of cells is not increased but the total volume of plasma is decreased. This is ordinarily the result of dehydration which is also produced by the fact that the animal patient is too weak to get to its source of water. Dullness, listlessness were some of the symptoms observed in he rats that were fed with E. uniflora which could imply that these animals were so weak from intoxication, to be unable to get to their drinking water which could produce dehydration and hemoconcentration. It is however, significant to note the hypoalbuminaemia produced by E. uniflora. Since albumin is reported to be important in the maintenance of colloid osmotic pressure of the blood plasma (Kaneko 1980) the clinical implication of reduced serum albumin level is obvious. The regulation of the distribution of water between the intravascular and interstitial tissue compartments are affected. A marked shift of intravascular water into the interstitial tissues in the E. uniflora poisoned

animals could therefore reduce the plasma volume and therefore the polycythemia that was noted.

L. leucocephala and D. madagascasiense did not produce any significant changes in the erythron (Table 8, 14,). The inconsistent changes in the total RBC of the rat produced by D. madagascasiense may be due to abnormal responses of some of the individual rats within group since such inconsistencies were not observed in the goats which were feeding on the fresh leaves of D. madagascasiense.

All the plants did not affect the blood coagulation time in the rats or in the goats. Plant poisoning generally influence blood coagulation by causing severe liver damage e.g. H. europauem or aflatoxicosis (Doerr et al 1976, Bull et al 1961) Aflatoxicosis causes loss of two of the active sites of tissue thromboplastin and reduces the level of prothrombin and fibrinogen which are all required for the coagulation process (Doerr et al 1976). Plants may produce hypocalcaemia which removes the calcium required for the coagulation process e.g. H. glomeratus (Shupe and James 1969 or A. retroflexus (Buck et al 1966, Marshall et al 1967). Coagulopathies are also observed with plants which cause platelet disorders e.g. P. aquilinum (Rao et al 1988) or vitamin K deficiency and antagonism as observed with. M. alba poisoning (Prier and Derse 1962). The non observation of coagulation defects with the plants in this study may imply that they do not produce the effects

enumerated above. This observation may not be conclusive since chronic feeding of the plants were not carried out. Chronic feeding of the poisonous plants could produce cirrhosis which will affect blood coagulation by virtue of lowered coagulation factor synthesis in the liver.

4.3.2 Effects of the poison plants on leucotron in rats and goats

Toxic plants are not reported to produce a direct effect on the white blood cells, neutrophils, lymphocytes eosinophils, monocytes, or basophils. However plant substances which produce toxic aplastic anaemia are known to also produce hypoplasia of the bone marrow and the depression of the formation of granuloc- cytic leukocytes leading to a more or less severe agronulocytosis (Smith et 1974). For example P. aquilinum is reported to cause agranulocytosis because of its toxic action on the bone marrow (Rao et al 1988, Singh 1987) The plant is reported to have an aplastic anaemia factor.

In this study, S. torvum, E. uniflora, D.madagascasiense, L. leucocephala did not affect the level of WBC in the rats and goats. L. camara produced decreases in total WBC with some of the doses of the extract administered to the rats though such changes were inconsistent (Table 12; For example the lower doses of L. camara (200 and 400 mg/kg) produced changes which were significant when compared with untreated controls whereas such dose dependent decrease was not observable with the highest dose (600mg/kg) of L.

camara. Moreover there were no decreases observed within the goats which fed on the leaves of L. camara. Seawright (1965) observed no changes in the level of leukocytes in ruminant and guinea pig poisoned with L. camara although Sharma et al (1982) reported increases in the total WBC counts.

In the contrary T. terrestris produced dose dependent changes in the total WBC and neutrophil counts in this study. Other authors (Brown 1968, Tonder et al 1972) did not mention the effect of T. terrestris on leukocytes though the plant is reported to produce photosensitization, anaemia, liver and kidney lesions. The mechanism of reduction in leucocyte counts in this study therefore is difficult to predict. The need for experimentation in other animals may therefore be a pertinent step in elucidating the leucocyte level changes associated with feeding of T. terrestris in rats.

4.4. Clinical signs, gross and histopathologic effects of poisonous plants

The effects of poisons which are most often looked for, in plant toxicology in animals are their gross and histologic actions on the tissues. The lesions of poisoning are rarely characteristics; nevertheless, the finding at autopsy can provide definite clues to the nature of the poison. (Smith, et al 1974).

