

**CLINICO-BIOCHEMICAL AND THERAPEUTIC STUDIES
ON LANTANA TOXICOSIS IN CATTLE**

THESIS

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Submitted to



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PALAMPUR-176062 (H.P.) INDIA**

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OF

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2003

*Is there anything I can say
Anything I can give
Or do for you?
(Because all that I'm
A [[that I have
I owe to you.*

*Affectionately dedicated to
9\iy parents
who sacrificed their present
to make my future better*

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CERTIFICATE -1

This to certify that the thesis entitled, "**Clinico-biochemical and therapeutic studies on Lantana toxicosis in cattle**" submitted in partial fulfillment of the requirement for the award of the degree of **Master of Veterinary Sciences** in the subject of **Veterinary Medicine** of Choudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, is a record of *bonafide* research work carried out by **Miss Jayta Sharma** (V-2001-30-17) under my guidance and supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been duly acknowledged.

Dr. Des Raj Wadhwa
(Chairman)
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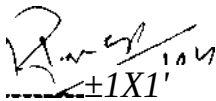
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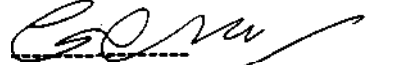
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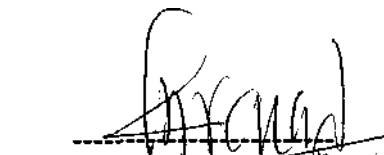
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(PMA^M(FU<il

(JaflaSharma)

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LIST OF ABBREVIATIONS USED

Abbreviation	Meaning
ALT	: Alanine amino transferase
AST	: Aspartate aminotransferase
BUN	: Blood urea nitrogen
AP	: Alkaline phosphatase
dl	: deciliter
eg.	: Example gratia(For example)
g	: gram
Kg	: Kilo gram
mg	: milli gram
hr	: Hours
ie	: idest (That is)
ml	: milliliter
°C	: Degree Celsius
°F	: Degree Fahrenheit
%	: Per cent
M	: micro liter
m ²	: meter square
cm	: centi meter

PH	: Negative log of H ⁺ activity
b.i.d.	: Twice a day
Wk	: Week(s)
b.wt.	: Body weight
yr.	: year
<i>etal.</i>	: et alii (and others)
Viz	: Videlicit (namely)
Bull.	: Bulletin
PP	: pages
min	: minute (s)
mEq	: milli equivalent
<i>w.r.t.</i>	: with respect to
Inj.	: injection
IU/l	: International unit per lit
Nm •	: nano meter
@	: at the rate of
IM	: Intramuscular
IV	: Intravenous
S.No.	: Serial number
fl	: femto liter
Pg	: picogram
Fig.	: Figure
Hb	: Haemoglobin

PCV	: Packed cell volume
TEC	: Total erythrocyte count
TLC	: Total leukocyte count
SAT	: Sedimentation activity time
GFT	: Glucose fermentation time
TVFA	: Total volatile fatty acid

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INTRODUCTION

CHAPTER I

INTRODUCTION

The dairy industry is one of the fast developing industries in India having about one fifth of the world's cattle population and contributing nearly 15 per cent to the gross national income. Still the animal production is much lower than the countries with lesser animal population. Improvement in animal productivity will be possible only through a coordinated approach of scientific breeding, improved feeding, management and animal health care. Disease problem due to ingestion of poisonous plants is one of the important constraints affecting livestock and profitable livestock production.

Among agriculture based states of India, Himachal Pradesh has about 91 per cent rural population that depends directly or indirectly upon animal husbandry, agriculture and horticulture. Maximum of livestock population sustained on natural pasture and grass lands. This is because of shortage of quality fodder round the year. A lot many type of plants, considered as weed, are also accidentally ingested by the animals and produce toxicity in them. These poisonous plants contain certain chemical constituent which if introduced into the body of animal in even small quantity, acts deleteriously and may cause serious impairment in bodily function or even death.

One of such noxious plants is *Lantana camara*. It is an attractive member of the Verbenaceae family. Lantana is an aggressive and gregarious weed occupying extensive area of the Indian plains and hills. It was introduced from tropical America to India in the first half of 19th century, probably as an

ornamental plant from Ceylon (Matthew, 1972). It is considered as one of the world's ten worst weeds. Now it has become the major intractable problem for agriculture, animal husbandry and forestry. The plant when once introduced in an area soon spreads and may get naturalized if the conditions are suitable. It grows in a wide range of climate and soils and in low lying region as well in high slopes up to a height of 4500 ft. above sea level. There are about 150 species of lantana mostly from the central and south America. Lantana plant regenerates quickly after pruning and form a dense impenetrable thicket. It can propagate readily from stumps or cutting and from seeds disseminated by birds through their dropping (Gujral and Vasudevan, 1983). The flower of lantana are small with auxiliary head and exhibit variety of color ranging from white, yellow, pink, red, violet, yellow red and crimson to scarlet. Lantana plant of yellow red variety is the most wide spread and most poisonous in this part of the country (Sharma *et al.*, 1981a).

In India, the plant is most abundant in scrub type jungles of deccan plateau, parts of Vindhya, Chotta Nagpur and Assam. The plant has now spread to almost all the states but is more common in Rajasthan, Punjab, Uttar Pradesh, Haryana, Bihar and parts of Jammu and Kashmir and Himachal Pradesh (Katiyar, 1981; Sharma and Makkar, 1981 and Singh *et al.*, 1988). In Himachal Pradesh, the road side and ghasni are covered with lantana plant in abundance in the districts of Kangra, Hamirpur, Solan, Sirmour, Bilaspur, Una and Mandi.

The livestock used to graze in lantana infested pastures, but don't consume it as matter of choice but do so in the event of drought and floods. Lantana menace to livestock is at least three pronged. It causes hepatotoxicity, allelopath/c action on grass and fodder resources and infestation of potential pastures. The prominent compounds present in lantana leaves whose toxicity has been

established are lantadene A and Lantadene B. Lantadene C has also been isolated from the leaves of the plant (Sharma *et al.*, 1992). Lantadene A is considered as most hepatotoxic among these. A new compound, ursethoxy acid has been recently reported from the aerial parts of *Lantana camara* (Begum *et al.*, 2002).

Field cases of lantana toxicity have been frequently observed from India which have accounted for high morbidity and mortality and causing substantial economic losses (Lai and Kalra, 1960; Yadava and Verma, 1978; Sharma and Makkar, 1981; Singh *et al.*, 1988 and Singh *et al.*, 2003). In Himachal Pradesh, incidence varies from sporadic to heavy outbreaks reported from Hamirpur, Una, Bilaspur, Rampur-Bushair and Kangra district/ areas. In natural intoxication after browsing or in experimentally induced lantana toxicity, it has been observed that animal go off feed and becomes severely constipated within 48 hours and by 3rd day, animal become icteric. There is lacrimation, sloughing and cracking of skin, ear become pendulous and swollen. Photosensitization due to accumulation of photodynamic substance resulting in cholestasis is common.

Significant decrease in haemoglobin and erythrocyte count, increase in blood clotting time was reported in lantana poisoned animals. Significant increase has been observed in the activities of enzymes aspartate amino transferase, alkaline phosphatase, glutamate dehydrogenase and gamma glutamyle transpeptidase. (Dwivedi *etal.*, 1971; Sharma *etal.*, 1981a; Ahmed and Roy, 1991 and Mandial and Randhawa,1999)

There is no specific treatment available against this toxicosis, resulting in heavy toll of livestock every year causing considerable economic loss to the farmers. Efforts have been made to treat the cases symptomatically with

glucose saline together with liver protectants, rumenotorics with or without activated charcoal (Hari *et al.*, 1973; Kaushal, 1994; Mandial and Randhawa, 1996 and Singh *et al.*, 2003).

A lot of experimental work has been done in different animal species to study various aspects of lantana toxicosis. But systematic study on natural clinical cases of lantana toxicity is lacking. Considering all these facts, the present study was undertaken with following objectives-

- To study the detailed clinical appraisal of animals with lantana toxicosis
- To study the physical and microbial changes in rumen liquor
- To study the hematological and biochemical alteration in diseased animals
- To formulate suitable therapeutic measure against this toxicosis



REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Lantana camara locally known as lantana, Panchphooli booti, Lai phulanoo or Bara phulanoo is a wild shrub growing luxuriantly in many temperate and sub temperate part of the world up to an elevation of 6000 ft above the mean sea level (Seawright, 1965). The plant was first described by Linnaeus in 1853 and has spread to about 50 countries. It has capacity of thriving even in adverse soil and climatic conditions (Holm *et al*, 1977). *Lantana camara* Linn, a photodynamic plant is well known for its toxic property to cattle and buffalo. It is considered to be one of the ten most obnoxious weeds of the world (Holm and Herberger, 1969). When domestic animals happen to consume it accidentally, or other wise, develop symptoms of poisoning terminating in death.

2.1 BOTANICAL CHARACTERISTICS

Lantana camara is an attractive member of the family Verbanaceae. Lantana includes about 150 species mostly from South and Central America. Some of the important species of Lantana are *Lantana camara*, *Lantana indica*, *Lantana crenulata*, *Lantana trifolia*, *Lantana lilacina*, *Lantana involucrata* and *Lantana sellowianae* (Sharma *et al*, 1981a). Among these red flowers variety *Lantana camara* Var *aculeata* is most prevalent.

Smith and Smith (1982) in a taxonomic study of naturalized *Lantana camara* complex in Australia described 29 taxa which were recognized on the basis of flower colour, corolla limb morphology and vegetative character. Ojha and Dayal (1994) reported the occurrence of polyhydroxy flavonoid and methoxy

flavonoids in lantana indicating its advanced affinity amongst the Verbanaceae. Chemotaxonomically, *L. camara* ecotype IV (from Jammu) and *L. montevidensis* appeared to be the most advanced and *L. Camara* ecotype II (from Varanasi) the most primitive one.

It is a compact shrub of less than 1 meter to as high as 5 meters or even more, but the average height varies in different locations (Gujral and Vasudevan, 1983). The stem is pubescent, covered with short stiff hairs. Its branches can spread to cover an area of about 1m². The leaves are 5-9 cm long, rough and are easily identifiable by the emittance of characteristic verbene like pungent scent, when rubbed. The flower is small, axillary head and exhibit variety of color ranging from white, yellow, red, pink, violet, yellow red and crimson to scarlet (Seawright, 1965; Gujral and Vasudevan, 1983 and Sharma *et al.*, 1981a). The plants can propagate rapidly by the means of stumps or cutting. The natural propagation appears to be from seeds disseminated by birds through their dropping or by the faeces of moving flocks of sheep and goat (Gujral and Vasudevan, 1983).

2.2 GEOGRAPHIC DISTRIBUTION

The genus was first described by Linnaeus in 1853. The distribution of lantana in European colonies started as early as 1800 and exotic species of lantana were recorded in South Africa. It is a principle weed in twelve countries (Holm *et al.*, 1979) and a serious weed in ten countries, spreading over Oceania, Asia, Africa, South America and North America.

In India, the Plant was first introduced in the first half of the 19th century (1809) as an ornamental plant (Gujral and Vasudevan, 1983). In India the plant is most abundant in scrub type jungle of the Deccan plateau, parts of

Vindhayas, Chotta Nagpur and Assam and sparsely distributed in the deltaic region, particularly those on the Eastern coast. It has been spread to all states in India, but more common in Rajasthan, Punjab, Uttar Pradesh, Himachal Pradesh, Assam, Bihar and Kashmir as a wild shrub. In different regions it is known by different names (Lai and Kalra, 1960 and Gujral and Vasudevan, 1983).

<u>Language</u>	<u>Vernacular name</u>
English	Lantana, wild saga
Hindi	Punchphulibuti
Telgu	Pulikampa
Kannada	Natahugida, Hesika Kakka
Tamil	Unnachadi
Malayalam	Arripu
Marathi	Ghaneri

In Himachal Pradesh, this plant is found as a wild shrub mainly in the district of Kangra, Shimla, Bilaspur and Hamirpur. The red variety of lantana is incidentally more toxic to the livestock in these districts of Himachal Pradesh (Dhillon and Singh, 1965; Sharma *et al*, 1981a).

Thirunavukkarasu *et al.* (2000a) reported that the red flower variety of *Lantana camara* brought from Himachal Pradesh and Nilgiri hill of Tamil Nadu were more toxic in comparison to yellow pink variety of the same in experimentally intoxicated calves.

2.3 TOXICITY IN ANIMALS

The first field report of lantana toxicity was observed in 1910 by Tucker in Townsville (Australia). There after toxicity in animals due to ingestion of lantana

foliage has been reported in different species of animals from at least nine countries spread over five continents (Sharma *et al.*, 1988c).

Lantana camara L. is poisonous to most of the domesticated ruminants. Natural incidence of lantana toxicity has been observed in sheep, cattle, buffalo and goats and is a major cause of livestock mortality and morbidity (Yadava and Verma, 1978; Sharma and Makkar, 1981). Brito and Tokamia (1995) compared the *Lantana camara* var *aculeata* in cattle and sheep and concluded that the plant is equally toxic for both species whereas goats appear to be tolerant to this plant and find it unpalatable (Sharma and Makkar, 1981; Lin *et al.*, 1985). Lantana toxicity has also been reported in wild animals. Hepatotoxicity and secondary photosensitization were seen due to ingestion of *Lantana camara* in three red Kangaroo in Texas (Johnson and Jensen, 1998). The intoxications take place all round the year but the incidence is higher during the summer months of April-June (Sharma and Makkar, 1981).

Livestock do not consume the plant as a matter of choice, but do so in the event of drought, flood or feed scarcity or during road transportation (Yadava and Verma, 1978; Gujral and Vasudevan, 1983 and Sharma *et al.*, 1988c). Field reports of toxicosis in sporadic to heavy outbreaks have been recorded in India and abroad. Black and Carter (1985) reported the first field incidence of lantana toxicity in cattle in New Zealand. Tokarnia *et al.* (1984) recorded the outbreak of lantana toxicity in cattle in Brazil with high mortality. Fourie *et al.* (1987) recorded an out break of lantana toxicity in South Africa. Frisch *et al.* (1984) in Australia reported an out break of lantana toxicosis in *Bos taurus* and *Bos indicus* cattle and their crosses. No difference in the incidence of toxicosis in different breeds of cattle was observed but parasitic infestations might be the predisposing factor.

Riet *et al.* (1984) reported poisoning of cattle by *Lantana glutinosa* in Santa Catarnia state of Brazil, where 93 out of 255 Holstein Friesian cattle died after grazing on a lantana infested pasture.

In India, lantana is a serious problem in the hilly terrain parts (Sharma *et al.*, 1988c). Yadava and Verma (1978) reported a heavy out break of lantana in animals during 1971 floods. As many as 400 animals were reported to have died and thousands of cattle showed symptoms of toxicity including jaundice and photosensitization. Sharma *et al.* (1981a) reported sporadic cases of lantana toxicity in Kangra Valley of Himachal Pradesh. Aloni (1979) reported 8 cases of photosensitization in indigenous cattle after ingestion of *Lantana camara* in Nagpur. A serious outbreak of lantana poisoning occurred in migratory sheep flock in Uttar Pradesh leading to 60 percent mortality (Srivastava and Sinha, 1980).

Katiyar (1981) reported an outbreak of lantana toxicosis in sheep and goats from Rampur-Bushair area of Himachal Pradesh in which out of 170 adult sheep, 80 were affected and 58 succumbed to the disease. Sharma and Makkar (1981) conducted random survey of lantana toxicosis in Kangra valley and reported heavy mortality in summer months. Out of 214 animals affected with poisoning, 93 were cattle and 77 were sheep. They further reported that poisoning was maximum in cattle (43.5 per cent) followed by sheep (35 per cent), buffaloes (11 per cent) and goats (6 per cent). Out of all the affected animals, only 14 cases survived.

Sreenivasulu *et al.* (1987) reported 40 clinical cases in sheep from year 1983 to 1985 and morbidity and mortality were highest among young animals. Pal *et al.* (1997) reported 8 clinical cases of lantana toxicity in cattle and were treated symptomatically. Mandial *et al.* (2000) also reported the occurrence of 39 sporadic

clinical cases of lantana poisoning in cattle in Palam Valley of Himachal Pradesh and evaluated the therapeutic measures provided.

