"Pathological Studies On The Toxicity of
Camara camara Linn var. aculeata
In Sheep".

By
G. Sri Ranga Reddy,

November, 1964
“Pathological Studies On The Toxicity of Lantana camara Linn var. aculeata In Sheep”.

A Thesis
Submitted to the Faculty of Veterinary Science,
Magadh University,
In Partial fulfilment of the Requirements For the Degree of Master of Science (Veterinary)

By
G. Sri Ranga Reddy,
1964
I certify that this Thesis has been prepared under my supervision by C. SRI RANGA REDDY, a candidate for the M.Sc. (Vet.) with Pathology as major subject, 1964, and that it incorporates the results of his independent study.

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D/19 Novr. '64.
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ACKNOWLEDGMENTS.

The author gratefully acknowledges his indebtedness to Sri P.B. Kuppuswamy, B.A., G.M.V.C., B.V.Sc., P.C. (New Zealand), M.S. (Missouri), Professor & Head of the Department of Pathology and Bacteriology, for suggesting the problem, constant encouragement, helpful advice and guidance.

I am also thankful to Sri S.A. Ahmad, B.Sc. (Ag.), M.R.C.V.S., F.C. (Denmark), Principal, Bihar Veterinary College, Patna, for the facilities provided for carrying out the experiment.

My thanks are also due to Sri S.K. Mukherjee, G.B.V.C., M.S., Assistant Research Officer, Yellow Disease, I.R.S., Patna, for his suggestions in preparing the extracts used in the experiment.

I am also thankful to Sri R. Bhusan, College Artist, for his help in the photographs.

... AUTHOR.
Chapter (1)

INTRODUCTION.

Wide variations in climatic, meteorological and topographical conditions prevailing due to the vastness of the country, make India the repository of perhaps the most varied and luxuriant flora growing anywhere on the surface of the earth. All types of exotic plants can be grown without difficulty in one part or the other of India. Our country abounds in all kinds of good plants of spices, perfumes, timbers, gums and plants containing powerful active principles having medicinal properties. But there are also plants classified as "poisonous plants" which are of great value but are dangerous to livestock.

Mortalities in livestock can be attributed to a number of causes, but there is probably nothing more annoying and puzzling to stock owners, than to experience losses as a result of stock feeding upon plants which possess poisonous properties. A poisonous plant is a plant, which when consumed in such quantities as will
be taken by animals or men over short or prolonged periods, exerts harmful effects on the system, or causes death by virtue of toxic substances normally contained in that plant (Steyn, 1934). There is no doubt that occurrence of harmful herbage in grass lands and other localities open to grazing causes death by poisoning in many domestic animals.

Animals of the herbivora class depend upon plants for their sustenance, growth and development and it is a peculiar paradox of Nature, that some plants should possess harmful properties when consumed. Unfortunately, there are no statistics available which would show the extent of the animal losses suffered by the livestock industries owing to poisoning. These losses are undoubtedly very considerable and very care should be taken to reduce such losses to a minimum, particularly as in most of all cases, they are due to carelessness and lack of minimum protective measures.

It is quite true that animals instinctively avoid toxic plants and that all classes of livestock are not equally susceptible, but it is also true that the animals acquire a depraved appetite for harmful plants, especially when fodder supply is scarce, a condition not uncommon in our country. Clippings
and trimmings from gardens and shrubberies, have proved a more or less common cause of livestock poisoning, such material being too often carelessly thrown out for animals to pick over. The habit of overgrazing which is commonly practised in different parts of the country causes the animals to eat unwholesome and harmful plants due to limited quantities of palatable fodder available. By far the greater proportion of mortalities in stock due to plant poisoning, occur in moving stock due to the fact, that they do not have the selection of plant life to eat on unfamiliar surroundings. Moving stock are usually hungry and when they encounter a lush growth of a grass, they graze it ravenously with fatal results. Animals which have become familiar with the plant by long association do not usually eat it and therefore are seldom affected. Poisoning of livestock is frequently set with in early rainy season when grass is just coming up or in late summer when it is dried up or in seasons of drought.

According to Chopra et. al. (1949), there are about 40 species of the genus Lantana (R.O. Verbenaceae), tropical or subtropical. Among these, the species usually responsible for livestock poisoning is Lantana camara L. var. acumina L. It is an exotic photodynamic plant, which has been introduced into India by an
Australian who planted it as a hedge shrub (Pillai et al. 1930). At present, *Lantana camara* L is a very common weed growing all over waste lands, pastures and village surroundings throughout India. Often cases of poisoning in livestock are attributed to eating of this plant. The condition is responsible for loss due to mortality, abortions, loss of milk production in dairy cows and chronic wasting in beef cattle. A very high proportion of clinically affected animals dies. So it was thought that a thorough study of toxicity of *L. camara* L in animals should be made and, with this object in view, the present work namely "Pathological studies on the toxicity of *Lantana camara* L in sheep" was undertaken, so that proper diagnosis of the condition could be made and suitable control measures evolved.
Chapter (2)

REVIEW OF LITERATURE ON LANTANA POISONING.

GENERAL

Cattle are most commonly affected by lantana poisoning, but sheep and goats are equally susceptible (Seawright, 1963). Goats were reported developing icterus, but rarely photosensitization in suspected lantana poisoning and a buffalo was also found showing icterus with skin lesions (I.C.A.R. Technical Programme of Yellow Disease Scheme, 1964-65). Lese (1927) noticed several cases of fatal poisoning in camels, attributable to lantana. But natural lantana poisoning of domestic animals other than cattle, sheep and goats is not well substantiated, and it seems unlikely that it occurs.

Tucker (1910) and Pound (1913) established the toxicity of the Lantana camara L by carrying out feeding trials. Further feeding trials and reports confirming the toxic nature of the plant were done by Seddon et. al. (1927-28 and 1929), McIntosh and White (1935), Legg (1941), Knott (1955), Bull (1961) and Hall (1964) in Australia, by Sanders (1946), Walter and Muenscher (1951) and Case (1957) in America, by Steyn et. al. (1941) in South Africa,
and by Pande (1934-39), Kehar (1936-39), Kehar et al. (1960), Lal and Kalra (1960), Prasad et al. (1961), Negi et al. (1961), Sharma (1961), Agarwal et al. (1962), Sastry et al. (1963) and Sastry and Mahadevan (1963) in India.

Isolation of the toxic principle of Lantana camara L was reported by Louw (1943 and 1948). He isolated two crystalline triterpenoid substances from alcoholic extract of leaves which he named Lantadene A and Lantadene B. The former was toxic when fed to sheep in a dose of 2 grams per os, but the latter was not. Besides the Lantadene A and Lantadene B, Eigan et al. (1957) reported the presence of another crystalline product of lactonic nature provisionally designated as "lancamarenene" which is a fish poison. Barton et al. (1954c and 1956) made chemical studies on extracts of Lantana camara L and demonstrated the chemical structures of Lantadene A and Lantadene B. (See page 7).

Negi et al. (loc cit) and Sastry and Mahadevan (loc cit) could not prepare the silage from plant material as processing did not reduce the toxicity of the plant. Sanders (loc cit) and Kehar et al. (loc cit) observed the cattle developing the typical symptoms of lantana poisoning following ingestion of 12-16 ounces of lantana leaves.
Lantadene A.