The poisonous plants cause an array of different symptoms since often, the effect of the poison is no more than one body system. Clinical evidence is reported to be only limited value. There are only nine systems of organs in the body that are capable of being affected, so the permutations and combinations of clinical signs are extremely limited. In addition there is extraordinary variability shown by different individuals in the symptoms caused by the same poison and not every symptom is known to appear on each occasion (Zook and Gilmore 1967).

The absence of lesions may be as important as their presence as it serves to exclude various toxic agents. The skin and visible mucous membranes may have a characteristic discoloration. Jaundice is a frequent sign of hepatic damage which has been noted in various plant poisoning such as Aspergilus flavus, Heliotropium europaeum (Smith et al 1974), A cherry red or pink color is seen in cyanogenetic plant poisoning (Van der Watt 1944). Methaemoglobinaemia due to nitrates and nitrites impart a brown coloration.

4.4.1. Gross and histologic lesions associated with L.leucocephala

In these studies no clinical symptoms were observed in the rats fed extracts of L. leucocephala. This contrasts with the findings of other authors who reported of hyperactivity in cattle (Falvey 1976 emaciation and drooling of saliva (Jones and Hegarty 1984) Severe bilateral oedematous palpebral conjunctivitis in cattle

(Akpokodje and Otesile 1987). Most of the other authors consulted gave different symptoms connected with L. leucocephala intoxication, These variations have been associated with respects to the rate of consumption and environmental factors related to the plant. (Jones and Hegarty 1984).

Apart from the clinical signs that were reported, several authors did not describe post mortem or histologic lesions. The congestion of the kidney and the paleness of the liver of rats which were fed extracts of L. leucocephala was indicative of pathologic changes in these organs. Akpokodje and Otesile (1987) reported of swelling of the face with oedema of cattle that consumed L. leucocephala. The perivascular lymphocytic cuffs that were observed around the central veins and portal vessels in some of the rat livers may be associated with a secondary infection probably resulting from the effect of the plant extract. However the degenerative and necrotic changes characterized by cellular swelling and clumping of the cardiac tissue could be associated with the effect of L. leucocephala. This compares with the swelling of muscle fibers observed with the poisoning of Cassia occidentalis (Henson et al 1965) In contrast however, the cardio myodegeneration observed with C. occidentalis intoxication also involved the skeletal muscle (O'Hara et al 1969).

4.4.2 Gross and histologic changes associated with S. torvum

The Solanaceae, the family of plants to which.S. torvum

belongs consist of several genera associated with poisoning due to the nature of the active principles they contain. All parts of these plants especially S. dulcamara and S. nigrum are reported dangerous (Clarke and Clarke 1975, Woodward 1980) No clinical signs were produced in the rats that were fed extracts of S. torvum though symptoms were observed in experimental animals fed other species of Solanum. Pienaar et al (1976) in their experimental studies with Solanum in cattle reported of loss of balance with transient epileptiform seizures, with these symptoms being precipitated by exercise, handling or fright. Contrary to these observations, other authors reported of depression, apathy,narcosis and paralysis in animals poisoned with Solanum species (Andrade 1960, Buck, Dollahite and Allen 1960).

The vasculitis with moderate perivascular lymphocytic and macrophage infiltration was not a common occurrence in all the experimental rats and may thus not be associated with the toxic effect of S. torvum.

The non observation of central nervous system effects in these studies which is associated with the Solanines (Pienaar et al 1976, Andrade,1960) might be related to the level of Solanine in the plant. For example the alkaloid content and hence the toxicity of S. nigrum is reported to varies enormously with soil, climate and season. Clarke and Clarke 91975) reports that in some areas S. nigrum, is reported to be harmless and deadly

in others. Therefore the absence of a serious organic damage produced by the extract of S. torvum may be due to the low concentration of Solanine in the variety around Nigeria or that the content of Solanine was low at the season at which the plants were collected for extraction.

4.4.3 Lesions associated with E. uniflora

E. uniflora represents one of the poisonous plants for which there is less literature as regard its toxicity. Its toxic effect is corroborated by local farmers. However the gross and histologic lesions of congestion of the kidney and moderate fatty change is indicative of its less severe toxic effect. The restriction of lesions to the kidney and liver may be due to the role there two organs play in the excretion and metabolism of the poisons from these plants. The plant is reported to have a high tannin level (Irvine 1961) The fact that it produced lesions on Subacute consumption means the plant could adversely affect livestock production when they are continuously exposed to it.