Singh *et al.* (2003) reported the occurrence of lantana toxicity in sheep flock from Rampur district of Uttar Pradesh. Due to fodder scarcity, sheep were forced on browsing shrubs readily available in the area. The overall incidence of poisoning in flock was 32.67 per cent (49 out of 150) and 5 severely affected animals died within 3 days suggesting case fatality rate of 10.20 per cent.

2.4 EXPERIMENTAL TOXICITY IN ANIMALS

Various workers have induced the toxicity in different species of animals with varying dose rates of dried lantana leaves or crude extracts of lantana toxins. Variation in the dose requirement for the causation of experimental toxicity could be attributed to the season, susceptibility of animal, age, parasitic infestation, stress, quantity of lantana ingested and concentration of the toxic content in the plant (Yadava and Verma, 1978).

Dhillon *et al.* (1970) induced lantana toxicity in buffalo calves by giving *Lantana camara* powdered leaves at dose of 125 and 250 g suspended in water for consecutive 4 days to study the haematological changes. Dwivedi *et al.* (1971) studied the clinico-biochemical changes in induced lantana toxicity in calves. The toxicity was induced by dried lantana leaves of the plant having pink flowers at dose rate of 6 g per kg. b.wt. Hari *et al.* (1973) and Kalra *et al.* (1984) also induced lantana toxicity in buffalo calves by dried lantana leaves at dose of 4 g per kg b.wt. as a single dose and 200 g leaves in water for 3 days, respectively.

Kaushal (1994) and Mandial and Randhawa (1996) induced lantana toxicosis in cow and buffalo calves, respectively by giving lantana leaves at dose

rate of 4 g (cow calves) and 6 g per kg b. wt. (buffalo calves) to study the haemato-biochemical changes and efficacy of various drug combinations.

Seawright and Hrdlicka (1977) demonstrated oral toxicity for sheep of triterpene acid isolated from lantana leaves. Pass *et al.* (1979) demonstrated toxicity in sheep with I/V injection of triterpene acid lantadene A, isolated from lantana leaves. Lantana toxicity could be induced in sheep with crude lantadene obtained from lantana leaves @ 50 and 100 mg per kg b.wt. orally (Uppal and Paul, 1978 ; Nauriyal and Gupta, 1983).

Susceptibility to lantana toxicosis has been established in goats through lantana leaves by different dose rates (Lin *et al.*, 1985; Obwolo *et al.*, 1991 and Ali era/., 1995)

Experimental lantana toxicosis has also been reported in rabbits and Guinea pigs (Sharma era/., 1988a & 1989).

Recently, lantana toxicity was experimentally induced in calves @ 20g per Kg b.wt. to study the hemato-biochemical and pathological alterations along with electrocardiographic evaluation of lantana intoxicated calves (Thirunavukkarasu *et al.*, 2000b,c&2001a,b).

2.5 NATURE OF TOXINS

Clinical investigation on nature of lantana toxins was started in early 1940s. Louw (1943) isolated a crystalline compound from the leaves of *Lantana camara* at a melting point of (276-280°C) and named lantanin. But this name had already been given to an alkaloid isolated from *Lantana brasiliensis*, so the active principle was named as lantadene A (22 p-angeloyloxy-3-oxoolean-12-en-28-oic acid). Another compound isolated was named as lantadene B (22 p- dimethyl-acryloyloxy-3 oxoolean-12-ene-28 - oic acid). Out of these, lantadene A was found

to be more toxic. Brown *et al.* (1963) reported that lantadene A is not only icterogenic or cholestatic but it is the minuscule impurity in lantadene A that produce the effect.

Sastry and Mahadevan (1963) estimated that lantadene was 0.2 per cent in the month of March - April and 1.4-1.7 per cent in July -September. However Sharma *et al.* (1980) found the lantadene content to be 6-7 mg per g dry weight of leaves in July-August. They further reported that these toxins were not detected in the flower, fruit and tender shoots. A higher amount of Lantadene A (13.6 mg per g) was recovered from the leaves by the spectrophotometric method (Sharma *et al.*, 1983).

Hari *et al.* (1973) in a comparative study of triterpene of different taxa of *Lantana camara* observed that in toxic taxa, lantadene A & B are the major constituents while Betulonic acid (3-oxo-19-(20S)-en-28-oic acid) predominated the Helidon white. Reduced lantadene A (22-(3-angeloyloxy-3 β -hydroxyolean-12-en-28-oic acid) and reduced lantadene B (22-(3-dimethylacetyloxy-3 β -hydroxyolean-12-en-28-oic acid) were also present in *Lantana camara*. Common pink taxa are non toxic and do not contain lantadene A and B.

Sharma *et al.* (1988b) isolated a partially purified preparation of toxin isolated from *Lantana camara* L, red variety leaves called fraction C, in 2 crystalline forms. Only form II was icterogenic to guinea pigs.

Sharma *et al.* (1990) isolated a noble triterpenoid, lantadene D from the leaves of hepatotoxic plant *L. camara*. Earlier it was named as compound 7. Its structure has been elucidated as 22- p-iso butyryloxy-3-oxoolean-12-en-oic acid. Further Sharma *et al.* (1991) compared the lantadene content and toxicity of different taxa of lantana plant and found that out of 3 taxa of *Lantana camara* var

aculeate, taxa I (white-pink) taxa II (yellow-pink) and taxa III (yellow-red), lantadene A, B, C are the major lantadenes of taxa III. Ingestion of lantana leaf powder of taxa III by male guinea pigs caused severe hepatotoxicity. Taxa I produced mild hepatotoxicity, while taxa II was non-toxic.

The lantana toxins are pentacyclic triterpenoids (Morton, 1994) and the toxicity of Lantadene A, reduced Lantadenes, and Lantadene C has been established (McSweeney, 1988; Ahmed and Roy, 1991 and Sharma *et al.*, 1992).

Begum *et al.* (2002) isolated a new pentacyclic triterpene named ursethoxy acid from the aerial part of *L. camara* (Linn) and its structure elucidated as (3, 25-epoxy-3 alpha-ethoxy-urs-12 ene-28 oic acid).

2.6 CLINICAL SYMPTOMS AND PATHOGENESIS

Livestock reared in the area infested with lantana shrub normally do not consume it as a matter of choice but they do so during the scarcity of other green vegetation in the pasture (Sharma *et al.*, 1981a). After browsing over a couple of twigs animals go off feed and severe constipation sets in. Two forms of lantana poisoning are generally encountered. In acute form the animal becomes sluggish, weak and refuses to take feed. The faeces are usually soft and contain blood. Paralysis of limbs may occur and death occurs in 3-4 days in progressive cases.

When lantana leaves are consumed in small quantity for a longer period of time, toxic symptoms are characterized by dullness, depression, off feed, excessive salivation, constipation and icteric mucous membrane. Violent head shaking especially when animal is exposed to sun light. Unpigmented areas of skin become swollen, hard and painful. The skin mainly of the muzzle may peel, leaving raw area. The membrane of the mouth, eyelids, ears, nose, thigh (inner side), udder and tail may be involved. Ear may become pendulous and swollen

(Dwivedi *et al.*, 1971; Yadava and Verma, 1978; Tokarnia *et al.*, 1984 and Ahmed and Roy, 1991)

Body temperature, pulse rate and respiration rate were found to be within normal limits during the early stages of the toxicity but rise in body temperature as high as 105°F in the peak stage of toxicity may be seen. There is recumbency and subnormal temperature in the terminal stages of the disease (Dhillon and Paul, 1971; Sastry and Singh, 1979 and Ali *et al.*, 1995).

There is decrease in gastrointestinal motility and liver dysfunction leading to photosensitization dermatitis (Dwivedi *et al.*, 1971; Silva and Couto, 1971; Tokarnia *et al.*, 1984 and Mandial *et al.*, 2000). In chronic phases, continuous dysfunction of intestinal smooth muscle giving rise to chronic constipation, inappetance and low water intake leading to dehydration and terminal nephrotoxicosis, nephrosis and finally uraemia is set up (Seawright, 1963; Black and Carter, 1985).

Lantana toxicity is manifested in three phases: The release and absorption of toxins in the gastrointestinal tract. The hepatic phase resulting in cholestasis, hyperbilirubinaemia, hyperphylloerythinaemia and finally the tissue phase where cell injury result from accumulation of bilirubin and phylloerythrin (Sharma *et al.*, 1988c).

The main pathophysiological feature in the mechanism of 'antana poisoning include- small amount of triterpene acid absorbed from digestive tract, continuous and slow absorption, ruminal stasis, liver injury with absorbed toxins and injury to bile canalicular membrane (Pass, 1986).

Dhillon and Paul (1971) recorded decrease in ruminal motility in experimentally induced lantana toxicosis in buffalo calves. McSweeney and Pass

(1983a) experimentally induced lantana toxicity in sheep at the dose rate of 4g per Kg b.wt. and ruminal stasis appeared to be due to two influences. There was decrease in ruminal motility and no direct effect of lantana toxin on the stretch and tactile receptor on the reticulum, vagus nerve and gastric center. It was observed that stasis of rumen was due to reduced feed intake and inhibitory reflex from damaged liver. The decreased rumen motility causes toxic material to be released in the rumen and continuous absorption of toxins from the rumen maintained the disease (Sharma *et al.*, 1988c).

The absorbed toxins interact with the unknown receptor on the hepatocyte followed by cascade of reaction leading to the development of cholestasis, regurgitation of bile and hepatic injury (Pass, 1986 and Sharma *et al.*, 1988c).

Seawright (1963) intoxicated sheep with lantana leaves and reported distention of gall bladder muscle. Pass and Heath (1977) suggested in an experimental trial that taurocholic acid (bile salt) retained during hepatic insufficiency, together with lantadene A, may contribute to paralysis of gall bladder.

Pass *et al.* (1979) in an experimental study in sheep recorded that lantana toxins did not seem to cause intestinal paralysis. Photosensitization in animals was also due to damage to the bile canaliculi and there was inhibition of bile secretion. During cholestasis, phyloerythrin from chlorophyll of lantana leaves get accumulated in the body, which is not cleared from the liver after damage (Radostits *et al.*, 1994). It has been observed by many workers that photosensitization and jaundice were the main clinical findings in lantana intoxication (Aloni, 1979; Tokarnia *et al.*, 1984; Kellerman and coetzer, 1984; Lin *et al.*, 1985; Lau, 1990 and Mandial *et al.*, 2000).

Composition of extra cellular fluid is also affected by lantana toxicity. It was observed in sheep given grinded lantana @ 4 g per Kg b.wt that in the early stages of the disease, the plasma electrolytes remained unchanged but some major changes like increased plasma osmolarity and metabolic acidosis occurs in the terminal stages of the disease (McSweeney and Pass, 1983b).

Lantana camara was also thought to produce immunosuppression in animals. The cell mediated immunity of intoxicated sheep @ 200 mg per kg b.wt. was assessed with the help of 2, 4- dinitrofluorobenzene skin sensitivity test and graft verses host reaction and antibody forming potential by haemagglutination reaction. There was significant reduction in both humoral and cell mediated immunity (Ganai and Jha, 1991). Garg *et al.* (1997) also reported antilymphocytic and immunosuppressive activity in rats given different dose rates of *Lantana camara*.

Thirunavukkarasu *et al.* (2000c) suggested myocardial hemorrhages in induced lantana poisoning (red variety). The lantana toxins interfere with the conduction of heart and bioelectric activity of myocardium is disrupted leading to death of animals.

Singh *et al.* (2003) reported an out break of *Lantana camara* toxicity in sheep flock in Uttar Pradesh. The clinical manifestations were salivation, lacrimation, constipation, anorexia, icteric mucous membrane, dry skin lesion, dry muzzle, grinding of teeth, peeling of skin from different parts of body especially head and neck and lesion of photosensitization were also noted.

2.7 HEMATO-BIOCHEMICAL CHANGES

All hemato-biochemical alteration has been reported in the experimentally induced lantana toxicity. Dwivedi *et al.* (1971) in clinical, hematological and

biochemical studies, observed leukocytosis, reduction in erythrocyte and haemoglobin values. There were increased serum proteins, globulin, total bilirubin and serum glutamic oxaloacetic transaminase activity with slight decrease in A/G ratio.

.Dhillon and Paul (1971) and Silva and Couto (1971) examined the urine of intoxicated buffalo calves and reported increased alkaline pH, intense yellow colour after two days of toxicity, presence of bile pigment, no bile salt and presence of albumin on 4th and 5th day of toxicity.

Uppal and Paul (1982) recorded significant rise in hematocrit percentage and significant fall in erythrocyte sedimentation rate, total serum proteins and fibrinogen.

Sharma *et al.* (1981b) studied the osmotic fragility of erythrocytes in calves with induced lantana poisoning and reported increased fragility of erythrocytes. They observed that the hemolysis started even at 0.72 per cent saline and was complete at a saline concentration of 0.44 per cent.

The increase in indirect and direct reacting bilirubin indicated a haemolytic effect of Icterogenin, a toxic principle of *L. camara* which has been reported to produce partial haemolysis of blood (Hari *et al.*, 1973). Similar observations were also noted with rise in icteric index and serum bilirubin in cattle and sheep in induced lantana toxicosis (Agarwal *et al.*, 1962; Sastry and Singh, 1979; Uppal and Paul 1978; Kaushal, 1994; Mandial and Randhawa, 1999 and Thirunavukkarasu *et al.*, 2001a). Delayed coagulation time was observed in lantana toxicosis (Uppal and Paul, 1978; Hussain and Roychoudhury, 1992a and Bahadureef *et al.*, 1992)

There was marked decrease in serum aspartate aminotransferase, alkaline phosphatase, glutamate dehydrogenase, sorbitol dehydrogenase and notable increase in the mean elimination time of bromosulfalein (BSP $T^{1/2}$) dye (Nauriyal and Gupta, 1983; Kalra *et al*, 1984; Aguilera *et al*, 1986; Bahadure *et al*, 1992; Mandial and Randhawa, 1999 and Thirunavukkarasu *et al*, 2001a).

Mandial and Randhawa (1996) produced experimental lantana toxicity in buffalo calves and reported significant increase in mean total and direct plasma bilirubin. Plasma gamma glutamate and Alkaline phosphatase activities were significantly increased indicating obstructive jaundice. Increased activity of Arginase, AST and ALT were also recorded which were suggestive of hepatic degeneration.

It has been suggested experimentally that increased percentage of dye retention in Bromosulfalein (BSP $t_{1/4}$) test, increased alkaline phosphatase, arginase and γ - glutamate transaminase are sensitive diagnostic indicator in lantana toxicity (Uppal and Paul, 1978 ; Mandial and Randhawa, 1999).

Marked increase in serum protein, blood glucose level and blood urea nitrogen was observed in induced lantana toxicated in sheep and cattle (Hari *et al*, 1973; Sastry and Singh, 1979; Nauriyal and Gupta, 1983 and Bahadure *et al*, 1992). Increased blood urea nitrogen in induced lantana toxicosis has also been reported by Kaushal (1994) and Thirunavukkarasu *et al* (2001a).

Significant increase in conjugated bilirubin, unconjugated bilirubin and acid phosphatase was also observed in guinea pig by oral administration of lantana toxin @ 125 mg per kg b.wt. (Sharma *et al*, 1989).

Singh *et al* (2003) recorded markedly increased aspartate amino transaminase, total bilirubin, lactate dehydrogenase, blood urea nitrogen,

creatinine and serum alkaline phosphatase in an out break of *Lantana camara* toxicity in sheep.

2.8 RUMEN LIQUOR EXAMINATION

McSweeney *et al.* (1983) observed decreased number of rumen protozoa and number of bacteria, increased rumen liquor pH, increased concentration of Na^+ , increased ammonia level and decreased TVFA in the rumen liquor of sheep with induced lantana toxicosis. Similarly, Kaushal (1994) reported complete absence of protozoal motility, decreased glucose fermentation activity, increased sedimentation activity and significantly lowered VFA in induced lantana toxicity in calves.