Lantadene B.

Sastri et al. (loc cit) by carrying out feeding trials with different extracts of the leaves of the plant, evaluated the fraction responsible for producing toxic condition. They could not produce the poisonous condition with aqueous extract, essential oil, aqueous fraction of the alcoholic extract, recinuous fraction, colouring matter and with lantadenes suspended in water. They induced the toxic
condition by administration of alcoholic extract, chloroform soluble fraction of alcoholic extract and with lantadenes as alcoholic solution. They concluded that the toxic principles in Indian species of the plant are identical with those of South African species. They also evaluated the lethal dose of total lantadenes and the effect of chronic toxicity in albino rats. For Heikel et al. (1960) studied the aspects of liver dysfunction in the rabbit following the administration of lantadene A by intraperitoneal route.

Lantana poisoning in cattle has been listed as one of the important diseases of livestock in Fiji, according to the report for the year 1932 of the Chief Veterinary Officer of that country.

Clare (1952) in his admirable monograph on photosensitisation in diseases of domestic animals, listed L. camara as one of the plants causing hepatogenous photosensitisation following ingestion.

**CLINICAL SYMPTOMS:** The spectacular aspect of lantana intoxication is a disturbance of liver function which gives rise to hepatogenous photosensitisation and jaundice, the so-called pink nose of cattle and sheep.
Pande (loc. cit.), Kehar (loc. cit.), Kehar et al. (loc. cit.), Steyn et al. (loc. cit.), Lown (loc. cit.), Sanders (loc. cit.), Knott (loc. cit.), Sharma (loc. cit.), Agarwala et al. (loc. cit.), Negi et al. (loc. cit.), Sastry et al. (loc. cit.) and Hall (loc. cit.) recorded clinical symptoms due to lantana poisoning in cattle and sheep. There was loss of appetite, constipation, icteric condition of visible mucous membranes and photosensitization. The photosensitization was characterized by diffuse oedematous swelling of the muzzle and ears, thickening, cracking and sloughing of the skin of the different parts of the body, loss of hair, lachrymation and total blindness. But according to Seawright (loc. cit.), lesions of photosensitization in sheep were slight and consisted only of a slight transient conjunctivitis and excoriation of the hairless skin of the muzzle.

Lal and Kaira (loc. cit.) and Sastry et al. (loc. cit.) recorded salivation, subnormal temperature, weakness, staggering gait and dropping of head. Sanders (loc. cit.) noticed severe gastrointestinal disturbances accompanied by intestinal hemorrhages.

Kehar (loc. cit.) and Sharma (loc. cit.) noted photophobia and rhinorrhea. McIntosh and White (loc. cit.) and Knott (loc. cit.) recorded decrease in milk yield in lactating cows.
MACROSCOPIC PATHOLOGY

Pande (loc. cit), McIntosh and White (loc. cit), Kohar (loc. cit), Kohar et al. (loc. cit), Sanders (loc. cit), Sharma (loc. cit), Sastry et al. (loc. cit) recorded thickening, cracking and sloughing of the skin of the different parts of the body.

Kohar (loc. cit), Steyn et al. (loc. cit), Sanders (loc. cit), Sharma (loc. cit), Sastry et al. (loc. cit), Hall (loc. cit) observed yellow colouration of all internal organs. There was congestion and enlargement of liver. It was friable. Kidneys were congested and swollen.

Steyn et al. (loc. cit) recorded hydrothorax, hydroperitonium, subpleural and subepicardial haemorrhages.

Sharma (loc. cit) recorded inanition, cachexia and dehydration of carcass. The skeletal muscles were reported flabby, moist and seldom gelatinous and the intermuscular adipose tissue was considerably reduced. The lungs were congested. The portal and mesenteric lymph glands were enlarged and congested. The spleen was hyperaemic. Myocardium was slightly flabby and presented cocked appearance.
II.

Pande (loc. cit), Sharma (loc. cit). Seawright (loc. cit) noted distension of gall bladder. Seawright (loc. cit) noted paralysis of intestinal musculature and atony of alimentary tract and impaction of faeces in the colon. Knott (loc. cit) recorded congestion of gall bladder lining and gastro-enteritis.

HISTOPATHOLOGY:

Pande (loc. cit), Sharma (loc. cit) recorded toxic hepatitis in lantana poisoning. Sharma (loc. cit), Seawright (loc. cit) noted necrosis of central zonal parenchymal cells. Gastry et. al. (loc. cit) recorded diffuse areas of necrosis and fatty changes in liver. Sharma (loc. cit), Prasad et. al. (loc. cit), Seawright (loc. cit) observed accumulation of bile pigment in parenchymal cells. Sharma (loc. cit) noted intense congestion of central veins, disorganisation of structure of hepatic cords around the central veins, dissociation of hepatic cells. Hepatic cells were swollen and the cytoplasm was highly granular and eosinophilic. Sinusoids were distended and erythrodiapedesis into spaces of Disse was prominent. There was significant increase in the periportal connective tissue and the
proliferation of the bile ductules. In the portal triads, the bile ducts and blood vessels showed loss of structure and degenerative changes in the connective tissue in their walls.

Seawright (loc. cit) recorded inflammation and thickening of gall bladder wall.

Pande (loc. cit) recorded nephritis.

Prasad et al. (loc. cit) and Seawright (loc. cit) noted the presence of casts and dilation of tubules. Sharma (loc. cit) recorded increased cellularity due to endothelial hyperplasia and marked disorganization of glomeruli. In cortex, distal convoluted tubules and loop of Henle showed retrograde changes. In medulla, the straight tubules revealed gross dilation and compression of the tubular epithelium. The lumen of the tubules showed bilirubin pigmentation and the intertubular blood vessels were severely congested.

Sharma (loc. cit) recorded capillary congestion, granular degeneration and fragmentation of myocardial fibres. In spleen, the pulp was markedly hyperaemic and stromal cells were loaded with haemosiderin and bilirubin. The malpighian corpuscles were prominent and enlarged due to hyperplasia of the reticular cells. The lining endothelial cells of central arteries revealed hypertrophy. Lymphatic gland exhibited acute sinus
catarrh, congestion of subcapsular, trabecular and medullary sinuses. The lining littoral cells revealed hyperplasia and metaplasia into reticular histiocytes and free macrophages. The lymph nodules showed depletion of the cells of germinal centres of follicles. In skin, there was coagulative necrosis of the epidermal layers. The hair follicles and sebaceous glands were necrotic and disorganised. The papillary layer of dermis was disorganised and necrotic. The collagenous bundles were fragmented and fibrillated. In the reticular layer, there was hyperplasia of stromal and perivascular connective tissue. The mucosa of the tongue was ulcerated.

CLINICAL PATHOLOGY:

Kesar et al. (loc. cit) recorded haemoglobinuria and high icterus index in serum and plasma of the animals. Sharma (loc. cit) studied haematological and urinary changes in cattle, sheep and goats in lantana poisoning. He recorded fall in erythrocyte count, leucocytosis, neutrophilia, lymphocytopenia, anisocytosis, poikilocytosis and polychromasia. He observed Howell-Jolly bodies in free erythrocytes of bulls. He recorded positive biphasic Van den Bergh test with blood plasma.
In urine, he reported the presence of albumin, bile pigments, bile salts and blood in lantana poisoning.