4.4.4. Gross and histologic lesions due to T. Terrestris

Tribulus terrestris represents an important fodder plant in some parts of the world (Clarke and Clarke 1975). The depression and disinclination of the animals treated with T. terrestris to feed was an indication of the effect of the plant.This was shown by the post mortem lesions observed on the liver and kidney of experimental rats T. terrestris is reported to cause

photosensitization in sheep (Brown 1968, Tonder et al 1972). However photosensitization was not observed in the rats used in this studies. The absence of such a symptom in these rats may not be unconnected with the fact that the rats were not exposed to direct sunlight. Furthermore the degree of hepatotoxicity might have not been so severe as to allow the accumulation of the photodynamic substance phyloerythrin which is what is responsible for the development of photosensitivity in animals poisoned by such plants.

The hemorrhages observed on the liver and kidney indicates that T. terrestris causes damages to the circulatory system. The absence of histologic lesions in the liver from the rats treated with T. terrestris may be associated with the level of the active agent in the plant. Since it is reported that wilting seriously affects the degree of poisoning from this plant (Tonder et al 1972). The leaves used in this study were dried leaves. The lesions observed in the kidney may be due to the concentrating ability of the kidney so that the relatively reduced level of the active substance could be concentrated in the kidney to produce the lesions.

4.4.5. Lesions associated with Lantana camara

The ecchymotic hemorrhages observed in the heart kidney and liver indicates the damage due to L. camara to the blood circulating system (Smith et al 1974) However hemorrhages were not

observed in the tissues of the goats which fed on the plant. This observation may be due to the species or to genetic variation in response to L. camara toxicity that has been reported (Seawright 1965). The severity of the lesions are also reported to depend on such factors as starvation and dehydration (Seawright and Allen 1972).

The fatty degeneration of the liver observed in both the rats and goats supports the observation of other authors (Ahuja and Skewes 1971), Brown 1968, Sharma et al 1987). However the photosensitization which was reported in some animals poisoned with L. camara (Aluja and Skewes 1971 and Seawright and Allen 1972) was not deserved in the rats and goats used in these studies. Though the rats were not exposed to sunlight the goats grazed normally outside in sunshine after they were exposed to the plants. The non observation of photosensitivity reactions again may be associated with such factors as nutritional status and the degree of dehydration of the animals (Seawright and Allen 1972). Furthermore it may be due to the degree of hepatic damage and/or the level of the circulating photodynamic agent in the experimental rats and goats.

4.5 GENERAL DISCUSSIONS AND CONCLUSIONS

4.5.1 The problem of poisonous plants

The word problems according to Kingsbury (1979) is anthropocentric and mainly implies a human point of view. From that vintage

point, the problem of poisonous plants is not their direct toxicity to man and animal alone but also the inconvenie- nce and economic loss associated with the poisoning of domestic animals and the cost of preventing or reducing such happenings. It is worthy of note that plant poisons can either be accumulated in the animal or in certain organs or they are metabolized and excreted with milk (Liener 1969) By this food chain, toxins or metabolites thereof may become harmful to man (Habermehl 1987),

4.5.2 The toxic effects of Solanum torvum

Most of the cultivated members of the family Solanaceae to which S. torvum belongs are reported to be poisonous (Clarke and Clarke 1975) The leaves of S. torvum analyzed contained levels of phytin which was high enough to produce inhibition of essential minerals such as zinc and iron required for the nutrition of animals. The level of the nitrate and nitrite could constitute a source of methemoglobin formation if animals are exposed to large quantities of the plant alone. However the ethanolic extraction of the leaves which was performed to concentrate the toxic constituents of the plant did not cause expected symptom probably because the level of the toxic factor was low in the leaves or the extraction procedure did not achieve the expected outcome. Except for the histologic lesions in the lungs which could not be associated with the effect of the plants, no gross or histologic lesions were observed in the experimental animals dosed with the

leaf extract. The absence of gross or histologic lesions is reflected in the serum biochemical changes produced in the rats. The plant only produced dose dependent increase in the serum activity of alkaline phosphatase. ALP is used in characterizing bone and hepatic disorders (Kaneko 1980). Since it is a non liver specific enzyme it becomes difficult therefore to directly associate its increase with hepatic damage moreso when the more liver specific enzymes like alanine aminotransferase did not show increases in activity.