Along with these changes, toxicity in bovine resulted in change of rumen liquor color to deep brown, ammonical odour, viscous consistency followed by increased sedimentation activity time (SAT), increased methylene blue reduction time (MBRT), decreased glucose fermentation test (GFT) value and decreased total volatile fatty acid (Hussain and Roychoudhury, 1992b and Mandial and Randhawa, 1998).

2.9 PATHOLOGICAL CHANGES

2.9.1 GROSS PATHOLOGY

The carcass of lantana-intoxicated animals exhibited generalized jaundice, emaciated and congested streaks on sides of inguinal region. Seawright (1963) reported bile stained liver, greatly distended gall bladder with pale green bile, kidney was pale yellow and moist. Rumen content were dry, undigested, impaction of faeces in colon with hyperemic mucous membrane, colon wall was edematous and enlarged adrenal gland.

Dhillon and Singh (1965) and Dwivedi *et al.* (1971) reported similar changes along with yellowish discoloration of subcutaneous tissue, inflamed peritoneum and congestion from abomasum to rectum and necrotic foci on liver. Degenerated fat around the base of heart and petechiae and echymosis on the endocardium were also recorded.

Easily removable capsule of kidney, congestion of corticomedullary junction of kidney and congestion of lungs were also reported (Kalra *et al.*, 1984; Thirunavukkarasu *et al.*, 2001b).

2.9.2 HISTOPATHOLOGICAL CHANGES

Histopathology of intestine revealed desquamated epithelium, congestion and hemorrhages in mucosa and congested submucosa. In spleen white pulp was atrophied with hemosiderin pigment in splenic tissue. Liver showed hydropic degeneration with extensive vacuolization of cytoplasm and necrosis of parenchymal cell (Dhillon and Singh, 1965). Youssef *et al.* (1984) observed proliferation in epithelium of bile duct, biliary cirrhosis and formation of newly formed ductules. In the lungs, interstitial proliferation of mesenchymal elements were recorded in the later stages.

In kidney, there was tubular degeneration and necrosis, congestion in cortex and medulla, urinary tubules of cortex filled with eosinophilic hyaline cast, glomeruli were hyperemic and swollen (Dwivedi *et al.*, 1971; Kalra *et al.*, 1984).

In histopathology of gall bladder, there was interstitial edema with polymorphonuclear and mononuclear cell infiltration along with degeneration of smooth muscle fibers (Seawright, 1963; Pass *et al.*, 1979).

Similar histopathological changes were also recorded in kangaroos (Johnson and Jensen, 1998) and in cow calves by Thirunavukkarasu *et al.* (2001b).

2.10 THERAPEUTIC MANAGEMENT

Lai and Kalra (1960) suggested to discontinue feeding and to keep the animal in shade. They also suggested the use of saline purgative, glucose saline by intravenous or subcutaneous or rectal route. To restore liver function, gruel, linseed tea, milk etc. as demulcent for GIT mucosa and caffeine et sodii benzoas sub-cutaneously as cardiac and respiratory stimulant.

Aloni (1979) recommended the treatment of animals for a period of a fortnight. Animals recovered fully after stopping their grazing in pasture, giving them sodium thiosulphate 120 g per 100 Kg b.wt. He also tried Inj. Avil 5 ml I/M once, drenching of jaggary 250 g twice daily in water, dextrose sodium chloride (5 per cent) 1000 ml I/V, Inj. Benzyl penicillin @ 20 lacs I/M daily for 4 days, Inj. Prepalin forte @ 9000 IU on alternate days, Inj. Belamyl 5 ml I/M biweekly, ointment zinc oxide + boric acid topically.

Hari *et al.* (1973) studied the efficacy of certain drugs in lantana poisoning in buffalo calves dosed with varying doses of lantana powder. They observed that treatment with 20 per cent glucose saline along with antihistaminic and liver extract was better than that of 5 per cent glucose saline and antihistaminic only.

Sastry and Singh (1979) provided symptomatic treatment as saline purgative, general appetizer followed by I/V saline and molasses. Oral administration of molasses was found very effective and convenient under field conditions.

Pass and Stewart (1984) used activated charcoal @ 500g in sheep and 2-2.5 Kg to cow, along with 4 liter, and 20-30 liters electrolyte respectively. Supportive therapy was also recommended

As the toxic content remain in the rumen stasis and absorbed continuously. McSweeney and Pass (1982) were able to reverse the lantana toxicity in sheep by performing rumenotomy followed by removal of rumen content and replacing them with suitable substitute along with supportive therapy.

Pass (1986) recommended treatment with large quantity of activated charcoal into the rumen together with large volume of electrolyte in lantana poisoning in ruminants. Similarly McSweeney (1988) recommended treatment with activated charcoal @ 500g to sheep and 2.5 kg to cattle in 4 liters and 20 liters of electrolyte solution, respectively along with rehydration and photosensitization therapy.

McLennan and Amos (1989) studied the effect of activated charcoal in naturally poisoned cattle by administering activated charcoal @ 5 g per kg body weight in 10-20 liters of water plus multiple electrolyte solution by stomach tube immediately and after 9 days of diagnosis of lantana poisoning. The mean time of clinical recovery after early treatments was 11 days and calves treated after 9 days required electrolyte and procaine penicillin for photosensitive dermatitis.

McKenzie (1991) and Sharma (1998) compared the efficacy of bentonite and charcoal powder. Both were used @ 5 g per kg b.wt. Though the bentonite was cheaper alternative but activated charcoal was found to be better than bentonite.

Therapeutic efficacy of certain drug combinations namely activated charcoal, rumenotomics, electrolytes, liver extract, antihistaminic and saline

purgative was assessed in experimentally induced lantana toxicosis in cow calves (Kaushal, 1994). It was concluded that treatment with activated charcoal, rumenotorics along with 5 per cent glucose saline, liver extract and antihistaminic proved highly effective.

Ali *et al.* (1996) treated lantana toxicated goats with 25 per cent dextrose, injections of vitamin B-complex with liver extract with or without activated charcoal. They concluded that goats treated with activated charcoal along with other therapy recovered earlier.

Mandial and Randhawa (1996) treated experimentally induced lantana toxicated calves with combined therapy comprising of activated charcoal @ 5 g per Kg b.wt. with 5 liters of multielectrolyte solution along with liver extract and dextrose normal saline given at 12 hr post intoxication and concluded that treatment regimen provided, proved 100 per cent effective against this toxicosis.

Pal *et al.* (1997) reported that out of 8 clinical cases of lantana poisoning, 5 cattle responded well to combined therapy comprising 5 per cent dextrose given parenterally, liver extract with B-complex, antihistaminic drug along with oral administration of magnesium sulphate (2.5 g per kg b. wt.). Similarly Mandial *et al.* (2000) studied 39 cases of lantana poisoning and reported that 85 per cent cases were saved with activated charcoal along with supportive therapy.

Vaccination was also thought as a possible preventive mean in lantana toxicosis. The toxic triterpene acids lantadene A and lantadene B were isolated and conjugated to bovine serum albumin or haemocyanin. This was emulsified and injected into sheep and cattle. After inducing lantana poisoning, cholestasis was found less severe in vaccinated animals (Stewart *et al.*, 1988).

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

3.1 SOURCE OF ANIMALS

The present study was conducted on 30 cows with the history of lantana ingestion. These clinical cases were either presented in the Clinics of College of Veterinary and Animal sciences, Palampur or attended at nearby veterinary hospitals and dispensaries. The detail of the place and number of animals attended are shown in the Table 3.1

Table 3.1 Source of animals (n=30)

S.No.	Name of Village /Place	No. of cases
1	Clinic COVAS	9
2	Bhawarna	6
3	Patti	3
4	Tanda	3
5	Latwala	2
6	Mansimbal	2
7	Molichuk	
8	Paprola	
9	Dehan	
10	Khalet	
11 .	Maghemu	

These clinical cases of lantana ingestion were divided into two groups. The animals presented before 3rd day of lantana ingestion were considered as early

cases and those on 4 day and onwards were classified as late cases.(Table 3.2)

Table 3.2 Distribution of lantana toxicity cases

S. No	Day of presentation	Type of group	No. of animals
1	Upto 3 days	Early cases	13(43.3)
2	After 3 days	Late cases	17(56.66)

Figures in parenthesis indicate per cent

3.2 HISTORY, CLINICAL OBSERVATIONS AND EXAMINATION

A comprehensive history comprised of description of patient with respect to age, sex, breed, type of feeding (stall feeding or grazing), lantana infestation in the area, owner's chief complaint *about* the animal and the main symptom observed, time of onset of symptom, previous treatment if any and response thereof was recorded. Recent status of appetite, rumination, water intake, defaecation and urination were also observed.

Clinical examination included records of rectal temperature, heart rate, respiration rate, condition of conjunctival mucous membrane, muzzle and skin condition and any swelling on any body part. Assessment of degree of dehydration was done as per the clinical sign and on the bases of packed cell volume.

3.3 HAEMATOLOGICAL AND BIOCHEMICAL STUDIES

3.3.1 Blood Collection

About 10-12 ml of blood was collected aseptically from the jugular vein in a sterilized vial of 20 ml capacity with heparin as anticoagulant, 1 ml of blood was also collected without anticoagulant and used to fill at least 9-10 capillaries immediately to determine the blood clotting time. Immediately after the collection of blood, plasma was separated from about 8-10 ml of blood by centrifuging at

3000 rpm for 10 min and stored at -4°C (till further use). Remaining whole blood was utilized for haematological studies and to determine osmotic fragility of erythrocytes. Blood samples were also collected from the recovering animals after an interval of seven days.

3.3.2 Haematological studies: The following hematological parameters were determined immediately after collection of blood as per the methods described by Jain (1986) and Benjamin (1998).

A) Hemoglobin (Hb): Hb was determined by acid-haematin method and expressed in g/dl of blood.

B) Packed cell volume (PCV): PCV was estimated by microhaematocrit method and expressed in per cent.

C) Total erythrocyte count (TEC): TEC was determined by using haemocytometer and expressed in million / microlitre ($\times 10^6/\mu\text{l}$) of blood.

D) Total leukocyte count (TLC): TLC was determined by using haemocytometer and was expressed in thousand / microlitre ($\times 10^3/\mu\text{l}$) of blood.

E) Differential leukocyte count (DLC): Thin blood smear were prepared on clean grease free microscopic slides immediately at the time of blood collection. These were stained with Leishman's stain as per the standard procedure. The stained smears were observed and studied carefully under oil immersion lens of microscope to assess and appreciate the pattern of different types of leukocyte in the blood. Proportion of different leukocytic cell types were expressed as per cent.

F) Erythrocytic indices: The packed cell volume, erythrocytic count and haemoglobin concentration were used for calculation of erythrocytic indices as given below:

1. Mean Corpuscular Volume (MCV):

$$\text{MCV} = \frac{\text{PCV (\%)}}{\text{RBCs (millions/ (.il))}} \times 10^3 \text{ and expressed in femto litre (fl)}$$

2. Mean Corpuscular Haemoglobin (MCH):

$$\text{MCH} = \frac{\text{Hb(g/100 ml)}}{\text{RBCs (millions/^(^))}} \times 10^3 \text{ and expressed as picograms (pg)}$$

3. Mean Corpuscular Haemoglobin Concentration (MCHC):

$$\text{MCHC} = \frac{\text{Hb(g/dl)}}{\text{PCV (\%)}} \times 100 \text{ and expressed as (\%)}$$

G) Blood clotting time : Blood clotting time was determined by capillary tube method and expressed in minutes.

H) Erythrocyte fragility test:

Erythrocyte osmotic fragility test was done by standard method (Jain, 1986). 5 ml each of various concentration of phosphate buffered sodium chloride solution ranging from 0.85 to 0.0 per cent in 16 centrifuge tubes were taken. To each concentration 0.02 ml of heparinised blood was added. The tubes were then kept for 30 minutes at room temperature and then centrifuged for 5 min at 2000 rpm and optical density of supernatant was measured on spectrophotometer at 540nm. For calculating per cent hemolysis tube no. one was taken as blank and

tube no. 16 was considered having the 100 percent hemolysis. The per cent hemolysis was calculated as:

$$\% \text{ Hemolysis} = \frac{\text{Optical density of unknown}}{\text{Optical density of tube no. 16}} \times 100$$

3.3.3 Blood biochemical estimation

A) Aspartate aminotransferase (AST) : Levels of AST was determined by Reitman and Frankel's method (1957) using AST kit*. Readings were taken at 505 nm on spectrophotometer and values expressed in IU/l.

B) Alanine aminotransferase: ALT was determined by the Reitman and Frankel's method (1957) using ALT kit*. Readings were taken at 505nm on spectrophotometer and values expressed in IU/l.

C) Blood glucose : was estimated by GOD-POD method using glucose kit*. Readings were taken on spectrophotometer *'' at 505 nm. The values were expressed in mg / dl.

D) Total protein: was determined by biuret method using a total protein kit*. The readings were taken at 555 nm on the spectrophotometer and results were expressed in g / dl.

E) Albumin : Albumin was estimated by using the total protein kit*. The readings were taken on spectrophotometer at 630 nm and the values were expressed in g/dl.

F) Globulin : As per the method given in the total protein kit and the values of globulin expressed in g / dl.

G) A/G Ratio : A /G ratio was calculated as the ratio of the albumin (g / dl) and globulin (g / dl)

H) Blood urea nitrogen (BUN): was estimated by diacetyl monoxime method using BUN kit and using spectrophotometer at 520 nm. The values were expressed in mg/dl.

I) Total bilirubin : was estimated by modified Jendrossik and Grof method by using bilirubin kit *. Results were expressed in mg / dl.

J) Alkaline phosphatase: Alkaline phosphatase was done by kind and King's method by using alkaline phosphatase kit* and the results were expressed in IU/L.

K) Acid phosphatase : Acid phosphatase was done by King's method by using acid phosphatase Kit". Readings were taken on spectrophotometer at 510nm and the results were expressed in KA units.

L) Vandenbergh Test: The test was done as per the method described by Benjamin(1998). The result were expressed as direct positive (conjugated), indirect positive (unconjugated) and both positive (biphasic reaction).

* For all the biochemical estimations, kits used were manufactured by Sigma diagnostic (1) Pvt. Ltd. 321 GIDC estate, Por Ramangamdi, Distt. Baroda 391243, Gujrat.

Marketed by : Qualligens fine chemical, a division of Glaxo India Ltd., Dr. Annie Besant road, Warli, Mumbai - 400025.

** Acid phosphatase was done using Acid phosphatase Kit by Span Diagnostic Ltd. Plot No. 336,338,340. road No. 3, G.I.D.C..SACHIN 394 230 (Surat) INDIA.

*** Spectronic 20-D (Milton Roy Company, USA)

3.4 RUMEN LIQUOR EXAMINATION

3.4.1 Collection of rumen liquor

Rumen liquor was collected for investigating physical characteristic, microbial and biochemical changes. The collection was made from left lower para lumbar fossa by a 3 inches long needle of 16 gauge and a dry hypodermic 20 ml capacity syringe or by stomach tube in few cases. The parameters like pH, protozoal motility, glucose fermentation test, sedimentation activity test was determined immediately after collection while for others, rumen liquor **weyc** preserved using saturated mercuric chloride solution at the rate of 1 ml for 10 ml of rumen liquor.

3.4.2 Examination of rumen liquor:

- A) Physical Characteristics:** Physical characteristics viz; odour, color and consistency were determined as per the method described by Rosenberger (1979).
- B) pH of rumen liquor :** pH was determined by using narrow range pH indicator (BDH) paper strips.
- C) Protozoal Motility:** The rumen liquor protozoal motility was determined as per the method of Misra and Singh (1974). It was graded as plenty (+++), moderate (++) and scanty (++).
- D) Glucose Fermentation Test (GFT):** Glucose fermentation test was performed by using Sacchrometer as per the method of Rosenberger (1979) and expressed as ml/hr.
- E) Sedimentation Activity Time (SAT):** SAT was performed as per the method described by Nicholas and Penn (1958) and the result was expressed in min.