Agarwala (loc. cit) recorded high icterus index and high serum bilirubin levels in cattle and sheep intoxicated with *L. camara* L.

Seawright (loc. cit) recorded that with the exception of decreased thrombocyte count and a slight transient elevation of neutrophils and hematocrit, no change in hematological criteria in lantana poisoning in sheep. He also recorded no change in the blood biochemical contents except of a slight terminal drop in plasma sodium and great elevation of blood urea. He also recorded low water content of faeces.
CHAPTER (3)

MATERIALS & METHODS.

A. Experimental animals:

From the viewpoint of economy and ease of handling, young sheep of either sex were used as experimental animals in this study. Ten white healthy sheep of about 5 months old and of average live body weight of 35 pounds were used. All the sheep were sheared, numbered with tags and were dewormed and accommodated in hygienic and well ventilated sheds, which were cleaned daily in the morning. All the sheep were maintained on the same standard ration comprising of half-a-pound of gram, one pound of bhusi, half-an-ounce of salt and ad libitum quantities of greens and water. The temperature of all the sheep was recorded in the morning and evening and the animals were exposed to sunlight for three to four hours daily throughout the period of experiment.

3. Intoxicating material:

Lantana camara Linn (N.O. Verbenaceae).
Tel. - Pulikampa; Tam. - Arippu.
Hn. - Panchphuli; Local name - Putus.
Hooker’s (1886) and Coven’s (1950) description
of the plant is furnished here with slight modifications. *Jasminum camara* L. (Fig. 1) is about 3 - 8 feet high, perennial, rambling prickly shrub. The branches are ridged and angular, oppressively sebroid pubescent or prickly and towards the tips densely hairy. The leaves are simple, ovate, 4 - 8 cm. long with serrate margin, petioled, opposite, tip-pointed and underneath many prominent veins giving a wrinkled effect. The spikes are pedunculated, capitatis, ovoid or cylindrical, the bracts conspicuous, exceeding the calyx, bracteoles 0. Calyx is gamosepalous, small, membranous, truncate or sinuous toothed. Corolla is gamopetalous, tubes slender and cylindrical. The lobes are 4 - 5, spreading, pink, yellow and white. Stamens are 4, didynamous and the anthers are broadly oval. Ovary is superior, two celled, two ovuled and the style is short. Stigma is oblique and subcapitate. The fruit is a drupe, purple in colour, fleshy or nearly dry, containing two bony, one celled pyrenes. Seeds are without albumin and radicle is inferior.

The leaves of the plant in the flowering and seeding stages were collected from the campus of Bihar Veterinary College, Patna, during the months of May and June. The material i.e. leaves was dried
under shade for five to six days, powdered and used for the extraction purpose.

(a) Alcoholic extract: - The material was extracted with boiling 96% alcohol under reflux for four hours and filtered. The residual powder was extracted two more times using fresh alcohol each time and the combined filtrate, obtained by extracting the same quantity of leaves for three times, was distilled to obtain the residue, free from the solvent (Gastry et. al., 1963).

(b) Aqueous extract: - The material i.e. leaves was soaked in water for overnight and it was then boiled with the same water for three hours and filtered. The extraction was repeated using fresh alcohol, filtered and the residual powder was squeezed through a muslin cloth. The combined filtrate was then concentrated.

(c) HEMATOLOGY: - About 7 c.c. of blood was collected aseptically with a sterilized record syringe from the jugular vein of the sheep under basal conditions. From the blood thus collected, a drop of
blood was transferred on to each of two clean slides and uniform smears were made for the differential leucocyte count of the sample.

The rest of the blood was transferred into a clean test tube containing appropriate quantity of anticoagulant, potassium and ammonium oxalate mixture (Wintrobe and Landsberg, 1935), evaporated to dryness in the tube on a water bath previously. The tubes were shaken gently for about three minutes to ensure a thorough mixing of the blood and anticoagulant.

About 5.5 cc. of the oxalated blood was centrifuged for one to one-and-half an hour to obtain plasma. The plasma was used for performing Van den Bergh test and icterus index.

The rest of the oxalated blood was used for the examination of the total erythrocyte count, total leucocyte count, haemoglobin percentage, erythrocyte sedimentation rate, packed cell volume (Coffin, 1963) and for chloride estimation.

(a) Differential leucocyte count:— The blood

smears prepared with unoxalated blood were stained with Leishman’s stain. Four hundred cells were counted in each examination and were classified as lymphocytes, monocytes, neutrophils, eosinophils and basophils.
(b) Haemoglobin determination: Haemoglobin percentage of the sample was determined by Färsthab Hæmometer, following the directions for its use.

(c) Total erythrocyte count: Total erythrocyte count was determined by using haemocytometer. Hayes's fluid was used as diluting fluid. Another diluting fluid, originally described by Vallarino (1941), but modified by Sastry and Bhanda (1962) for use in veterinary practice was also employed. With the new fluid, the red blood corpuscles were staining brown and were settling in the counting chamber in short time without any clumping.

Formula of modified fluid:

- Iodium ... ... 0.3 gms.
- Potassium iodide ... 0.4 "
- Sodium citrate ... 2.0 "
- Distilled water ... 100 c.c.

(d) Total leucocyte enumeration: White blood cell count of the sample was determined using Turk's fluid as a diluent.

(e) Erythrocyte sedimentation rate (E.S.R.) and Packed Cell Volume: Clean and dry Wintrobe hematocrit tubes of 11 c.m. length and 2.5 m.m. bore
and calibrated with centimeter and millimeter scale were used. One such tube was filled with the blood up to the mark 10 c.c. with loading pipette and allowed to stand vertically. The E.S.R. was recorded directly from the tube in millimeters at the end of one hour, one-and-half an hour and two hours and it was then calculated per hour (Wintrobe, 1933).

The tube was then centrifuged at 3,100 revolutions per minute for about one-and-half an hour, till a constant reading was obtained. The cell volume was thus directly read from the tube and expressed in percentage.

(f) Estimation of blood chloride: Blood chloride of the sample was estimated with the help of Fisher Clinical Electrophotometer, adopting the technique given in the manual for colorimetric clinical analysis.

(g) Van den Bergh test: Excess of bilirubin in the blood was tested by Van den Bergh test, using plasma and diazo reagent. Both direct and indirect tests were performed (Whitby and Britton, 1957; Kapler and Das Gupta, 1945).

(h) Icterus index: Bilirubin content of the blood was also measured by determining the icterus index. The colour of the plasma was compared with
the standard solution of 1 in 10,000 potassium bichromate, which was taken as the unit. The diluting fluid used was normal saline (Napier and Das Gupta, 1945).

D. URINE EXAMINATION:

The urine was collected early in the morning and was examined for colour, consistency, reaction, sediment, glucose, albumin, acetone, blood, bile pigments, bile salts, urobilinogen (Coffin, 1953; Hauk et al., 1954) and for porphobilinogen.

Reaction of the sample was tested with litmus paper and urinary sediment was examined after centrifuging the sample in the tube for 15 minutes at 1,000 r.p.m.

Presence of glucose in the sample was determined by Benedict's test.