The Solanaceae family include members with active alkaloids related to atropine solanine, nicotine which are mostly substances which affect the central nervous system. Pienaar et al 1976, Gilman et al 1980) A subgroup however are reported to contain a compound related to 1, 25- dihydroxycholecalciferol which is responsible for the calcinosis which are reported in many animals which were maintained on for example S. malacoxylon and Cestrum diurnum (Samson et al 1971, Krook et al 1975, Peterlik et al 1976)

It is preposterous to conclude that the species of S. torvum used in this study is not poisonous because the glycoalkaloids which are reported to be responsible for the toxic nature of this group is known to be concentrated especially in the berries (Woodward 1985) The observation of less toxic effects in this study might be due to the low level of the glycoalkaloids in the leaves or environmentally related factors. (Clarke and Clarke 1975).

4.5.3. The toxic effects of Lantana camara.

The toxic nature of L. camara is widely reported and the spread of the plant into pastures, forest areas and gardens are also reported (Sharma et al 1971, Sharma et al 1981, Achhireddly and Singh 1984). However since the degree of toxicity is environmentally related and there are no experimental evidence of toxicity in Nigeria regarding this plant it was auspicious to find out the toxic nature of the plant in Nigeria.

The L. camara used in this study was found to contain relatively high levels of nitrate and nitrite. However since it is reported that it is difficult to make a categorical statement as to the toxic dose of nitrate as it depends on the rate at which the substance is consumed, it is hasty to say that the effects observed were due to these substances. The production of dose dependent decrease in the serum total protein in both the rats and goats is a reflection of the toxic damage to the liver at the cellular level by the plant. This observation is further augmented with the dose dependent increases in the activities of the serum enzymes that were measured mainly alanine aminotrasfe- rase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). ALT among other enzymes is reported to be one of the excellent markers of hepatocellular damage (Kaneko 1980). With the additional increase in the serum activities of ALP and AST which though are not liver

specific enzyme, it is appropriate to confirm the production of hepatocellular damage by L. camara.

This observation is supported by the gross and histologic lesions that were observed in the liver of the rats and goats that were used in this study.

The level of toxic inorganic substance assayed in this plant were not very high so that they cannot be associated with the damages observed in the tissues of the experimental animals. A substance known as lantadene A, has been connected with the damage which is usually observed in the animals which consumed L. camara (Sharma et al 1980 Alfonso et al 1982, Sharma et al 1988). Using the guinea pig, Sharma et al (1982) described Lantana toxicity as an acute occurrence resulting in the death of the animal. It was mentioned that the plant produces intrahepatic cholestasis associated with dispersal and fragmentation of endoplasmic reticulum. Furthermore, Sharma et al (1982) also observed that Lantana toxicity caused obstructive jaundice, photo sensitization and a rise in serum bilirubin. In this study no observation was made of the hypertrophy of the hepatocytes nor intrahepatic cholestasis. The level of the total bilirubin was seen to rise in the goats used in the experiment though jaundice was not noticed. Photosensitization in the goats was also not noticed though the goats were allowed free access to the sunshine after been fed their ration of the leaves of L. camara. However since the level of

conjugated bilirubin increased in the treated goats it could not be ruled out that the animals might develop jaundice if the feeding of the Lantana leaves had continued beyond the twenty one days in the goats.

The Lesions produced by L. camara in other organs of the animal were non consistent. This is because apart from the pale kidneys that were observed in the goats used in this study and have also been mentioned with other animals (Sharma et al 1980, Alfonso et al 1982, Achhireddy et al 1985, Sharma et al 1988) lesions in the gastrointestinal tract, spleen, lungs, heart were not a constant finding in the animals poisoned by L. camara.

The variations observed in Lantana toxicity in goats and rats as compared with the observations in other countries may be connected with environmental or climatic factors reported to influence the toxicity of plants from one area to another. Similarly such variations could be related to the season at which animals were exposed to Lantana camara.

4.5.4. Toxic effects of Leucaena leucocephala

A considerable variation has been mentioned about the toxicity of L. leucocephala in different parts of the world (Falvey 1976) Mullenax 1963). In this study, the level of phytin and cyanogenetic glycoside analysed from the leaves of L. leucocephala used, were high enough to be mentioned as one of the causes of poisoning by this plant. Since the hydrocyanic acid

present in form of cyanogenetic glycoside determines the toxic nature of this compound (Vander Watt 1974) it means the nature of the HCN from the plant will have to be verified inorder to ascertain its role in influencing L. leucocephala toxicity.