F) Total Volatile Fatty Acid (TVFA) : The TVFA concentration in rumen liquor was determined by using Markham's MicroKjeldahal distillation apparatus as per the method of Annison (1954)

3.5 THERAPEUTIC MANAGEMENT

These lantana intoxicated animals (both early and late cases) were treated with combined therapy for at least 3 to 4 days. The therapy comprised of:

(1) Activated charcoal*: given @ 5 g per Kg b.wt. orally once at the day of presentation of case in both groups.

(2) Magnesium sulphate: given @ 300 to 400 g orally in luke warm water two times on alternate days.

(3) Inj. Belamyl:** given @ 5-10 ml I/M (depending on body weight) for three days continuously and then 3 more injection on alternate days .

(4) Inj. Avil Vet*:** given @ 5-10 ml I/ M for 3 days.

(5) Bolus Rumentas**:** Given @ 2 boli bid for 3 days.

(6) Inj. Mifex***:** given @ 100-150 ml slow I/V once in all cases and 50-100 ml by S/C route and further in some cases depending upon the condition.

* Marketed by S.D. Fine Chemical Ltd., Mumbai.

"Marketed by Sarabhai Zydus Animal Health Ltd., Vadodara and contains Thiamine hydrochloride 10 mg, Riboflavin 3 mg, Niacinamide 100 mg, crude liver extract 0.66 ml / ml (having vit. B12 activity equivalent to not less than 2 meg of cynocobalamine /ml) and phenol 0.5 %.

***Marketed by Intervet (India) Ltd., Chennai. It contains pheniramine maleate 22.75 mg/ml.

**** Marketed by Intas-Neovet Pharmaceutical Ltd., Ahmedabad, It contain Antimony Potassium tartarate 2 g, Ferrous sulphate 2 g, Copper sulphate (anhydrous) 50 mg, Cobalt chloride 100 mg.

***** Marketed by Novartis India Ltd., Animal Health Sector, Mumbai. It contains Calcium borogluconate 25 % w/v, Magnesium hypophosphite 5% w/v and Dextrose anhydrous 20 % w/v.

Evaluation of treatment

The therapeutic efficacy of the regimen followed was evaluated on the bases of following:

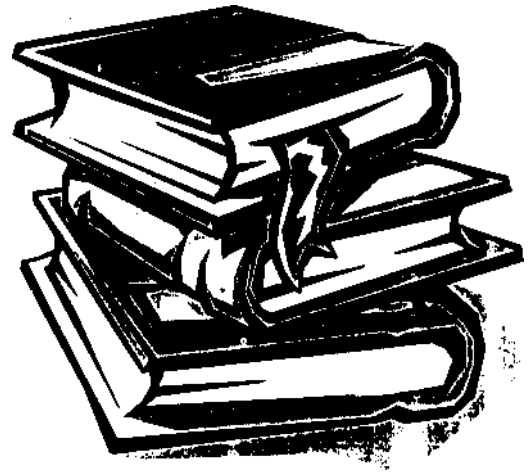
- Post treatment haemato-biochemical profile
- Improvement in the clinical parameter and condition
- Resumption of appetite

3.6 CONTROL GROUP

Total of ten clinically normal cattle were taken as control animals. Normal clinical parameters, haematology, rumen liquor examination and blood biochemical profile were obtained from this group.

3.7 STATISTICAL ANALYSIS

The data obtained were subjected to one way analysis of variance and Turkey - Kramer multiple comparison tests at 1% and 5 % level of significance and Dunnet test at 5 % and 1 % level of significance by using computer software Instate from Graphpad software, San Diego, USA (Russell, 1990).



RESULTS

CHAPTER IV

RESULTS

4.1 OBSERVATIONS ON CONTROL GROUP ANIMALS

To compare the results of various aspects of study on the clinical cases of lantana toxicosis, 10 healthy adult female cattle of Govt. Dairy Farm, Banuri were selected, which served as the control group. Their clinical observations, haematology, rumen liquor examination and biochemical profile are as under:

4.1.1 CLINICAL OBSERVATIONS

All the cattle taken as control group were inspected and examined. They were clinically found healthy, active, alert and had normal appetite, defecation and urination.

Table 4.1 Clinical observations in normal cattle (n= 10)

S. No.	Parameters	Values (Mean ± S.E.)
1	Rectal temperature (°F)	100.68 ±0.14
2	Heart rate (per min)	67.50 ±2.05
3	Respiration rate (per min)	20.9 ±1.23
4	Conjunctival mucous membrane	Pinkish
5	Skin coat condition	Normal
6	Muzzle	Wet
7	Urination and defecation	Normal
8	Faecal examination	NAD

The mean values of rectal temperature, heart rate and respiration rate were 100.68 ± 0.14 F, 67.50 ± 2.05 per min and 20.90 ± 1.23 per min respectively. The

conjunctival mucous membrane was pinkish in appearance. These animals were screened for the presence of parasitic infestation by direct smear examination and all were found negative (Table 4.1).

4.1.2 HAEMATOLOGICAL EXAMINATION

The results of the haematological parameters are given in the Table 4.2. The mean values of haemoglobin, packed cell volume, total erythrocyte count and

Table 4.2 Haematological parameters in healthy cattle (n=10)

sTisTor	Parameters	Values (Mean ± S.E.)
1	Blood clotting time (min)	6.67 ± 0.55
2	Hb (g/dl)	10.26 ±0.20
3	PCV (%)	37.30 ± 0.35
4	TEC($\times 10^6$ /pI)	7.99 ± 0.32
5	TLC($\times 10^3$ /MI)	7.50 ± 0.77
6	MCV (fl)	47.25 ± 2.48
7	MCH (pg)	12.89 ±0.43
8	MCHC(%)	27.85 ±1.22
9	Lymphocyte (%)	68.0 ±1.62
10	Neutrophils (%)	25.0 ±1.36
11	Monocyte (%)	3.50 ±0.58
12	Eosinophils (%)	3.80 ±1.74

total leukocyte count were recorded to be 10.26 ± 0.20 g / dl, 37.30 ± 0.35 %, 7.99 ± 0.32 $\times 10^6$ / pi) and 7.50 ± 0.77 $\times 10^3$ / pi respectively. Differential leukocyte count revealed 25.0 ± 1.36 per cent neutrophils, 68.0 ± 1.62 per cent lymphocyte, 3.50 ± 0.58 per cent monocyte and 3.80 ± 1.74 per cent eosinophils.

The mean values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

were observed as 47.25 ± 2.48 fl, 12.89 ± 0.43 pg and 27.85 ± 1.22 per cent respectively. Mean blood clotting time was 6.67 ± 0.55 min.

4.1.3 OSMOTIC ERYTHROCYTIC FRAGILITY TEST

The osmotic fragility of erythrocyte of cattle at various concentrations of hypotonic saline solution is presented in the Table 4.3. The fragility increased as

Table 4.3 Osmotic fragility of erythrocyte in healthy cattle (n=10)

S. No.	% NaCl solution	Per cent hemolysis
1	0.85	-
2	0.80	-
3	0.75	0.86 ± 0.44
4	0.70	1.11 ± 0.64
5	0.65	3.45 ± 1.72
6	0.60	10.45 ± 2.14
7	0.55	19.92 ± 4.24
8	0.50	31.31 ± 4.91
9	0.45	64.38 ± 5.65
10	0.40	79.50 ± 3.84
11	0.35	96.55 ± 2.58
12	0.30	99.81 ± 2.93
13	0.25	100.0 ± 3.43
14	0.20	-
15	0.10	-

the concentration of saline solution decreased. The osmotic fragility of erythrocyte showed sigmoid curve when per cent hemolysis was plotted against various concentrations of hypotonic saline solution (Fig.4.1). The mean corpuscular fragility (concentration of NaCl solution producing 50 per cent hemolysis) was found to be 0.47 per cent NaCl solution. The minimum resistance (initial hemolysis) was at 0.70 per cent saline solution and complete hemolysis (maximum resistance) was at 0.25 per cent saline solution.

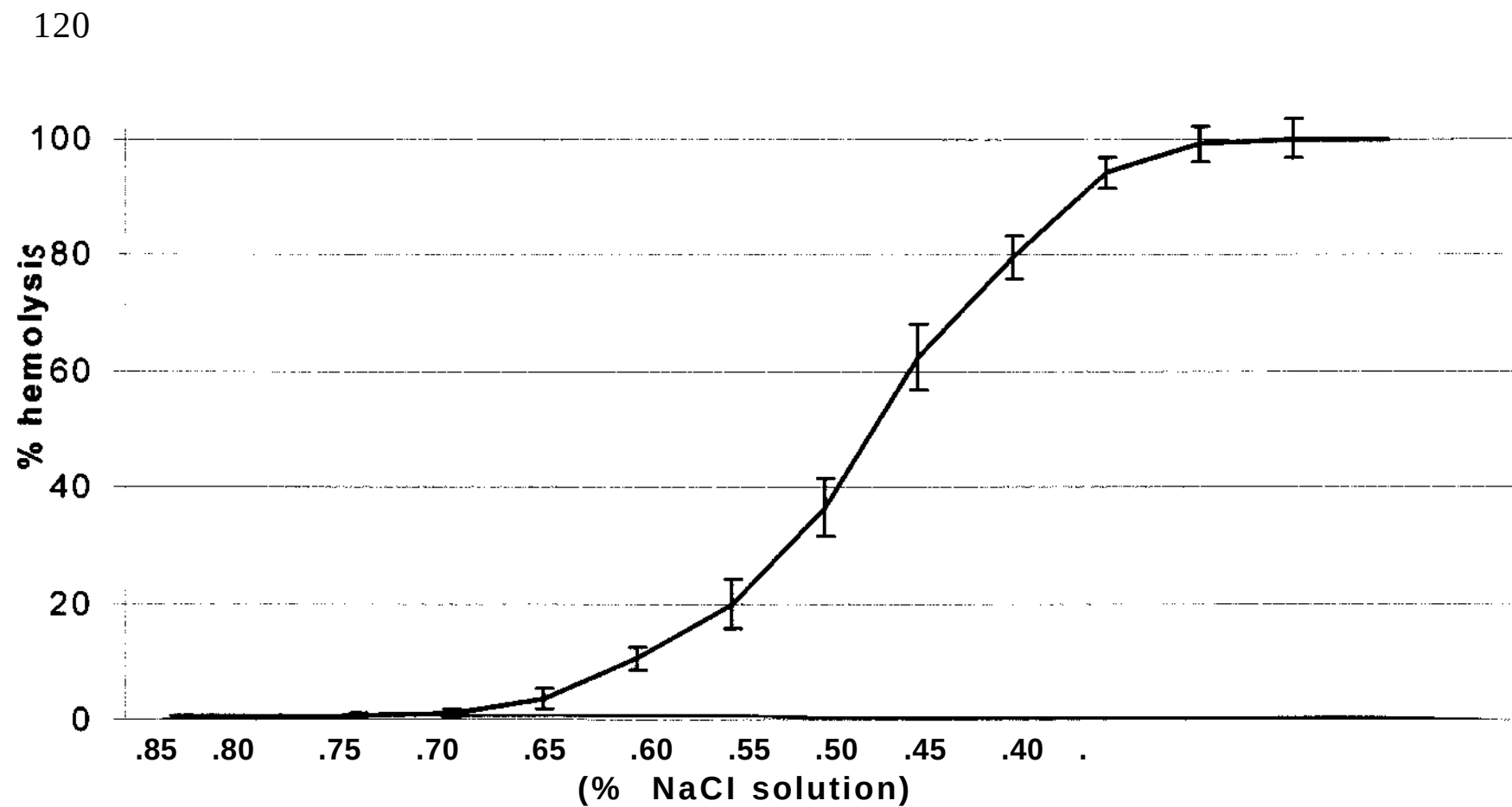
4.1.4 BLOOD BIOCHEMICAL PROFILE

The results of blood biochemical studies have been presented in the Table 4.4. Mean blood glucose, blood urea nitrogen and total bilirubin values were recorded as 50.96 ± 1.76 mg/dl, 15.84 ± 1.25 mg/dl and 0.42 ± 0.02 mg/dl respectively. The mean values of total protein, albumin, globulin and A/G ratio were 6.64 ± 0.35 g/dl, 3.08 ± 0.08 g/dl, 3.56 ± 0.15 g/dl and 0.86 ± 0.02 respectively.

Table 4.4 Blood biochemical profile in healthy cattle (n=10)

S. No.	Parameters	Values (Mean $\pm$ S.E.)
1	Blood glucose (mg/dl)	50.96 ± 1.76
2	Total protein (g/dl)	6.64 ± 0.35
3	Albumin (g/dl)	3.08 ± 0.08
4	Globulin (g/dl)	3.56 ± 0.15
5	A/G ratio	0.86 ± 0.02
6	AST (IU/l)	60.40 ± 4.76
7	ALT (IU/l)	35.70 ± 2.10
8	BUN (mg/dl)	15.84 ± 1.25
9	Alkaline phosphatase (IU/l)	44.95 ± 4.00
10	Acid phosphatase (KA units)	1.82 ± 0.23
11	Total bilirubin (mg /dl)	0.42 ± 0.02

Fig.4.1 Osmotic fragility of erythrocyte in hea



The mean values of aspartate aminotransferase (AST), alanine aminotransferas (ALT), alkaline phosphatase (AP) and acid phosphatase were 60.40 ± 4.76 IU/1, 35.70 ± 2.10 IU/L, 44.95 ± 4.00 IU/I and 1.82 ± 0.23 KA units respectively.

4.1.5 RUMEN LIQUOR EXAMINATION

Physical and microbial examination of rumen liquor of control animal revealed pH value of 7.08 ± 0.11 with greenish colour, aromatic odour, slightly viscous in consistency and protozoal motility was ++ to +++ . The mean values c sedimentation activity time, glucose fermentation test and TVFA were 6.38 ± 0.6 min, 2.14 ± 0.21 ml/ hr. and 87.08 ± 4.73 mEq /1 respectively (Table 4.5).

Table 4.5 Rumen liquor evaluation in healthy cattle (n=10)

S. No.	Parameters	Values (Mean $\pm$ S.E)
1	PH	7.08 ± 0.11
2	Colour	Greenish
3	Consistency	Slightly viscous
4	Odour	Aromatic
5	Protozoal motility	+ + to + + +
6	SAT (min)	6.38 ± 0.63
7	GFT (ml /hr)	2.14 ± 0.21
8	Total VFA (mEq /l)	87.08 ± 4.73

4.2 OBSERVATIONS ON DISEASED ANIMALS

4.2.1 DISTRIBUTION OF CASES

The clinical cases of lantana toxicity were presented at different intervce (days) after the ingestion of lantana (Table 4.6). Out of total 30 cases, 13 case (43.33 per cent) were presented before 3 days of lantana ingestion and thu:

classified as early cases, where as 17 cases (56.67 per cent) were presented on 4th days onward and so constituted late cases (Table 4.7).

Table 4.6 Presentation pattern of lantana toxicity cases

S. No.	Day of presentation of case	No. of animals	Per cent
1	1 st	3	10.00
2	2 nd	6	20.00
3	3 rd	4	13.33
4	4 th	5	16.67
5	5 th	3	10.00
6	6 th	2	6.67
7	7 th	1	3.33
8	8 th	3	10.00
9	9 th	1	3.33
10	>10 th	2	6.67

4.2.2 OBSERVATIONS IN RELATION TO AGE, SEX AND BREED

The occurrence of lantana toxicity in relation to age, sex and breed is summerised in Table 4.7, Figs. 4.2, 4.3 and 4.4. Maximum occurrence was recorded in younger age group. As many as 53.33 per cent cases (16 cattle) were below 1 year of age and remaining 14 (46.67 per cent) were between 1 to 7 years of age (Table 4.7, Fig. 4.2).The incidence of toxicity was much more in female cattle (83.33 per cent) than

males (Fig.4.3). Breed wise, maximum cases were recorded in Jersey cross (15 cases, 50.00 per cent) followed by local cattle (13 cases, 43.33 per cent) and only two animals (6.67 per cent) were of Holstein breed (Table 4.7 and Fig.4.4).