Albumin in the sample was detected by nitric acid ring test (Feller), boiling test and by sulfoalicylic acid method.

Acetone was determined by nitroprusside test.

Bile pigment in urine was tested by filter paper test. Bile salts were determined by Hay's test.

Guanic test was employed to detect the presence of blood in urine.
Urobilinogen in urine was tested by using Ehrlich's reagent (Wallace and Diamond, 1925).

Presence of porphobilinogen in urine was tested by using Ehrlich's p-dimethylamine benzaldehyde reagent and saturated sodium acetate solution (May and Marrack - 6th Edn.).

E. Histopathological procedures:

Necropsies were conducted immediately after death or destruction of the animals. Tissues for histopathological examination were fixed in 10% formal saline, washed in running tap water, dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin wax. Sections cut to the thickness of 5 microns with Spencers rotary microtome were stained with Harris' alum hematoxylin and eosin. Stein's technique for bile pigment and Comori's silver impregnation method for reticulin fibres were also employed (Culling, 1957; Manual of histologic and special staining techniques, Armed Forces Institute of Pathology, 1957, Washington).

F. Feeding procedures:

Sheep used in this experiment were divided into four groups. Group 'A' consisted of 5 animals, numbered 5, 6, 7, 8 and 9. Group 'B'
consisted of two animals, numbered 1 and 2. Group 'C' consisted of two animals, numbered 3 and 4. Animal No.10 was grouped under 'D'.

(a) Alcoholic extract:
Varying doses of alcoholic extract were administered to five animals of group 'A'. The residue from the alcoholic extract corresponding to 200 gms. of dried leaves was made into a thin paste by adding 10 to 12 c.c. of alcohol and the paste was suspended in about 300 c.c. of distilled water. On 1.6.64, two sheep numbered 8 and 9 were drenched each with extract made from 200 gms. of dried leaves. Sheep No.6 was drenched with alcoholic extract made from 125 gms. of dried leaves on 2.6.64 and again on 8.6.64 with alcoholic extract made from 75 gms. of dried leaves. On 1.7.1964, sheep numbered 5 and 7 were drenched with alcoholic extract made from 155 gms. of dried leaves.

(b) Feeding of Lantana leaves and fruits and flowers:
About 2 lbs. of chaffed Lantana leaves, fruits and flowers were given mixed with concentrates, salts and greens to each of the two sheep numbered 1 and 2 of group 'B' from 1.6.64 to 4.6.64. The sheep took only little amounts of mixed feed. From 5.6.64,
they were given only lantana leaves, flowers and fruits till they died. The two sheep consumed little amounts of the offerins on 5.6.64 and 6.6.64. Later on, they stopped taking lantana leaves.

(c) Aqueous extract: Extract, corresponding to 200 gms. of dried leaves was concentrated to about 200 c.c. Two sheep numbered 3 and 4 of group 'C' were drenched daily for 30 days from 1.6.64 to 30.6.64 each with aqueous extract corresponding to 200 gms. of dried leaves and were further kept under observation from 1.7.64 to 12.7.64.

Sheep No.10 was maintained as a healthy control animal until 12.7.1964.
CHAPTER (4)

RESULTS

The sheep No. 2 and No. 1 which were fed with leaves, flowers and fruits of *Lantana camara* L. were destroyed on 16.6.64 and 17.6.64 respectively, when the symptoms of jaundice were disappearing and the animals were lying in a comatose condition.

The sheep No. 3 and No. 4, which were drenched daily with aqueous extract made from 200 grammes of dried leaves, for 30 days and kept under observation for 12 days, were active and healthy throughout the experimental period. They did not show any symptoms of poisoning and they were destroyed along with the control sheep No. 10 on 13.7.1964.

Among the sheep intoxicated with varying doses of alcoholic extract, sheep No. 8 and No. 9, which were drenched on 1.6.64 with extract made from 200 grammes of dried leaves, died on 2.6.64 and 3.6.64 respectively, without showing any symptoms of jaundice. Sheep No. 6 was drenched with alcoholic extract made from 125 gms. of dried leaves, on 2.6.64 and again on 8.6.64, with extract made from 75 gms. of dried leaves. It developed
symptoms of jaundice. But it was destroyed on 13.6.64, when the jaundice was disappearing.

Sheep No. 7 and No. 5, which were drenched each with alcoholic extract made from 155 grammes of dried leaves on 1.7.64, developed jaundice and died on 3.7.64 and 7.7.64 respectively.

**Clinical symptoms:**

Symptoms were noticed after about 24 hours following drenching with alcoholic extract.

In animals, which were fed on leaves and flowers of the plant, symptoms were observed only after about 6 days. The animals which developed toxic symptoms were dull and depressed. Inappetence was observed at an early stage and the consumption of concentrates and greens was completely stopped. Sclera and visible mucous membranes, i.e. conjunctiva, buccal mucous membranes and in females, vaginal mucous membrane were yellow tinged and the intensity of yellow colouration increased as the disease progressed. There was increased salivation and copious yellow nasal discharge. There was progressive weakness and emaciation and usually the animals were weak on their hind quarters. There were symptoms of staggering gait and the animals were unable to stand even with support and were lying flat on the side in a moribund condition.
for a short period preceding death. There was subnormal temperature at the time of approaching death. In some sheep, severe constipation was also observed.

None of the animals exhibited photosensitization, although all the white sheep used in the experiment, were sheared and daily exposed to sun-light for three to four hours, throughout the period of experiment.

**Macroscopic pathology:**

Post mortem examination of sheep No. 3 and No. 4 which did not develop symptoms of jaundice following the administration of aqueous extract, and of healthy control sheep No. 10, revealed no abnormality.

In other animals, carcasses were emaciated and dehydrated. Visible mucous membrane, subcutaneous fat was also scanty, gelatinised and icteric. The skeletal muscles were flabby and moist and the intermuscular adipose tissue was reduced considerably. The liver was slightly enlarged, swollen, friable, congested and icteric. In some animals, lobular markings were prominent. The gall bladder was invariably distended, 7 to 8 times the normal size and contained dark green thick viscid bile. Its wall oedematous and the bile duct
was patent. The kidneys were congested and the medulla of the kidney was icteric and the fat was absent. The urine in the urinary bladder was turbid and yellowish. The lungs were severely congested. The myocardium was slightly flabby and the right side of the heart was dilated. Serous membranes of rumen, reticulum, omasum and abomasum, intestines and mesentery were icteric. The mucous membranes of the intestines were congested and oedematous. In some animals, intestines were severely congested and the caecum and rectum contained constipated faeces as pellets. Spleen was hyperaemic and lymph glands showed slight congestion. In females, vaginal mucous membrane was icteric.