L. leucocephala in this study, produced decreased level of the blood total protein which was reflected as a decrease in blood albumin. This means the plant is capable of damage to the liver. This observation is further buttressed with the increased level of the blood area nitrogen. However the effect on the liver may be mild since the serum enzymes which were assayed to determine tissue damage in this work, did not show increased activity. It was only the 200 mg/kg dose of the extract which produced an increase in the serum activity of alkaline phosphatase. Since ALP is non liver specific enzyme, the leakage of this enzyme could have taken place from any tissue in the body of the rats (Kaneko 1980).

The gross and histologic lesions that were observed further confirms the fact that L. leucocephala produces mild toxicity. The swollen liver with the perivascular lymphocytic cuffs around the central veins and portal vessel which is suspected to be associated with a secondary infection could account for the decreased blood total protein and albumin which were earlier mentioned. Contrary to these observations Jones and Hegarthy (1984) observed emacipation drooling of Saliva and vomiting in animals that were fed L.leucocephala. Jones (1977) had earlier mentioned the

observation of enlarged thyroid and death of offspring in cattle that were exposed to L. leucocephala.

A relatively common observation in animals that were poisoned with L. leucocephala was the loss of hair which did not occur all over the body. Akpokodje and Otesile (1987) noticed loss of hair from only the tail switch and swollen face. Hill (1971) reported of hair loss in a young women who consumed the seeds of the plant. The experimental rats used in this study did not show hair loss from any part of the body. The important chemical component of L. leucocephala which has been incriminated as the caused of hair loss is mimosine (Jones 1979, Jones and Hegarthy 1984). Since L. leucocephala has been chosen as an important plant in alley forming as is presently practiced at the International Institute for Tropical Agriculture, Ibadan, (Akpokodje and Otesile 1987) it is very important that the true toxic nature of L. leucocephala in this country be thoroughly ascertained. This is necessary because variations in the effect of the plant have been noticed from different areas (Jones 1984). Furthermore the level of mimosine needs, to be evaluated since its excretion in cattle fed with the plant could be consumed to produce the typical hair less observed with it (Hill 1971).

The observation of an increase in total white blood cell count which was reflected as an increase in lymphocyte count could either be the result of secondary infection or that the

plant contain lectins which act as lymphocyte mitogens (McPherson 1979). If the plant acts as a lymphocyte mitogen, then it may be important in maintaining the immune status of the animals fed on the plant most especially during challenge by pathogens.

4.5.5. Toxic effects of Tribulus terrestris

The toxic nature of T. terrestris in animals has long been documented in other parts of the world most especially in South Africa where it is given various types of names, though it is doubtful whether its the plant which solely produces the associated symptoms or that other factors are equally involved (Clarke and Clarke 1975, Amjadi et al 1977). Since it is an introduced plant and it is very widespread in Nigeria, the toxic nature had to be evaluated in our local livestock.

T. terrestris harvested for this study was observed to contain the highest levels of nitrate, nitrite and phytin. This means that these in organic constituents could contribute to the symptoms of poisoning in animals most especially during the dry season when the water content of the plant is low and the plant tissues are able to concentrate more of the secondary chemical constituents coupled with poor feeding or fasting animals (Winter and Hokanson 1964).

T. terrestris was the only plant among the other poisonous plants studied which produced clinical symptoms in the experimental animals. The hemorrhages which were produced in the liver and

kidneys of these animals, is an indication of the toxic injury to the capillary endothelium or a disorder in the blood clothing mechanism of the animals (Smith et al 1974, Jain 1980). However since the plant did not produce significant changes in the blood coagulation time of the animals it is doubtful whether the cause of the hemorrhages was due to effect on the coagulation mechanism. The implication of the hemorrhages produced by T. terrestris is that prolonged exposure to this plant will result in hemorrhagic anaemia. The reduced total red cell count encountered in the rats dosed with the extract of T.terrestris could therefore have a link with the hemorrhages produced.

The plant also showed liver damage in the rats by producing a decreased blood total protein as well as increases in the activities of the liver enzymes that were measured mainly alanine aminotransferase and aspartate aminotransferase. The damage to the kidney could be responsible for the increase in blood area nitrogen. The photophobia, jaundice, swelling and acute dermatitis that have been associated with poisoning by this plant (Amjadi et al 1977) were not observed in this study. Since photophobia, jaundice and dermatitis may be the aftermath of liver damage by the plant it could be that these observations were not made in this study because the degree of liver damage was minimal due to the poor level of the poison in the plant or that the length of exposure to the plant was short.