Table 4.7 Incidence of lantana toxicity *w.r.t.* age, sex and breed

Parameters		No. of animals	Per cent
Age	< months 6	6	20.00
	6 to 12 months	10	33.33
	1 to 4 years	5	16.67
	4 to 7 years	7	23.33
	> 7 years	2	6.67
Sex	Young male	1	3.33
	Young female	15	50.0
	Adult male	4	13.33
	Adult female	10	33.33
Breed	Local breed	13	43.33
	Jersey cross	15	50.00
	H.F.	2	6.67

Fig. 4.2 Age wise incidence of lantana toxicity in cattle

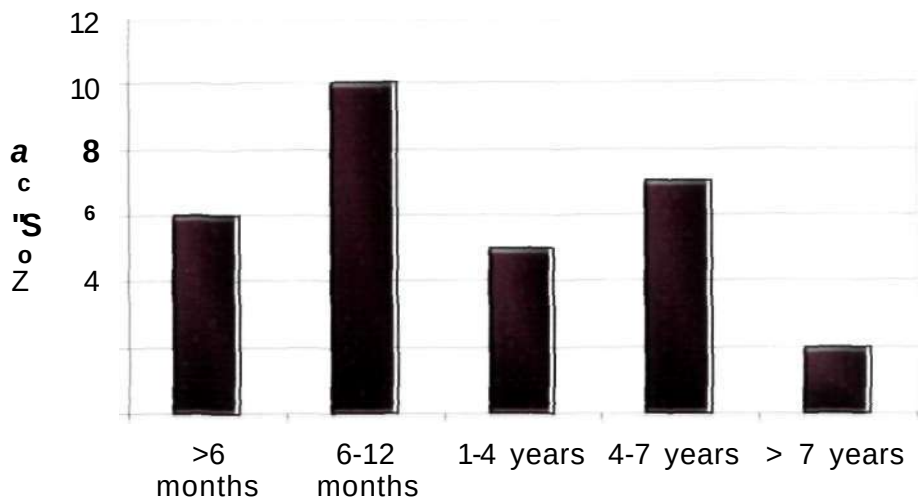


Fig. 4.3 Sex wise incidence of lantana toxicity in cattle



Fig. 4.4 Breed wise incidence of lantana toxicity in cattle



4.2.3 CLINICAL MANIFESTATIONS AND OBSERVATIONS

Difference was there in clinical manifestations of early and late cases (Table 4.8).

A) Signs in early cases

There was reduced appetite on first day of lantana ingestion and anorexia aflor 2nd day unwinds, cunslipatud fuocus, lacrimal discharge, swelling of eye lids

Table 4.8 Clinical observations in lantana intoxicated animals.

S. No.	Clinical signs	Early cases	Late cases	Total no. of animals
1	Eye lid swelling	4	3	7
2	Lacrimal discharge	2	1	3
3	Swelling of ear base and ear pinna	5	2	7
4	Drooping ears	3	7	10
5	Cracks over the ear skin	-	1	1
6	Redness of pinna	-	1	1
7	Cracks on muzzle	-	3	3
8	Dehydration (skin tenting)	2	15	17
9	Conjunctival mucous membrane			
	Pinkish	3	-	3
	Icteric	7	13	20
	congested	-	5	5
10	Recumbency	-	4	4

males (Fig.4.3). Breed wise, maximum cases were recorded in Jersey cross (1 cases, 50.00 per cent) followed by local cattle (13 cases, 43.33 per cent) and on two animals (6.67 per cent) were of Holstein breed (Table 4.7 and Fig.4.4).

Table 4.7 Incidence of lantana toxicity *w.r.t.* age, sex and breed

Parameters		No. of animals	Per cent
Age	< months 6	6	20.00
	6 to 12 months	10	33.33
	1 to 4 years	5	16.66
	4 to 7 years	7	23.33
	> 7 years	2	6.66
Sex	Young male	1	3.33
	Young female	15	50.0
	Adult male	4	13.30
	Adult female	10	33.33
Breed	Local breed	13	43.33
	Jersey cross	15	50.00
	H.F.	2	6.67

and ear base, decreased water intake, slight decrease in urination with dark yellow colour, muzzle started drying after 2nd day of ingestion. The animal examined on 3rd day revealed slight yellowish conjunctival mucus membrane. Rumen motility was nil after 2nd day of lantana ingestion, animals were dull, lethargic and preferred to be in shade

B) Signs observed in late cases

There was anorexia, decreased water intake, decreased urination with darker yellow colour urine. Cessation of urination on 8-9th day was also noted. Constipated faeces in all late cases and in few cases faeces was mucous coated. Conjunctival

Table 4.9 General clinical observations in lantana toxicity (Mean $\pm$ S.E)

S. No.	Parameters	Control	Early	Late	All cases
1	RT(F)	100.68 $\pm$ 0.14	102.76 $\pm$ 0.16**	100.16 $\pm$ 0.39*	101.90 $\pm$ 0.27**
2	HR (per min)	67.50 $\pm$ 2.05	72.0 $\pm$ 1.24	70.70 $\pm$ 3.58	71.56 $\pm$ 2.02
3	RR (per min)	20.90 $\pm$ 1.23	21.31 $\pm$ 1.50	25.50 $\pm$ 4.12	22.10 $\pm$ 1.26
4	Conjunctival mucous membrane	Pinkish	Pinkish to slightly pale	Congested to icteric	Icteric
5	Hair coat	Normal	Normal	Rough	Rough
6	Muzzle	Wet	Slightly dry	Completely dry	Dry to cracked
7	Dehydration status based on skin tenting	Normal	normal	+ to ++	+

* Significant at 5% level (P< 0.05) ** Significant at 1% level (P< 0.01)

mucous membrane was icteric (76.7 per cent). Cracks on muzzle, sloughing of skin over ear was also observed in these cases and 41.9 per cent cases also showed drooping of ears. Dehydration was there in animals suffering from lantana toxicity. The hair coat was rough in all the cases.

The mean values of rectal temperature, heart rate and respiration rate of animals suffering from lantana toxicity are given in the Table 4.9. The mean rectal temperature

Fig. 4.5 Value of Rectal temp, in different groups of lantana toxicity in cattle

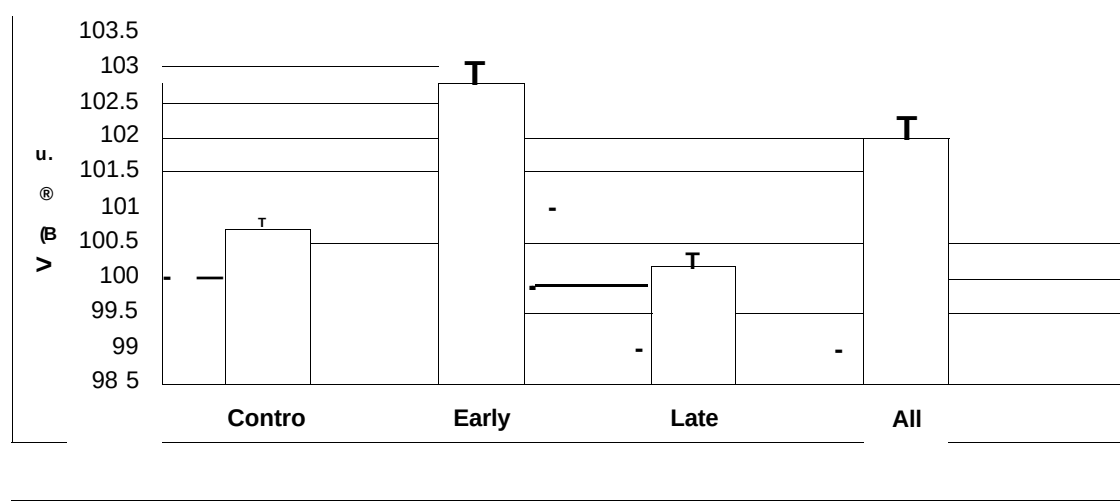
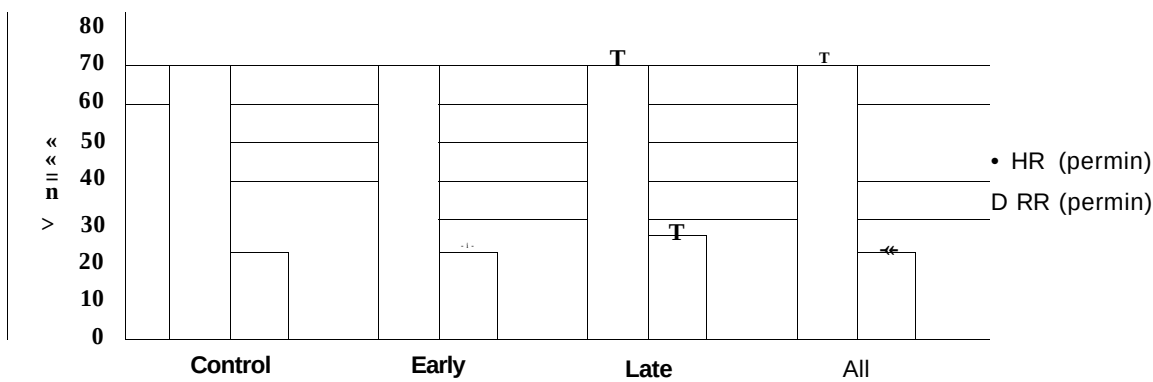


Fig. 4.6 Heart and respiration rate in lantana toxicity



was recorded to be $101.90 \pm 0.27^{\circ}$ F which was significantly ($P<0.05$) higher than the control group (Fig. 4.5). Heart rate was insignificantly increased in early ($72 \pm$

1.24 per min) and in late cases (70.70 ± 3.58 per min.) than control group animals (67.50 ± 2.05 per min). Similarly mean respiration rate (22.10 ± 1.26 per min) of all the affected cases showed no significant change than the control group (Table 4.9, Fig. 4.6).

Dehydration status in lantana toxicity

Among all the 30 cases, 17 cases (56.66 per cent) showed different degrees of dehydration (Table 4.10). On the basis of PCV per cent, in early cases only 2 animals

Table 4.10 Dehydration status in lantana toxicity cases

S. No.	Skin fold tenting (sec)	PCV (%)	Per cent dehydration	No. of early cases	No. of late cases
1	-	40-45	4-6	1(7.69)	7(41.17)
2	2-4	45-50	6-8	1(7.69)	3(17.64)
3	6-10	50-55	8-10	-	4(23.52)
4	20-45	55-60	10-12	-	1(5.88)

Figures in parenthesis indicate per cent

(15.38 %) were showing the dehydration; One with mild dehydration (+) and other with moderate dehydration (++). In late cases 7 animals (41.17 per cent) were having mild (+), 3 animals (17.64 per cent) with moderate (++) and 4 animal (23.52 per cent) were severely dehydrated (+++). While one animal (5.88 per cent) was having very severe dehydration (++++)

e 4.11 Haematological profile in lantana toxicity (Mean $\pm$ S.E.)

S. No.	Parameters	Control	Early (n=13)	Late (n = 17)	All diseased cases (n = 30)
1	Hb(g/dl)	10.26 $\pm$ 0.20	11.31 $\pm$ 0.47	11.96 $\pm$ 0.53	11.68 $\pm$ 0.53
2	PCV (%)	37.30 $\pm$ 1.35	35.46 $\pm$ 1.43	45.24 $\pm$ 2.16**	40.86 $\pm$ 1.61
3	TEC(X 10 ⁶ / JJl)	7.99 $\pm$ 0.31	8.39 $\pm$ 0.58	9.39 $\pm$ 0.91	8.94 $\pm$ 0.57
4	TLC(X10 ³ /ul)	7.51 $\pm$ 0.77	7.88 $\pm$ 0.45	9.74 $\pm$ 0.78*	8.93 $\pm$ 0.50
5	MCV (fl)	47.26 $\pm$ 2.48	45.65 $\pm$ 3.24	50.13 $\pm$ 3.25	48.68 $\pm$ 2.34
6	MCH (pg)	12.89 $\pm$ 0.43	14.09 $\pm$ 0.86	14.12 $\pm$ 1.11	14.11 $\pm$ 0.72
7	MCHC(%)	27.85 $\pm$ 1.22	31.64 $\pm$ 1.65	27.29 $\pm$ 1.36	29.18 $\pm$ 1.11
8	Neutrophils (%)	25 $\pm$ 1.36	20.85 $\pm$ 4.15	18.76 $\pm$ 2.54	20.13 $\pm$ 2.28
9	Lymphocyte (%)	68 $\pm$ 1.62	70.85 $\pm$ 4.09	77.23 $\pm$ 2.67	74.73 $\pm$ 2.70
10	Monocyte (%)	3.50 $\pm$ 0.58	2.92 $\pm$ 0.41	3.80 $\pm$ 0.57	3.43 $\pm$ 0.35
11	Eosinophil (%)	3.80 $\pm$ 1.74	3.44 $\pm$ 0.63	1.83 $\pm$ 0.47	3.06 $\pm$ 0.48
12	Blood clotting time (min)	6.67 $\pm$ 0.55	10.14 $\pm$ 1.19	15.88 $\pm$ 1.89**	13.75 $\pm$ 1.28*

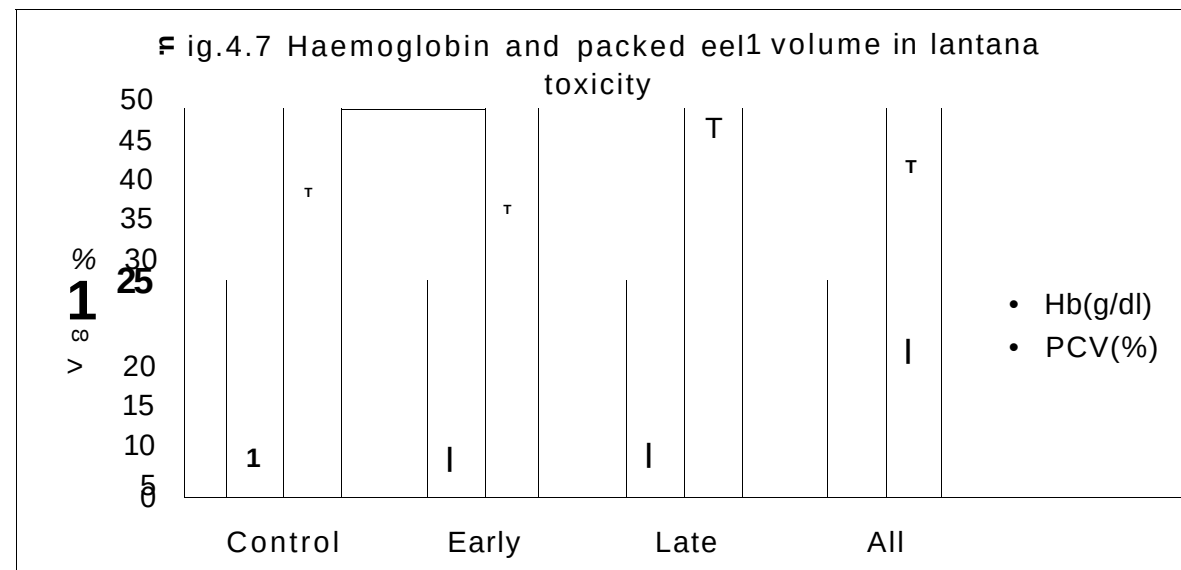
Significant at 5% level (P< 0.05) ** Significant at 1% level (P< 0.01)

4.2.4 HAEMATOLOGICAL EVALUATION

The haematological values of cattle suffering from lantana toxicity are depicted in the table 4.11 and Figs. 4.7 to 4.9.

1) Haemoglobin

The respective mean values of haemoglobin in early and late groups were 11.31 ± 0.47 g per cent and 11.96 ± 0.53 g per cent with over all mean of 11.68 ± 0.36 g per cent. Their values were non-significantly higher than control group (Fig.4.7). Twenty per cent cases (6 out of 30) were having haemoglobin more than 13 g per cent and haemoglobin level as high as 18 g per cent was also recorded in one cattle.



2) Packed cell volume

The mean values of hematocrit in early, late and all cases were 35.46 ± 1.43, 45.24 ± 2.16 and 40.86 ± 1.61 per cent respectively. In late cases the packed cell volume was significantly (P<0.01) increased than control as well as early cases, where as it was non-significantly higher in all lantana cases. Seven cattle had hematocrit value more than 50 per cent.