**Clinical pathology:**

As the experimental animals died at varying intervals following intoxication, materials such as blood, urine and plasma could not be examined at same intervals in all the animals. Three sheep No. 7, 8 and 9 among the five which were drenched with alcoholic extract, died by third day following the administration of extract and in these, blood, urine and plasma were examined only before administering the plant material. The initial values of the haematological and urine examination were obtained by taking average of three
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Tag No.</th>
<th>Sex</th>
<th>Urine tested for</th>
<th>Initial values i.e. before feeding</th>
<th>Average of three observations</th>
<th>Intervals of examination after feeding the plant material</th>
<th>Day of death of destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Female</td>
<td>(a) Glucose</td>
<td>-</td>
<td>-</td>
<td>7th day</td>
<td>17th day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(b) Albumin</td>
<td>-</td>
<td>-</td>
<td>12th day</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>(c) Bile salts</td>
<td>-</td>
<td>-</td>
<td>16th day</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(d) Bile pigments</td>
<td>-</td>
<td>-</td>
<td>17th day</td>
<td></td>
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<td></td>
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observations of each test, made prior to poisoning with the plant material. The results of the blood, urine and plasma examination, of all the experimental animals were incorporated in tables (I), (I-A), (I-B) and (I-C); table (II), (II-A), (II-B), (II-C) and (II-D).

From the tables, it is apparent that there was no change in observations of blood and urine examination in sheep No.3 and No.4 which did not develop jaundice and in control animal No.10.

In other animals which showed jaundice, there was no significant change in R.B.C. count, haemoglobin percentage and packed cell volume (P.C.V.) percentage, except in one animal, in which the R.B.C. count, Hb% and P.C.V% had come down to 8.8 mill/c.mm, 8.0 gms% and 27.5 % respectively from 9.65 mill/c.mm, 9.1 gms% and 30.7%. In respect of total leucocyte count, there was distinct leucocytosis in all the affected animals as shown in sheep Graph "A".

In one animal, the count had gone as high as 26,350/c.mm from 13,710/c.mm. Leucocytosis was characterised by distinct neutrophilia, accompanied by lymphocytopenia (Graphs "B" and "C"). And there was marked "shift to the left" as 40% to 50% of the neutrophils were immature band forms.
There was no significant change in the blood chloride content of the affected animals (Graph "B").

Van den Bergh test with plasma was positive for both direct and indirect tests, following intoxication. The icterus index recorded showed a marked rise over the initial value. The plasma of all the sheep before administering the plant material, was almost colourless. The highest values for icterus index recorded following poisoning with alcoholic extract and by feeding the leaves and flowers, were 69.0 and 33.0 respectively (Graph "B").

In urine examination, there was increase in the quantity of urine excreted by the animals. The colour of the urine had changed from straw to yellow and it was turbid. There was no change in the reaction of the urine. The onset of albuminuria and bilirubinuria were found to be simultaneous. Urobilinogen was detected in all the animals before and after intoxication except in animal No.5 in whose urine it was absent in the terminal stages. The tests for sugar, acetone, blood, bile salts and porphobilinogen were found negative. There was no change in the quantity of sediment and the microscopic examination of the same revealed the presence of few crystals of triple phosphates and renal epithelia, both before and after intoxication.
Histopathology:

Histopathological examination was made of organs which revealed gross abnormalities. Histopathological changes were prominent in liver and kidney. Animals which received alcoholic extract presented more severe lesions than animals which were fed with leaves and flowers.

Liver: The microscopic examination of liver revealed acute or subacute toxic hepatitis, characterised by diffuse necrosis, spreading from the area of central vein (Fig. 2 & 3). Central veins were severely congested and dilated (Fig. 4). There was marked disorganisation of hepatic cord structure and the hepatic cells were dissociated. The cells revealed various changes leading to necrosis. They were swollen, margins irregular, cytoplasm eosinophilic and highly granular. The nuclear changes were characterised by pyknosis, karyorrhexis and karyolysis. In some cases, there was complete loss of cell outline and the cells had lost differential staining ability. The sinusoids were distended and contained increased number of erythrocytes (Fig. 5). Kupffer cells were swollen, pyknotic and were present in increased numbers.

In the portal area, portal veins were severely congested and there was increase in the number of non-functional bile ducts (Fig. 6). There was moderate infiltration
of lymphocytes in the periportal connective tissue and there was also increase in number of fibroblasts (Fig. 7).

Sections stained by Stein's method, does not reveal the presence of bilirubin in liver.

Gomori's silver impregnation method of staining reticulin fibres, revealed slight damage to the reticulin fibres which were found fragmented at some places (Fig. 8).

Gall bladder:— Submucosa of gall bladder was oedematous and there was desquamation of epithelium, accompanied by round cell infiltration in the mucosa (Fig. 9 & 10).

Kidney:— In kidney, both the corpuscles and tubules were severely affected. In glomeruli, there was increased cellularity due to proliferation and swelling of capillary endothelium (Fig. 11). There was diapedesis of erythrocytes. There was also proliferation of glomerular and capsular epithelium. Bowman's capsule was distended and there was exudate in the subcapsular space. In tubules, both the convoluted and collecting, the epithelium showed varying stages of retrogressive changes and several tubules have undergone coagulative necrosis (Fig. 12 & 13).
Tubules contained exudate and casts. There was congestion of blood vessels. There was haemorrhage in the intertubular space (Fig. 14). In arteries, the lumen was narrowed due to fibrous proliferation of intimal layer. There was also round cell and fibroblast infiltration in the intertubular space.

Spleen:— In spleen, the pulp was hyperaemic and there was evidence for the presence of pigment in some areas, in sections stained by haematoxylin and eosin and also Stein's technique for bilirubin (Fig. 15 & 16). Few Malpighian corpuscles showed rarefaction of the lymphoid tissue around the central artery and few showed hyperplasia. The central arteries of corpuscles showed slight thickening, due to proliferation of endothelial lining. The capsule and trabeculae were prominent and there was increase in the fibrous connective tissue.

Lymph glands:— In lymph gland, germinal centres revealed necrosis (Fig. 17). Blood vessels were congested and medullary area was inflamed and the cells appearing as macrophages and histiocytes were present (Fig. 18).

Heart:— Muscle fibres were fragmented and the nuclei were hyperchromatic (Fig. 19). There was slight capillary congestion.
Intestines:— Submucosa was congested and oedematous (Fig. 20). Mucosa was desquamated accompanied by round cell infiltration resulting in formation of minute ulcers (Fig. 21).

Lungs:— Lungs were severely congested. In some cases, pneumatic areas were noticed due to which were found to be due to secondary organisms (Fig. 22).
CHAPTER (5)

DISCUSSION & CONCLUSION.

In the present study, three different methods were tried to intoxicate sheep with *Lantana camara* L., a photodynamic plant. Sheep No. 3 and No. 4 drenched daily with aqueous extract made from 200 gms. of dried leaves, for thirty days and kept under observation for 12 days, did not exhibit any poisonous symptoms. This observation is in consonance with that of Sastry et al. (*loc. cit*), who also couldn't induce toxicity in sheep with water-extract. But it differs from the observation of Sharma (*loc. cit*) who reported the development of toxic condition in sheep, following the administration of aqueous extract. It is to be noted in this connexion that Low (loc. cit) reported the insolubility of lantadene A. the active principle of *L. camara* in water. The inability of aqueous extract to produce toxic symptoms in sheep in the present study may be due to the insolubility of lantadene A. in water.
Feeding trials, conducted by Prasad et al. (loc. cit) in cattle and by Lal and Kalra (loc. cit) in buffaloes, sheep and goats, by allowing the animals to eat the plant material, were found to be nontoxic. Although extreme emaciation observed towards the terminal stages may be due to initial withholding of normal ration, the two sheep numbered 1 and 2, which were allowed to consume lantana leaves and flowers, developed symptoms of jaundice. This result is in accordance with that of Sanders (loc. cit) in cattle and Kehar et al. (loc. cit) in cattle and goats. Prasad et al. (loc. cit) reported that nontoxic condition of the plant at that particular period when experiments was conducted may be the factor responsible for their failure in producing the diseased condition.