4.5.6. Toxic effect of Dichapetalum madagascasiense

Various authors have reported about the toxic effects of some of the species of members of the family challetiaceae to which D. madagascasiense belongs (Irvine 1961, Watt, Breyer Brandwijk 1962, Van Dijk et al 1972, Vickery and Vickery, 1971, Nwude et al 1977. However D. madagascasiense in this study has produced very minimal toxic effects.

The level of the inorganic substances analysed in the leaves of D. madagascasiense were relatively low and can therefore not be associated with the poisoning by the plant. This means species of Dichapetalum contain other factors which could be responsible for the effects. The substance which was mentioned as possible cause of toxicity by other members of the family was fluoroacet- ate (Van Dijk et al 1972, Nwude et al 1977). However a prolonged exposure of animals to the plant could increase the amount of phytin which gets into the gastrointestinal tract inorder for some antinutritional effects to be produced.

D. madagascasiense did not affect the blood cellular components of the rats and goats. This is because the plant did not produce any changes in the level of the total RBC, WBC and other hematological parameters. This means the plant in this study did not have any direct effect on the blood cellular elements or the hemopoietic tissue.

Though no gross lesions were observed in the rats and

goats used, the histologic lesion seen in the rat tissues but not observed in the goats is an indication of the mild toxic nature of D. madagascasiense. The plant was however observed to increase the total blood protein. It also increased the serum activities of the enzymes alanine aminotransferase and aspartate aminotransferase in rats and goats. These are indications of the toxic effect of the plant at the subcellular level (Kaneko 1980). It is therefore possible that the plant can produce gross and histologic lesions on chronic exposure in goats. Alternatively it could be that D. madagascasiense does not have enough fluoro fatty acids which is alluded to undergo oxidation into the fluoroacetic acid which is responsible for the toxic effects of these plants (Vickery and Vickery 1971). Variations in the degree of toxicity of fluoroacetate has been reported (Watt and Breyer Brandwijk 1962).

The palatable nature of the leaves of members of the plants in this family has been reported (Van Dijk et al 1972) This observation was noted by the voracious nature by which the goats which were given the leaves of D.madagascasiense consumed them whenever they were fed.

4.5.7. Toxic effect of Eugenia uniflora

E. uniflora is quite a widespread plant in Southern Nigeria and it is quite a common occurrence to see groups of animals browsing on the plant. This means that the plant is potentially dangerous and the type of symptoms caused by it have to be

determined. In this study, the plant did not produce gross lesions in the tissues of the rats dosed with the extract of the plant though histology showed moderate fatty change in the liver. Except for the level of phytin which was relatively high, and could be antinutritional, the level of nitrate, nitrate cyanide or oxalate analysed from E. uniflora were very low. Toxicity produced by this plant could therefore be associated with other secondary chemical factors in the plant.

On the hematology of the rats, E. uniflora produced increases in the packed cell volume haemoglobin concentration and total RBC levels which could be an evidence of the increased requirement for oxygen by the animals due to the effect of the plant on cellular respiration (Swenson 1975).

The increases in the serum activities of AST and ALT can be associated with the fatty change observed in the liver of he rats fed on the extracts of E. uniflora.

CLINICAL SYMPTOMS AND SUGGESTED TREATMENTS OF POISONING IN ANIMALS

Clinical symptoms of poisoning by poisonous plants, are hardly observed in the affected animals (Clarke and Clarke, 1975). This is because the animals do not consume enough quantity, or that by the time they are observed they are dead or comatose.

Acute toxicity studies in rats using extracts of D. madagascasiense, S. torvum and E. uniflora produced irritability,

excitement and tremor until the animal went recumbent and then died 2 - 5 days after administration of the extract.

Tribulus terrestris, L. camara and L. leucocephala produced anarexia, somnolence with evidence of pain with the animals dying between 6 - 11 days.

Treatment of plant poisoning is done symptomatically. Prevention of further absorption can be done using stomach gavage and if the active component of the plant is known a specific antidote can be used.

The excitement and tremor produced by D. madagascasiense, S. torvum, and E. uniflora can be treated with pentobarbitone (10 to 30mg/kg) given intravenously. The somnolence produced by L. camara, L. leucocephala and T. terrestris can be treated with amphetamine or methylamphetamine (0.25mg/kg, subcutaneously).

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