3) Total erythrocyte count

No significant difference was observed in any group as compared to control value. Although the value showed an increasing trend in the late cases (Table 4.11 and Fig. 4.8).

4) Total leukocyte count

Total leukocyte count of 7.88 ± 0.45 , 9.74 ± 0.78 and $8.93 \pm 0.50 \times 10^3 / \mu\text{l}$ were observed in early, late and all cases, respectively. The leukocyte count of late cases was significantly ($P < 0.05$) higher than control as well as early cases (Table 4.11, Fig. 4.8). Forty per cent lantana toxicity cases (12 cases) had TLC value more than $10 \times 10^3 / \mu\text{l}$.

5) Erythrocytic indices

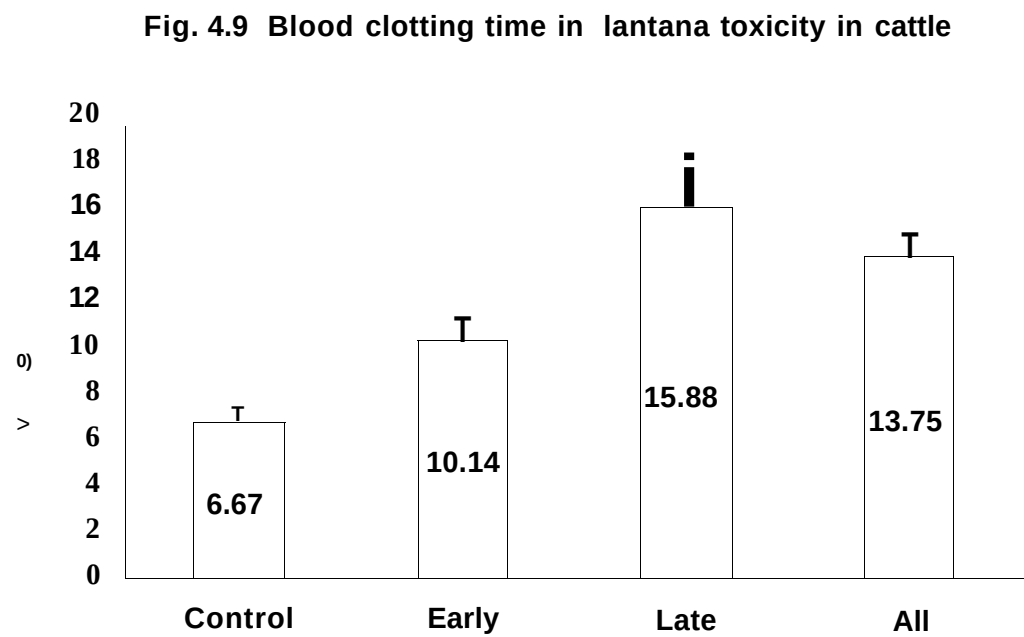
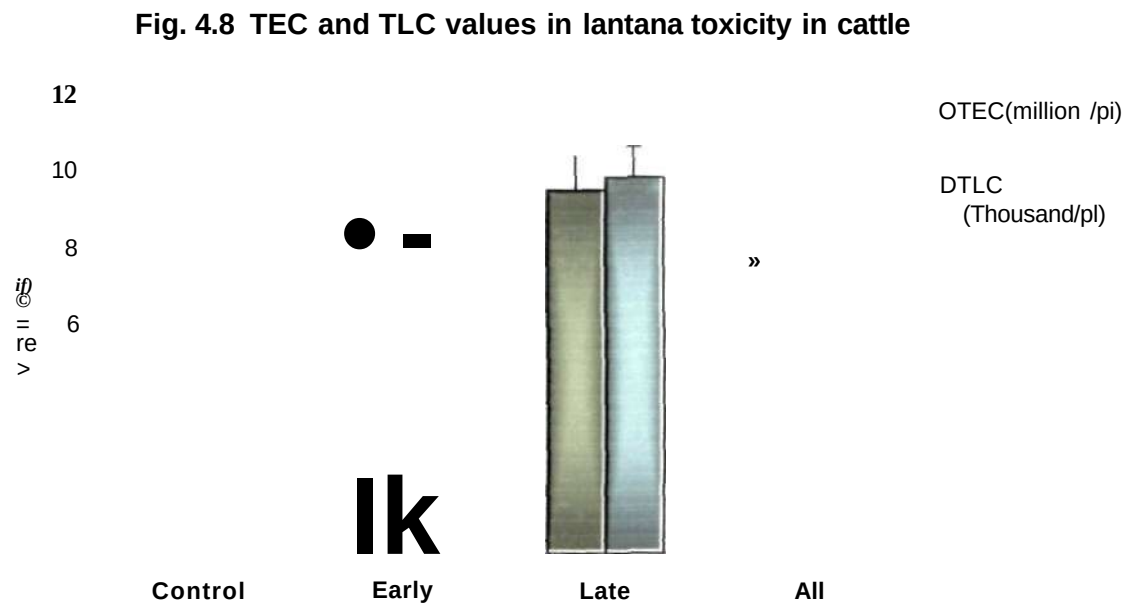
The mean value of erythrocytic indices *i.e.* mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of all the diseased groups (early, late and all cases group) were comparable to the respective control values (Table 4.11).

7) Blood clotting time

Blood clotting time of 10.14 ± 1.19 min in early cases group was significantly ($P < 0.05$) higher than control value of 6.67 ± 0.55 min. It was further increased to 15.88 ± 1.89 min in late cases which was significantly higher than control and early cases. Mean value of 13.75 ± 1.28 min observed in all cases was also significantly ($P < 0.05$) higher than control group (Fig. 4.9). In the animals presented after the 4th to 6th day, blood clotting time was in range of 15 to 18.5 min and in one case reported on 13th day, it was 27 min.

4.2.5 OSMOTIC FRAGILITY OF ERYTHROCYTE

The results of erythrocytic fragility test are depicted in Table 4.12 and Fig. 4.10 The result revealed that hemolysis of erythrocytes was significantly higher at each concentration of buffered saline solution in early and late presented cases as compared to the healthy control animals. The osmotic fragility of erythrocyte



showed a sigmoid curve when per cent hemolysis was plotted against different concentrations of hypotonic solution of sodium chloride. The hemolysis started (minimum resistance) at 0.80 per cent NaCl concentration in both the early and

Table 4.12 Osmotic fragility of erythrocytes in early and late cases of lantana toxicity (Mean $\pm$ S.E.)

S. No.	% NaCl solution	Per cent hemolysis		
		Control group	Early group	Late group
1	0.85	00	00	00
2	0.80	00	0.74 $\pm$ 0.58	1.40 $\pm$ 0.48**
3	0.75	0.86 $\pm$ 0.44	1.08 $\pm$ 0.57	2.53 $\pm$ 1.04**
4	0.70	1.11 $\pm$ 0.64	4.35 $\pm$ 5.35	10.42 $\pm$ 2.83*
5	0.65	3.45 $\pm$ 1.72	15.72 $\pm$ 5.36	21.4 $\pm$ 2.81*
6	0.60	10.45 $\pm$ 2.14	25.16 $\pm$ 7.70	36.2 $\pm$ 5.94
7	0.55	19.92 $\pm$ 4.24	46.56 $\pm$ 13.27	54.92 $\pm$ 6.70
8	0.50	31.31 $\pm$ 4.91	65.03 $\pm$ 11.99	79.34 $\pm$ 4.71**
9	0.45	64.38 $\pm$ 5.65	82.25 $\pm$ 10.94	92.17 $\pm$ 3.49*
10	0.40	79.50 $\pm$ 3.84	99.85 $\pm$ 7.25	100.0 $\pm$ 2.77**
11	0.35	96.55 $\pm$ 2.58	100.45 $\pm$ 7.95	
12	0.30	99.81 $\pm$ 2.93	—	
13	0.25	100.0 $\pm$ 3.43	—	---
14	0.20		—	—
15	0.10	—	—	—

*Significant at 5% level ($P < 0.05$) ** Significant at 1 % level ($P < 0.01$)

late presented cases of lantana ingestion. The complete hemolysis (maximum resistance) was at 0.35 and 0.40 per cent solution in early and late cases group respectively, showing that in lantana toxicated cattle, complete hemolysis occurred at much higher concentration of saline solution. The mean corpuscular fragility was found to be 0.51 and 0.53 per cent NaCl solution respectively early and late cases. Their concentrations were significantly higher than control group (0.48 per cent).

4.2.6 BLOOD BIOCHEMICAL PROFILE

The results of biochemical parameters in cattle suffering from lantana toxicity are presented in the Table 4.13 & 4.14 and Figs. 4.11 to 4.15.

A) Vanden bergh test

The results of type of reaction of vanden bergh test in clinical cases of lantana toxicity are depicted in Table 4.13. Lantana cases presented before 3rd day *i.e.* early cases showed direct, indirect and biphasic reaction in 10 (76.92 per cent), 8 (61.63 per cent) and 8 (61.63 per cent) cases. Whereas in late presented cases of lantana ingestion, 100 per cent animals showed direct, indirect and biphasic reaction. Over all 90.00, 83.33 and 83.33 per cent cases of lantana toxicosis showed direct, indirect and biphasic vanden bergh reaction (Table 4.13). Three animals presented on 1st and 2nd day with the history of very small amount of lantana ingestion, showed negative vanden bergh reaction. The Vandenberg test was graded as +, ++, +++, on the basis of the colour development and perusal of Table 4.13 revealed that most of the cases in late group showed + + to + + + reaction.

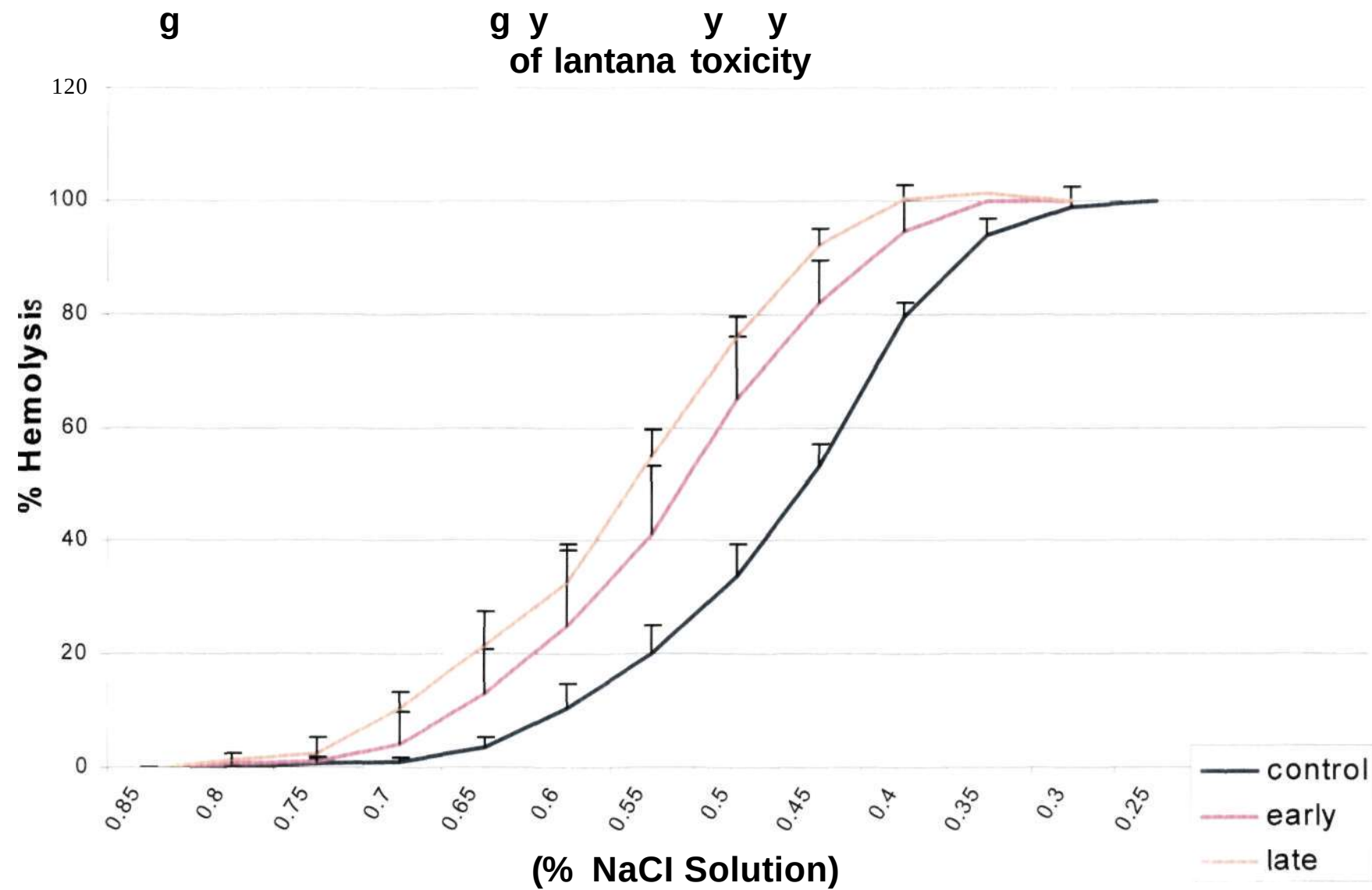


Table 4.13 Type of reaction of Vanden bergh test in lantana toxicity cases

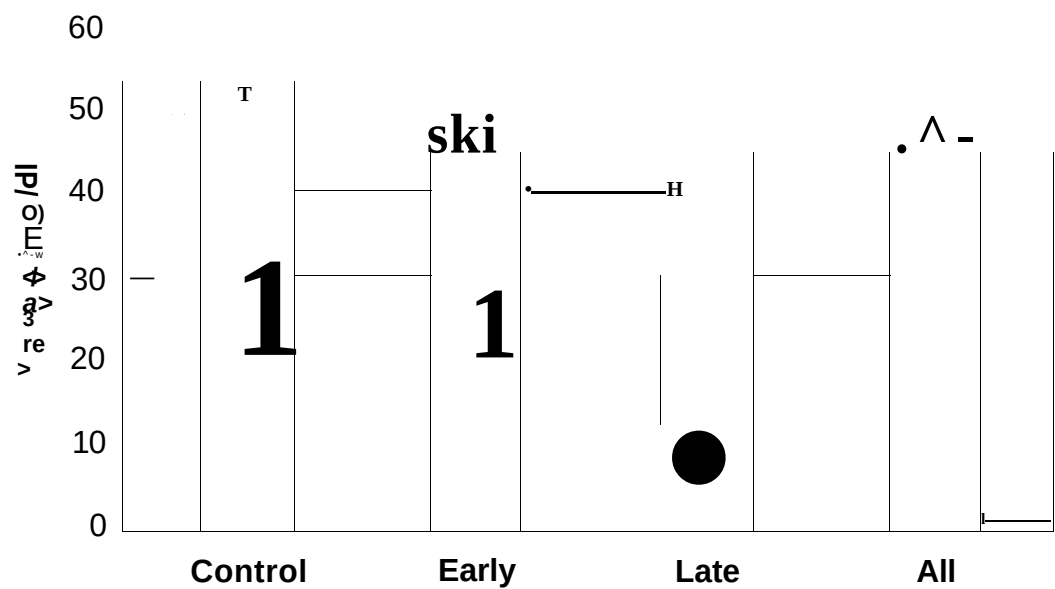
S. No.	Type of cases	Reaction type	Grades	No. of cases	Total
1	Early	Direct	- ve	3 (23.0)	10(76.92)
			+	7 (53.8)	
			++	3(23.3)	
			+++		
		Indirect	- ve	5 (38.48)	8(61.53)
			+	5 (38.48)	
			++	3 (23.3)	
			+++	-	
		Biphasic	+++	8	8(61.53)
2	Late	Direct	- ve	-	17(100)
			+	5 (29.41)	
			++	5 (29.41)	
			+++	7(41.17)	
		Indirect	- ve	-	17(100)
			+	2(11.76)	
			++	4 (23.52)	
			+++	11(64.76)	
		Biphasic	+++	17	17("100)
3	All	Direct	- ve	3	27(90.00)
			+	12	
			++	8	
			+++	7	
		Indirect	- ve	5	25(83.33)
			+	7	
			++	7	
			+++	11	
		Biphasic	+++	25	25(83.33)