Louw (loc. cit) reported the solubility of lantadene A. in alcohol. It is, therefore, significant that in the present experiment, alcoholic extract was found to produce toxic condition in sheep. This observation confirms the observations of Steyn et al. (loc. cit), Sastry et al. (loc. cit) and Seawright (loc. cit), who also recorded toxic symptoms with alcoholic extract. Two sheep, numbered 8 and 9, which were drenched each with alcoholic extract made from 200 gms. of dried leaves, died by second and third day respectively following the administration of extract before jaundice
could develop. Sheep No. 6 was given alcoholic extract made from 200 gms. of dried leaves in two doses with an interval of 7 days and it readily developed symptoms of jaundice even after the first dose. But if was destroyed on 11th day when the symptoms of jaundice started disappearing. Sheep numbered 7 and 5, which were drenched each with alcoholic extract made from 155 gms. of dried leaves, developed jaundice but died on third and seventh day respectively. It may be recorded here that jaundice in animals drenched with alcoholic extract was found to be more severe than in the animals which consumed lantana leaves and fruits.

As stated earlier, the important aspect of lantana poisoning is a disturbance of liver function, which gives rise to hepatogenous photosensitization and jaundice. In this type of photosensitization, animals are sensitised by the accumulation of phylloerythrin, a break-down product of chlorophyll digestion. Phylloerythrin is normally excreted into the bile by the liver, but in certain types of diffuse liver damage, commonly associated with a variety of plant, bacterial and chemical hepato toxins, it is gradually absorbed into the circulation, until levels are reached that will produce photosensitization. (Clare, 1952).
Steyn et al. (loc. cit), Louw (loc. cit), Sharma (loc. cit) and Sastry et al. (loc. cit) recorded photosensitization in sheep intoxicated with *L. camara*. But in the present study, photosensitization was not observed in sheep, although they were showing jaundice and porphobilinogen could not be detected in urine. This observation does not differ from Seawright's (loc. cit) who also could not notice lesions of photosensitization in sheep in lantana poisoning.

The absence of photosensitization in this experiment, could not be attributed to insufficient sunlight exposure, as clear weather prevailed and the sheep were exposed daily for three to four hours to sunlight throughout the duration of the experiment. The absence of photosensitization appears to be due to insufficient consumption of greens by the diseased animals as greens are essential for the formation of phylloerythrin which in turn is responsible for producing photosensitization. It is to be noted that Heimington and Quin (1934) in their experiment on the photosensitizing agent in "goeldikkop" also observed that in the absence of chlorophyll from the diet, the experimental animals neither became photosensitive nor could phylloerythrin be isolated from the bile, serum or faeces. Quin et al. (1935) stated that even in the presence of plentiful quantities of
chlorophyll, break-down depends upon the richness and activity of ruminal microflora.

Agave lecheguilla, another photodynamic plant, causes hepatogenous photosensitization in sheep and goats. Matthews (1938) in some of his experiments with *A. lecheguilla* in sheep and goats could not produce photosensitization, although the usual icterus, liver and kidney lesions were noticed. He attributed this to development of resistance on the part of animals.

In the present experiment, lantana poisoning in sheep was characterised by loss of appetite, clinical icterus, bilirubinemia, positive direct and indirect Van den Bergh test (Biphasic reaction) and high icterus index. In some animals, there was terminal fall in R.B.C. count, haemoglobin percentage and Packed Cell Volume value. Leucocytosis was characterised by neutrophilia and lymphocytopenia. Urine contained increased amounts of protein and bilirubin. Gall bladder was distended. Liver and kidney presented severe retrogressive changes. Spleen and lymph glands were hyperemic. Intestines were congested. Caecum and colon contained semi-solid or constipated faeces. There was terminal subnormal temperature.

Lippia rehmanni, another species of verbenacae, was found to produce a syndrome, similar to lantana poisoning, when fed to sheep. *L. rehmannii* contains
two active terpenes, icterogenin and rhommonic acid, which are structurally similar to lantadene A. (Hartog et al. 1934a, b). Ewing et al. (1937) found that icterogenin, when fed to sheep in a dose of 1.5 gms. per os, produces bilirubinemia together with atony and stasis in the forestomachs and large intestine. Loss of appetite and distension of gall bladder was recorded by Shama (loc. cit) and Seawright (loc. cit) in sheep in lantana poisoning. Seawright (loc. cit) attributed the loss of appetite to paralysis of smooth muscles of intestinal wall, because normal alimentary motility is essential for the maintenance of appetite. Similarly, gall bladder was distended and unable to empty spontaneously due to paralysis of smooth muscles of intestine and gall bladder even when the usual stimulant, ingesta is present in the duodenum. Gall bladder and alimentary smooth muscle effects, were related because of their common embryological origin.

So the loss of appetite, distension of gall bladder and stasis of faeces in the large intestines observed in the present study, may be attributed to paralysis of smooth muscles of gall bladder and intestine wall, by the action of lantadene A. Loss of appetite was an important factor, for weakness and emaciation observed in the present study.
Regarding the terminal fall recorded in erythrocyte count, haemoglobin and packed cell volume percentages, it may be stated that various forms of intoxications and burns are constantly manifested by anaemia. MacFarlane (1958) attributed anaemia associated with burns to the release of cell damage products, which causes toxic inhibition of erythropoiesis or increased intravascular destruction of erythrocytes. Klotz and Holman (1911), Bull and Pritchett (1917) and Henry (1922) recorded progressive fall in erythrocyte count in cases of gas gangrene in human beings. Chamberlain (1933) recorded a marked reduction of red blood cells in peripheral circulation in sheep affected with enzootic toxic icterus. Gordon et al. (1940) observed fall in erythrocyte count and haemoglobin percentage in sheep intoxicated with Clostridium welchii type A. Mathews (loc. cit.) recorded marked fall in erythrocyte count in Lechagrilla poisoning in goats. Brown (1963) observed deficient function of erythrocyte methaemoglobin reductase, which lead to increased fragility of erythrocytes, an explosive haemolytic crisis in enzootic icterus and low grade intravascular haemolysis in "geeldikkop".

An anaemia from an imperfectly understood disorder of haemopoiesis occurs in association with nephritis,
possibly through direct toxic action of the retained waste products. Interference with the production in the kidney of a specific enzymic substance termed "erythropoietin" has been reported as an explanation (Smith and Jones, 1956).

Sharma (loc. cit) recorded oligocythaemia, marked hypochromic anaemia, anisocytosis, poikilocytosis and polychromasia in sheep poisoned with L. carinii. Seawright (loc. cit) recorded no change in R.B.C. count. The terminal oligocythaemia and fall in Hb. and R.C.V. recorded in this experiment were associated with renal lesions. So these observations may be due to some disorder of haemopoiesis, resulting from nephritis. Unfortunately, histological examination of bone marrow was not made, which could have thrown further light on the terminal it oligocythaemia observed in the experiment.