Figures in parenthesis indicate per cent

B) Blood Glucose

The mean values of blood glucose in early, late and all lantana cases were 47.06 ± 2.37 mg/dl, 43.25 ± 1.90 mg/dl and 46.21 ± 1.27 mg/dl respectively (Fig.4.11). The level of early and all cases group were non significantly ($P < 0.05$) decreased than

Fig. 4.11 Blood glucose levels in lantana toxicity in cattle

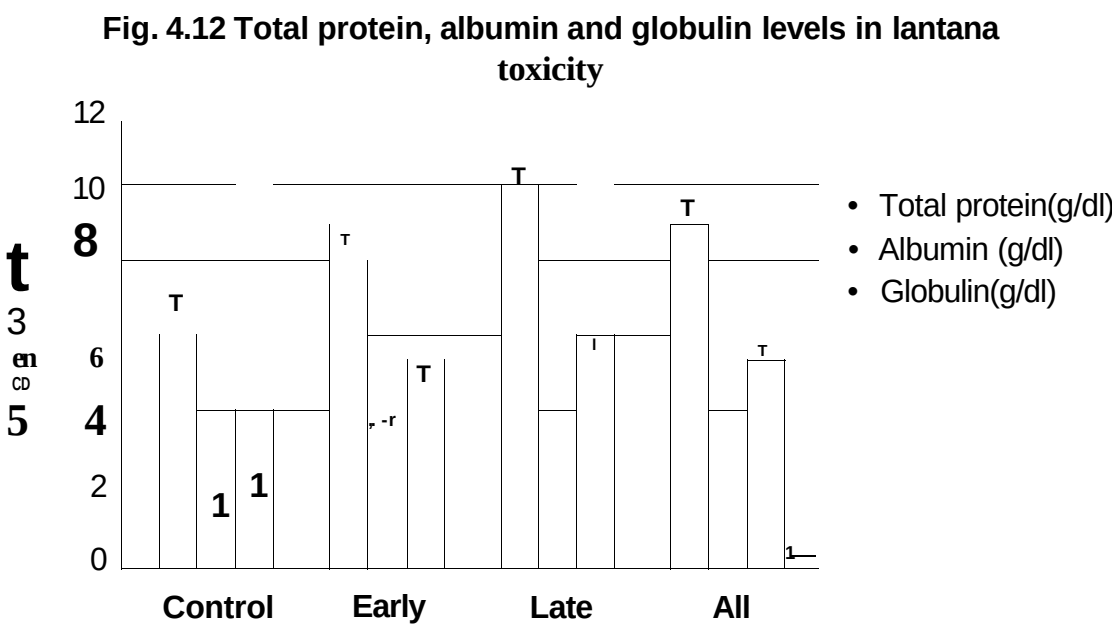


healthy control group but the blood glucose level was significantly ($P < 0.05$) decreased in cases presented after 3rd day of lantana ingestion (late cases).

C) Total protein, albumin and globulin and AG ratio

Total protein value of 8.44 ± 0.25 g/dl recorded in early cases was non-significantly higher than control value of 6.64 ± 0.35 g/dl. The level was further increased to 9.80 ± 0.58 g/dl in late presented cases and significantly ($p < 0.05$) higher than control as well as early cases (Fig. 4.12). Overall mean total protein value of ail cases were significantly ($P < 0.05$) higher (9.14 ± 0.36 g/dl) than healthy control group.

About 60 % cases (10 out of 17) in late group were having total protein level more than 9.00 g/dl and a total protein value of 14.56 g/dl was also recorded in one case presented at 13th day.



Globulin level was significantly ($P < 0.05$) higher in late group and all cases and it was non - significantly higher in early presented cases.

A/G ratio of 0.75 ± 0.08 , 0.73 ± 0.12 and 0.74 ± 0.04 were observed in early, late and all cases group and were significantly decreased than the control group ratio of 0.86 ± 0.24 .

D) Total bilirubin

Significant ($P < 0.05$) increase in the level of total bilirubin (1.73 ± 0.30 mg/dl) was observed in early cases and in the late cases, the level was further increased to 4.58 ± 0.83 mg/dl (Fig. 4.13) which reflects about 11 fold increase than control value of 0.42 ± 0.02 mg/dl. Bilirubin level as high as 3.03 mg/dl

.14 Blood biochemical profile in lantana toxicity cases (Mean $\pm$ S.E.)

S. No.	Parameters	Control	Early	Late	All diseased cases
1	Blood glucose (mg/dl)	50.87 $\pm$ 1.87	47.06 $\pm$ 2.37	43.25 $\pm$ 1.90*	46.21 $\pm$ 1.27
2	Total protein (g / dl)	6.64 $\pm$ 0.35	8.44 $\pm$ 0.25	9.80 $\pm$ 0.58**	9.14 $\pm$ 0.36**
3	Albumin (g/dl)	3.08 $\pm$ 0.08	3.61 $\pm$ 0.13	4.14 $\pm$ 0.13	3.86 $\pm$ 0.11
4	Globulin (g/dl)	3.56 $\pm$ 0.15	4.83 $\pm$ 0.39	5.66 $\pm$ 0.57**	5.28 $\pm$ 0.36*
5	A/ G ratio	0.86 $\pm$ 0.24	0.75 $\pm$ 0.08*	0.73 $\pm$ 0.12**	0.74 $\pm$ 0.04*
6	ALT (IU /l)	35.7 $\pm$ 2.10	39.26 $\pm$ 4.55	47.17 $\pm$ 2.15**	44.00 $\pm$ 1.51**
7	AST (IU /l)	60.40 $\pm$ 4.76	163.84 $\pm$ 18.54**	200.79 $\pm$ 18.39**	184.78 $\pm$ 13.38**
8	BUN (mg/dl)	15.85 $\pm$ 1.20	29.26 $\pm$ 4.54*	45.49 $\pm$ 4.00**	39.69 $\pm$ 3.29**
9	Alkaline phosphatase (IU/l)	44.95 $\pm$ 4.00	155.33 $\pm$ 8.85**	202.46 $\pm$ 25.20**	185.11 $\pm$ 15.85**
10	Acid phosphatase (KA units)	1.82 $\pm$ 0.23	4.05* $\pm$ 0.48	7.87** $\pm$ 1.16	6.22** $\pm$ 0.76
11	Total bilirubin (mg/dl)	0.42 $\pm$ 0.02	1.73* $\pm$ 0.30	4.58** $\pm$ 0.83	3.25** $\pm$ 0.44

* Significant at 5% level (P< 0.05) ** Significant at 1% level (P< 0.01)

Fig 4.13 Total bilirubin and A/G ratio in lantana toxicity in cattle

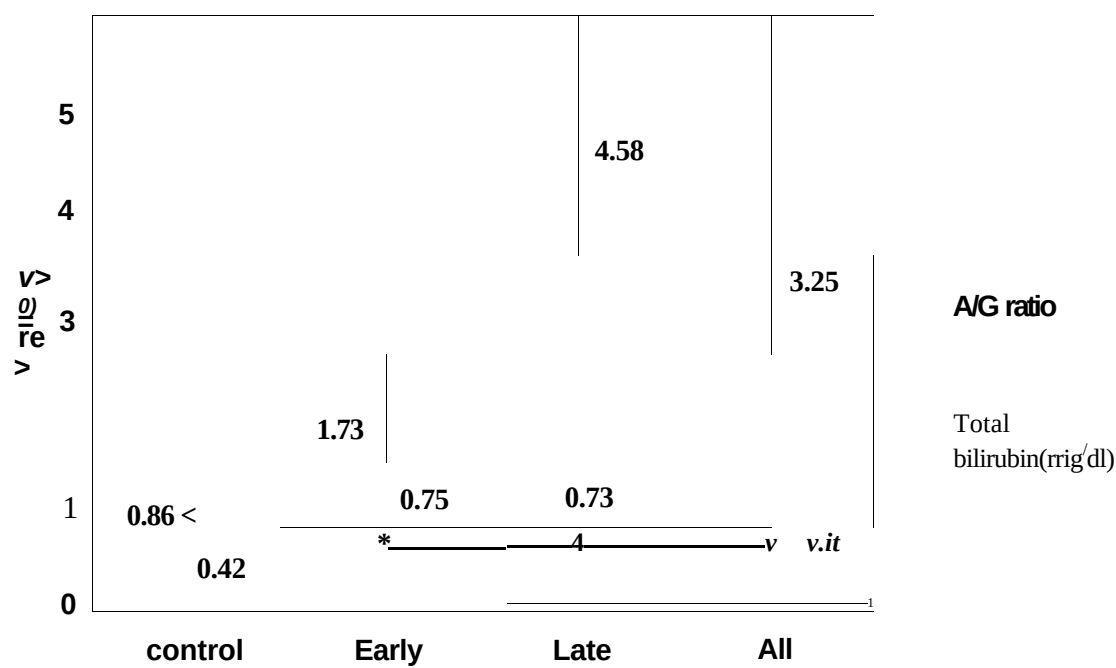
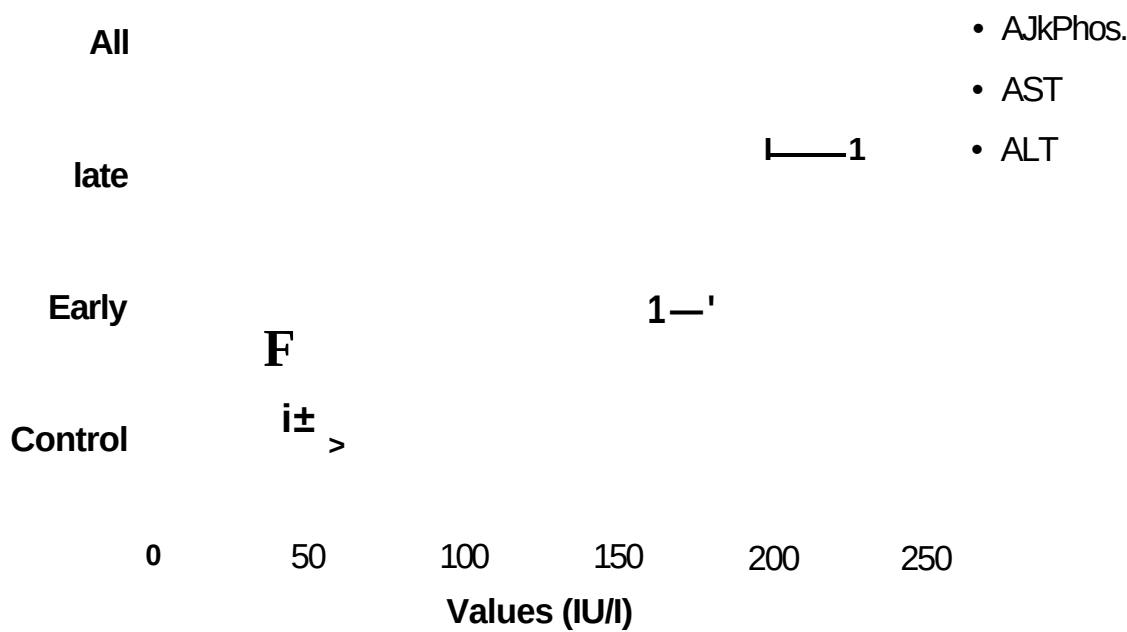


Fig. 4.14 Levels of ALT, AST and Alkaline phosphatase in lantana toxicity



(7 fold increase) in early group and 7.67 mg /dl (18 fold) in late cases was also observed. The overall mean bilirubin level of 3.25 ± 0.44 mg/dl was recorded in all lantana affected cases.

E) AST

Significant ($P < 0.01$) increase in the level of AST in early group as well as late cases was recorded. The AST activity of 163.84 ± 18.54 IU/l was recorded in early cases which was further raised to 200.79 ± 18.39 IU/l in the late cases (Fig. 4.14). AST activity as high as 326 IU/l was recorded in 2 case.

F) ALT

No significant difference was observed in ALT level of early group cases (39.26 ± 4.55 IU/l) than the level in healthy control group (35.70 ± 2.10 IU/l). However, in late group and all cases group, the level was significantly ($P < 0.01$) increased than the control group (Table 4.14, Fig. 4. 14).

G) Alkaline phosphatase

Mean value of alkaline phosphatase activity of 155.33 ± 8.85 IU/l was observed in early cases and it was markedly higher ($P < 0.01$) than the control group value of 44.95 ± 4.00 IU/l. The level was further increased to 202.46 ± 25.20 IU/l in cases presented after 3rd day (late group) and was significantly higher than control as well as early group (Fig. 4. 14).

H) Acid phosphatase

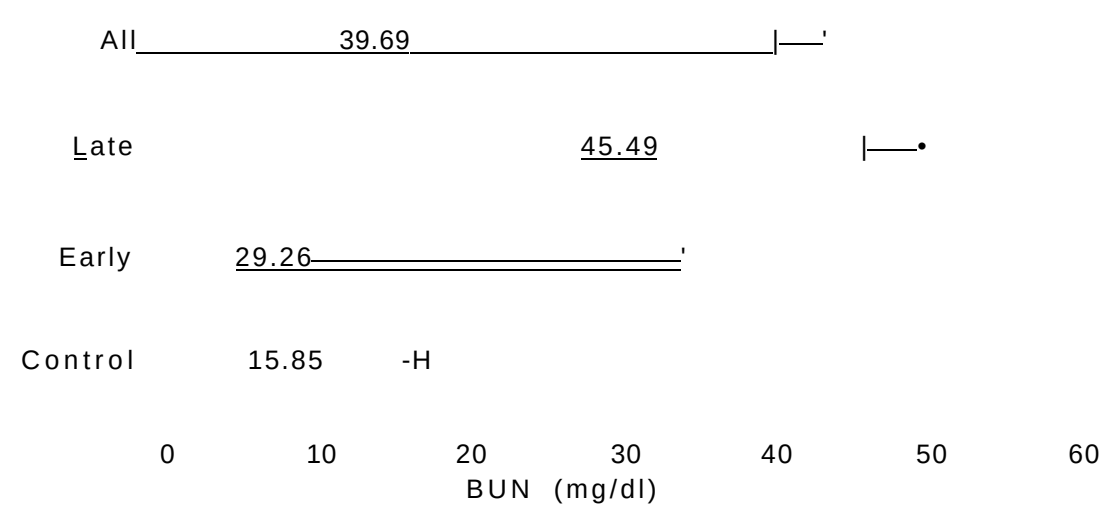
Overall mean acid phosphatase activity in lantana affected cases was observed to be 6.22 ± 0.76 KA unit which was significantly ($P < 0.01$) higher than the control. The activity in early group was 4.05 ± 0.48 KA units and was

significantly ($P<0.01$) higher than control group, which was further increased to 7.87 ± 1.16 KA units in late group cases

I) **BUN**

Mean BUN value of 29.26 ± 4.54 mg/dl was observed in early cases which were presented before 3rd day of lantana ingestion and the level was significantly ($P<0.05$) higher than the control value of 15.85 ± 1.20 mg/dl. BUN level was further increased to

Fig.4.15 **BUN** levels in lantana toxicity



45.49 ± 4.00 mg/dl (range of 40-76 mg/ dl) which was significantly ($P<0.01$) higher than the control levels as well as levels in early cases. The overall mean BUN in all the affected lantana cattle was recorded as 39.69 ± 3.29 mg/dl, which was also markedly ($P<0.01$) higher than the control value (Fig. 4.15).

4.2.7 RUMEN LIQUOR EXAMINATION

4.2.7.1 Physical examination

A) Colour, consistency and odour

In early cases up to 1-3 days of lantana ingestion the colour of rumen liquor was slightly changed to greenish brown from greenish colour in healthy animals. On first day

Table 4.15 Rumen liquor parameters in lantana toxicity cases (Mean ± S.E.)