Anaemia noticed at the terminal stages was not of haemolytic type was substantiated by the fact that the plasma was responding to biphasic Van den Bergh reaction.

As regards leucocytosis (shown in Graph No. A) observed in this experiment, it may be stated that a number of drugs and chemicals are known to have stimulating effect on the bone marrow and lymphoid tissue, causing the appearance of immature granulocytes in the
circulation. The stimulus for production of leucocytes in all cases is chemotactic. Production of polymorphs may be artificially stimulated by the injection of nucleic acid or its salts. The same stimulus is provided under pathological conditions where the nuclein products of tissue destruction acts as a definite stimulus. In the absence of infection, neutrophilic leucocytosis of varying degree are found in intoxications associated with cirrhosis of liver, poisoning with carbon monoxide, mercury, lead, camphor, adrenaline etc. (Whitby and Britton, loc. cit).

Henskin (1940, 1953 and 1955) reported that inflammatory exudates contain leucocytosis promoting factors which are polypeptide in nature. Collumbine and Rydon (1946), Beloff and Peters (1949) reported similar observations.

Silverman (1928), Moon (1939 and 1935) and Beloff & Peters (loc. cit), Moon and Tershakovec (1951) considered that the products of cell break-down directly stimulate bone marrow leucopoiesis.

It seems likely that leucocytosis which follows an injury whether by a living or non-living agent, is in part due to stimulation by nuclein substances derived from the injured tissues and part due to polypeptide leucocytosis producing substances present in inflammatory exudates.
Administration of corticotrophin or cortisone was found to result in a fall in lymphocytes and a rise in neutrophils (Hills et al. 1948; Herbert and deVries, 1949; Randolph and Rollins, 1950; Code et al. 1954).

Sharma (loc. cit.) recorded leucytosis, accompanied by neutrophilia and lymphocytopenia in cattle and sheep in lantana poisoning. Seawright (loc. cit.) recorded a slight transient elevation of neutrophils.

Matthews (loc. cit.) recorded leucocytosis with an increase in the percentage of neutrophils in lechuguilla poisoning in goats.

Lymphocytopenia and neutrophilia associated with "geeldikkop" in South African sheep were reported by Brown et al. (1960) and they attributed the changes to disturbances in the physiological functions of adrenal cortex. They also presented biochemical and histological evidence in support of their contention and stated that adrenal disturbances may be due to the action of plant constituents.

Clark (1941) recorded lymphocytopenia in ovine pregnancy toxaemia.

So leucocytosis accompanied by neutrophilia and lymphocytopenia observed in the present study may be due to the appearance of substances of general nature of leucotoxine in the damaged tissues. It may be also due to endocrinal disturbances. Unfortunately histological examination of endocrine glands was not
made in the present study.

No significant change in blood chloride content, could be recorded in the present experiment. This confirms the earlier observation of Seawright (loc. cit).

Clinical icterus observed in this study was also observed by Steyn et. al. (loc. cit), Louw (loc. cit), Sharma (loc. cit), Agarwala et. al. (loc. cit), and Sastry et. al. (loc. cit.) in sheep and by Pande (loc. cit.), Kohar (loc. cit), Kohar et. al. (loc. cit), Sanders (loc. cit.), Knott (loc. cit.), Negi et. al. (loc. cit.) and by Hall (loc. cit) in cattle. Plasma which was almost colourless in normal condition, has changed to dark yellow following intoxication due to its increased bilirubin contents.

Agarwala et. al. (loc. cit.) recorded increase in icterus index and serum bilirubin levels in sheep. Serum bilirubin has increased from 0.34 mg./100 ml. to 5.22 mg./100 cc. The icterus index recorded by them was as high as 123.9, with average of 64.0. Sharma recorded positive biphasic Van den Bergh reaction with plasma in Lanthum poisoning. In the present observation, plasma was positive for both direct and indirect Van den Bergh tests, which indicates hepatocellular damage, dysfunction leading to intrahepatic cholestasis.
An icterus index of 69.0 was recorded in the present study in one animal. Positive biphasic Van den Bergh reaction and patent bile duct observed in this study point out to intrahepatic or toxic jaundice. Continued excretion of urobilinogen in urine excepting in one animal, excludes the obstructive jaundice in this observation.

Subnormal temperature in lantana poisoning was recorded by Lal and Kaila (loc. cit.) in buffaloes, Sharm (loc. cit.) in cattle and sheep and by Gastry et. al. (loc. cit.) in sheep. McIntosh and White (loc. cit.) and Sanders (loc. cit.) recorded elevation of temperature. In the present experiment, there was no change in the rectal temperature except at terminal stages when it was subnormal.

Regarding albuminuria and bilirubinuria observed in the present study, it may be stated that mere presence of icterus will cause toxic tubular nephritis. But it is uncertain whether the injury to renal tissues is due to bile pigments or bile salts or associated hepatic damage (Smith & Jones, 1958; Anderson, 1957). Albuminuria associated cholemic nephrosis was reported by many, in humans (Walter and Parham, 1922; White, 1923; Fitze - Bugh, 1929; McKnight, 1930; Helwig & Schutz, 1932).
Thompson et al. (1940) observed choluria and nephrosis in dogs by ligaturing bile duct.

**Albuminuria** is a clinical sign of injury to the glomeruli and a serious renal malfunction (Smith and Jones, 1968).

Regarding the source for proteins in urine, it is now universally accepted that in albuminuria of any notable degree, by far the greater part of the urinary protein is derived from the blood. And the amount of protein derived from the tubular epithelium is evidently small in comparison to that coming from the blood. (Fishberg).

Sharma (loc. cit.) detected the presence of albumin and bile pigments, bile salts and blood in cattle and sheep in lantana poisoning. But in the present experiment only, albumin and bile pigments were detected in increased amounts in urine. The presence of albumin in urine can be explained by the fact that the damaged capillary tufts of glomeruli permitted the escape of proteins from plasma and were not reabsorbed by damaged tubules. Urobilinogen was detected in all the animals throughout the period of experiment, except in sheep No.5 in which it could not be detected in the terminal stages, which may be due to failure to excrete bile into the intestine, by the gall bladder whose smooth muscles were paralysed.
Incidentally it may be mentioned that the animal showed more severe icterus than others.

Histological examination, presented hyperaemia of pulp, depletion of lymphoid tissue in Malpighian corpuscles around the central arteries, in spleen. In some Malpighian corpuscles, there were hyperplasia. In lymph glands, there was necrosis of germinal centres and the medullary areas presented inflammatory changes accompanied by infiltration of cells, appearing free macrophages and histiocytes. Sharma (loc. cit.) recorded similar findings in lymph glands and spleen in lantana poisoning in cattle and sheep.

In intestines, submucosa was found to be congested and there was desquamation of epithelia accompanied by round cell infiltration. Knott (loc. cit.) also recorded gastroenteritis. Desquamation of epithelia observed in the present study may be due to irritant action of toxic principle of the plant.

Gall bladder was found distended and its wall was oedematous and there was desquamation of epithelium. Knott (loc. cit.) recorded congestion of gall bladder wall.

The liver lesion was manifested by diffuse necrosis spreading from central vein. Central veins were severely congested. Necrosis of central zonal parenchymal was recorded by Sharma in cattle and sheep and by Seawright (loc. cit.) in sheep in lantana poisoning. Sastry et al. (loc. cit.) reported diffuse necrosis and fatty changes of liver in sheep.
Hepatic cellular damage was also reported in sheep by Mathews (1941) in lechuguilla poisoning and by McFarlane et al. (1959) in facial eczema. Dunn et al. (1960) observed that the severity of liver damage was related to the extent of the increase in certain serum constituents including bilirubin.

Toxic hepatitis occurs in many poisonous conditions, the important poisons being carbon tetrachloride, tetrachloroethylene, mercury, copper; among plants, species of genus, senecio and phyllanthus causes toxic hepatitis (Smith & Jones, 1956).

Regarding the severity of necrosis around the central vein, it may be stated that it occurs in animals and humans, in etiologically different conditions. Altman (1949) reported by carrying out experiments with animals kept in low pressure chambers, that anorexia plays an important role in the development of centrilobular necrosis. Delorme (1951) observed centrilobular degeneration in about 5 hours, after the organ was perfused with venous blood.

Wallach and Pepper (1950) observed that when liver cells were injured there occurred a reflex contraction of the central vein and of the related venules, which together with a possible swelling of the central cells, causes a decrease in oxygen tension with a consequent impairment of detoxification mechanism. Cohrs (1923) and Belle (1933) reported that toxins are likely to accumulate around the
central veins, as the total cross-section of the sinusooids decreases towards the centre. Stoner et. al. (1957) reported that the toxic liver injury results from direct interference with the biochemical machinery of the liver cells by the toxic agent.

Discussing lantana poisoning in sheep, Seawright (loc. cit.) stated that the functional disturbance of liver, which gives rise to jaundice and photosensitization was not associated with necrosis of parenchymal cells, since there was no significant histological changes in some of the sheep. And the serum glutamic pyruvic transaminases did not rise. He also did not observe any change in serum alkaline phosphatase levels, which indicates that the rise in serum bilirubin is independent of extrahepatic biliary obstruction. But in the present study, jaundice was associated with hepatocellular damage. This may be due to direct toxic action of lantadene A, which reaches the liver through portal circulation, following absorption from intestines. Venous congestion observed may also play a part in degeneration of cells, due to anoxia.

Renal lesion was manifested by degenerative changes in the glomeruli and in the tubules, associated with albuminuria and bilirubinuria. These changes were accompanied by hepatocellular damage.
Kidneys are damaged by toxins that may be released from the damaged liver or that may accumulate in the blood in the absence of the hepatic route of removal. Unfortunately, there is inadequate information on pathology and cardio-vascular, renal and hepatic function, to afford a clear understanding of what is probably a mixed group of physiological disturbance (Smith, 1953). Bradley (1945) reported that renal and hepatic impairment may appear simultaneously in many intoxications, with substances like carbon tetrachloride, chloroform, dioxane etc.

As stated earlier, it is uncertain whether the circulating bilirubin, urobilinogen or some other substance, is the actual causative agent for renal damage in cholemic nephrosis. Wilensky (1927) reviewing the subject of "liver death" points out that the hepatogenous syndrome in addition to its incidence in biliary and liver diseases, it follows operations for other conditions such as carcinoma of breast, gastric ulcer and so on. He stresses the phylogenetic relation between the liver and kidney.

Helwig and Schatz (loc. cit.), Fitz-Rugh (loc. cit.), Helwig & Orr (1932), McKnight (loc. cit.), Walter and Parham (loc. cit.) and White (loc. cit.) have noted evidence of renal injury in diseases of and trauma to the liver. Helwig and Schatz (loc. cit.) believed that the damage was not due to an increase in the bile salts in the blood.
Boyce and McFetridge (1935), following a clinical and experimental investigation of the mechanism of so called "liver death" came to conclusion that the hepatorenal syndrome is due to the release of the common bile duct obstruction. This, they believed, permitted the escape of toxin which acting upon a overtaxed kidney, causes a break-down of the convoluted tubules. This thesis is also held by Selwig and Schutz (1935) and Schutz et. al. (1932). Seawright (loc. cit.) attributed, nephrosis as the factor responsible for eventual death of animal in lantana poisoning. Damage to the kidney occurs concurrently with the development of excretory dysfunction in the liver. Seddon and Earne (loc. cit.) believed the putrefaction of faeces in the large intestine to be important in the failure of an animal to survive.

So it may be stated that the renal lesions observed in the present study may be due to the irritant action of circulating bilirubin, or toxic substances liberated from the damaged liver or toxic substances originated from plant material which liver failed to detoxify. It may also be due to nephroticxic substances absorbed from the intestines.
CHAPTER (6)

SUMMARY

Available literature on the symptomatology and pathology, due to ingestion of Lantana camara L. in sheep and cattle was recorded.

Ten healthy young sheep of either sex and of five months old each and of thirty-five pounds body weight were used in the present experiment, to study the toxicity of L. camara. All the sheep were sheared and exposed to sunlight daily for 3 to 4 hours, throughout the period of experiment. Temperature of all the animals was also recorded daily. The plant material was collected from Bihar Veterinary College, Patna campus. Alcohol and water extracts of the lantana leaves were prepared. Alcoholic extract was administered to five sheep and water extract was drenched to two sheep. Two sheep were allowed to feed on lantana leaves, flowers and fruits and one healthy control was maintained throughout the period of experiment. Water extract did not produce the poisonous condition in sheep. Sheep that were drenched with alcoholic extract and sheep which were
allowed to feed on lantana flowers and leaves, exhibited toxic symptoms. It was also observed that jaundice in animals intoxicated with alcoholic extract was more severe than in animals which were allowed to eat lantana leaves and flowers, and the control animal, sheep No. 10, remained healthy throughout the experimental period.

Haematological examination and urine examination was made before and after intoxication in all the sheep. The significant blood dyscrasias observed in poisoned animals are, terminal oligocytæmia, fall in haemoglobin and P.C.V.% values. There was significant leucocytosis characterized by neutrophilia and lymphocytopenia. Plasma was positive for direct and indirect Van den Bergh tests. Icterus index of 69 was recorded. The urine was turbid and contained proteins and bilirubin following intoxication. Porphobilinogen could not be detected in urine.

The symptoms recorded in lantana poisoning are anorexia, salivation, icteric condition of visible mucous membranes, constipation, progressive emaciation and dehydration. But no symptom of photosensitization was noticed and this has been explained as might be due to the animals not taking enough greens.
Pathology of experimental intoxication of sheep with L. similis, was dominated by renal and hepatic lesions.

Liver lesion was manifested by diffuse necrosis spreading from central veins, congestion of central and portal veins, proliferation of Kupffer cells and lymphocytic infiltration in the perportal area. The gall bladder was found distended and its wall was oedematous.

Microscopic picture in the kidney presented severe degenerative changes in the glomeruli and tubules. There was increased cellularity in the glomeruli. Tubules contained casts and epithelium presented retrogressive changes.

In spleen, pulp was hyperemic. Malpighian corpuscles showed rarefaction of lymphoid tissue around the central vein.

Intestines were found congested and the mucosa was desquamated. Lungs were severely congested.

The clinical syndrome produced by the alcoholic extract was found to be more serious than that observed in animals fed with lantana leaves and flowers.

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