S. No.	Parameters	Control group	Early	Late	All diseased cases
1	Rumen motility (per 2 min)	2.50 ±0.17	0.78 ±0.17*	Nil**	0.33 ± 0.09
2	PH	7.08 ±0.11	8.05 ±0.39	8.82 ±0.21**	8.36 ± 0.23**
3	Colour	Greenish	Straw to green color	Straw colored	Greenish brown to straw color
4	Consistency	Slightly viscous	Viscous to watery	Watery	Viscous to watery
5	Odour	Aromatic	Slightly ammonical	Putrid	Putrid
6	Protozoal motility	+ + +	+	Nil	Nil
7	SAT (min)	6.39 ±0.63	17.61 ±3.35**	Nil**	7.63 ±2.15**
8	GFT (ml Per hr)	2.14 ±0.21	0.88 ±0.14**	Nil**	0.38 ±0.01**
9	Total VFA (mEq/l)	87.08 ±4.73	44.71 ± 4.47**	39.87 ± 4.87**	43.75 ± 3.68**

* Significant at 5% level (P< 0.05) " Significant at 1% level (P< 0.01)

of lantana ingestion the colour was found normal and then changed to brownish in late cases. The consistency of rumen liquor was not much changed in very early

cases but it became watery as the time elapsed after the lantana ingestion and in late group cases after 4th day, it was watery straw coloured. Odour was also found changed to ammonical in early 1-2 days but after that the odour become putrid (Table 4.15).

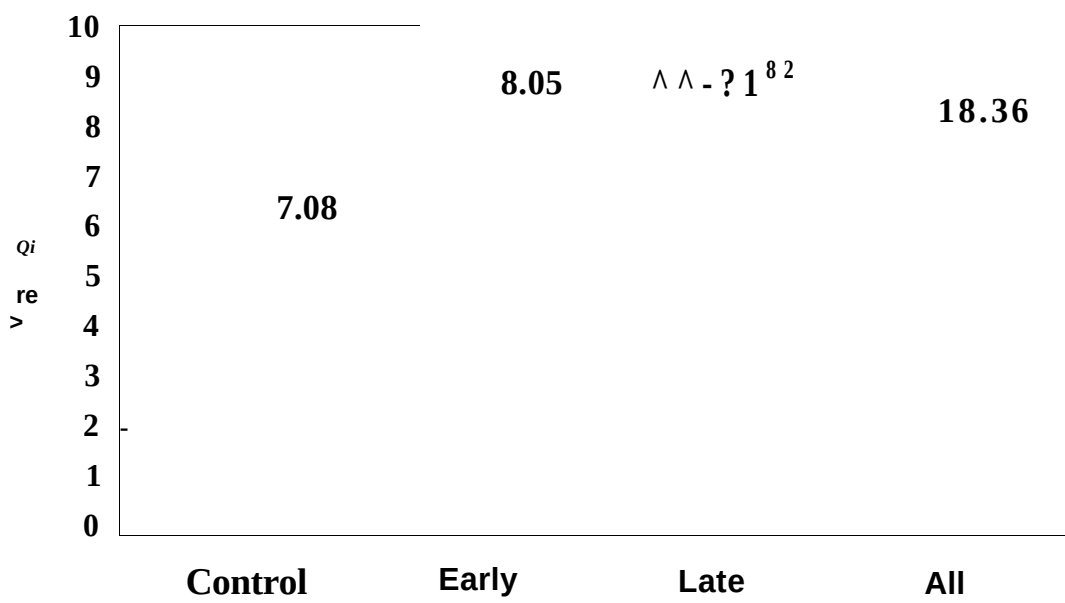
B) Ruminal motility

Significant ($P<0.05$) decrease in rumen motility (0.78 ± 0.17 per 2 min) was recorded in early cases (1-2 days). The motility was also decreased in amplitude. However, after 3rd day onward no rumen motility could be recorded.

C) pH of rumen liquor

As given in the Table 4.15 and Fig. 4.16, pH of all the groups was found significantly ($P<0.01$) increased than the healthy control group. The mean pH

Fig.4.16 Effect of lantana toxicity on rumen liquor pH



value of 8.05 ± 0.39 was recorded in early cases which were further increased to 8.82 ± 0.21 in late presented cases. Over all mean pH of 8.36 ± 0.23 was recorded in all lantana affected cases.

4.2.7.2 Microbiological changes

A) Sedimentation activity time

A significant difference in all the groups (early, late and all cases) was seen in comparison to control group animals as given in Table 4.15 & Fig. 4.17. Sedimentation activity time value was significantly ($P<0.01$) increased in the early presented cases. The mean SAT value of 12.54 ± 5.47 min and 14.82 ± 4.83 min was recorded in cases presented on 1st and 2nd day of lantana ingestion respectively. The mean SAT value of 17.61 ± 3.35 min was recorded for early cases group as a whole which was significantly ($P<0.01$) higher than control value of 6.39 ± 0.63 min. However SAT was found nil in late presented cases.

B) Glucose fermentation test

In early group, GFT value was 0.88 ± 0.14 ml/hr which was markedly ($P<0.01$) decreased than the control group value of 2.14 ± 0.21 ml/hr (Fig. 4.17). It was further decreased to nil in cases which were presented after 4th day of lantana ingestion (late group).

C) Total volatile fatty acid concentration

A significantly ($P<0.01$) decreased level of TVFA (44.71 ± 4.47 mEq/l) was recorded in early group cases than the healthy control group (87.08 ± 4.73 mEq/l). The level was further decreased to 39.87 ± 4.87 mEq/l in late group (Fig.4.18). Over all mean value of 43.75 ± 3.68 mEq /l was recorded for all the lantana affected animals.

Fig.4.17 SAT and GFT values in lantana toxicity

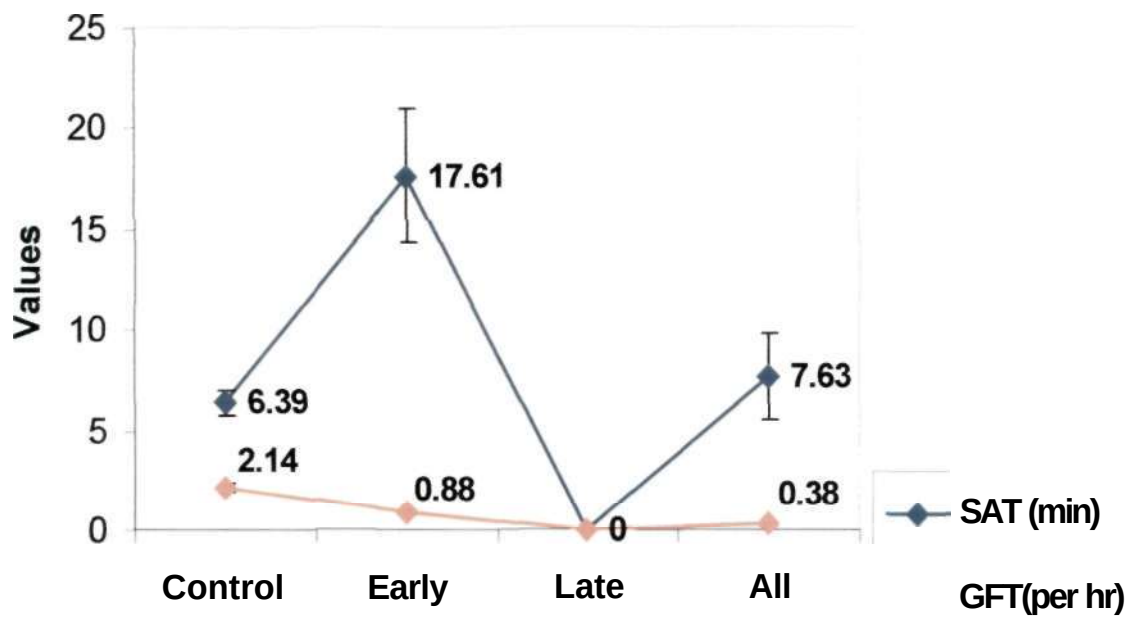
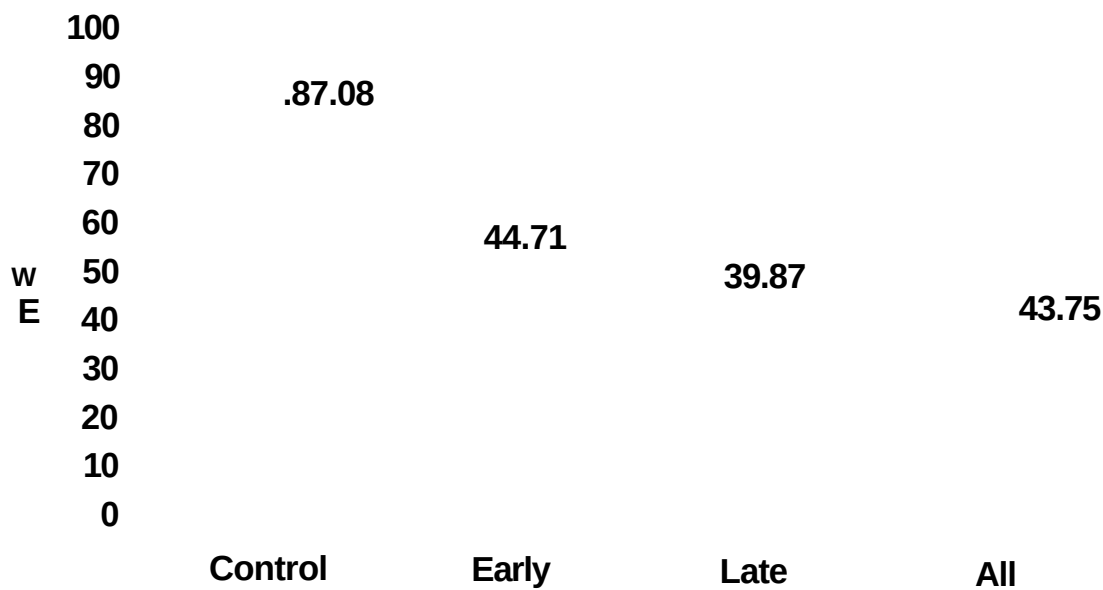


Fig. 4.18- Effect of lantana toxicity on TVFA concentration



4.2.8 THERAPEUTIC STUDIES

The mean values of various parameters in recovered animals were compared with their pre-treatment values. Post-treatment observations were recorded after 7th day of treatment. Various parameters recorded after the treatment are as follows:

4.2.8.1 Effect on Clinical and Haemato - biochemical observations

4.2.8.1.1 Post -treatment clinical observations

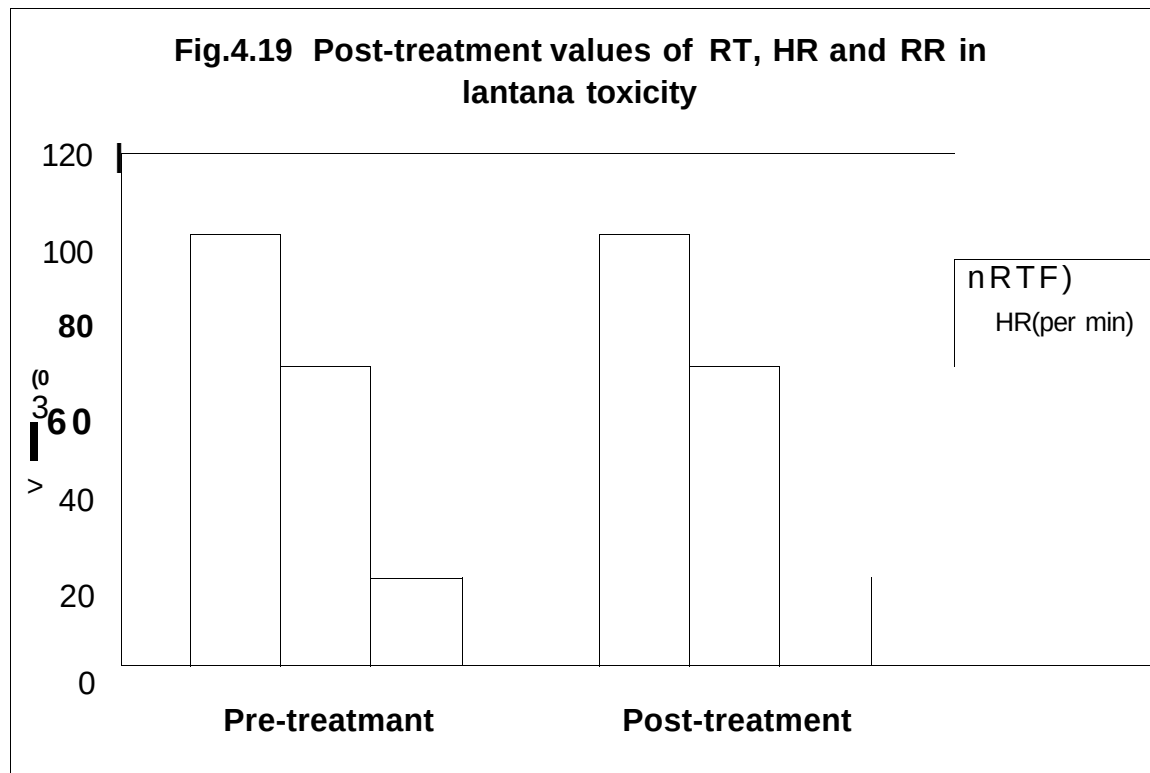
The animals were active, alert and there was improvement in appetite after the 2-3 days of treatment. Faeces become loose after 2nd day of treatment. Water intake and urination also increased. The colour of urine turned to normal. Animal started rumination on the 3rd- 4th day of treatment. There was no visible swelling of

Table 4.16 Post-treatment general clinical observations in lantana toxicity
(n=12, Mean ± S.E.)

S. No.	Parameters	Pre- Treatment Values	Post- Treatment Values
1	RT (°F)	101.42 ±0.23	100. 80 ±0.12**
2	HR (per min)	72.00 ±1.31	68.90 ±1.05**
3	RR (per min)	21.16 ± 1.42	18.00 ±0.71
4	Conjunctival mucous membrane	Icteric	White to pinkish
5	Hair coat	Rough	Normal
6	Muzzle	Dry to cracked	Wet
7	Dehydration status •based on skin tenting	+ to + +	Normal

* Significant at 5% level (P< 0.05) "sigin'ficantat 1% level (P< 0.01)
eye lids and ear base. Muzzle became wet on 3rd to 4th day of treatment Post-treatment heart rate, respiration rate and rectal temperature values recorded

before and after the treatment are given in the Table 4.16 and Fig. 4.19. Heart rate, respiration rate and rectal temperature were decreased toward normal limits after the treatment.



4.2.8.1.2 Post-treatment Haematological observations

There was significant ($P<0.01$) decrease in haemoglobin and total leukocyte count after the initiation of treatment (Table 4.17). Haemoglobin decreased to 10.12 ± 0.21 g per cent from pre-treatment value of 11.27 ± 0.51 g per cent. Where as the total leukocyte count decreased from $7.67 \pm 0.43 \times 10^3/\text{pi}$ to $6.63 \pm 0.26 \times 10^3/\text{pl}$ after treatment. PCV and TEC showed non-significant decrease. Differential leukocyte count changed little after the treatment. Blood clotting time was 11.12 ± 1.71 min pre- treatment and it was 8.30 ± 0.52 min post-treatment, showing a significant ($P< 0.01$) decrease following the treatment and the level was close to the control value (6.67 ± 0.55 min).

Table 4.17 Post- treatment haematological parameters in lantana (n=12, Mean $\pm$ S.E.)

S. No.	Parameters	Pre- Treatment	Post- Treatment
1	Hb(g/dl)	11.27 $\pm$ 0.51	10.12 $\pm$ 0.21*
2	PCV (%)	36.91 $\pm$ 1.54	32.18 $\pm$ 1.48
3	TEC($\times 10^6$ /ul)	7.94 $\pm$ 0.57	6.50 $\pm$ 0.34
4	TLC($\times 10^9$ /MI)	7.67 $\pm$ 0.43	6.63 $\pm$ 0.26*
5	MCV (fl)	46.97 $\pm$ 3.25	55.90 $\pm$ 2.42
6	MCH (pg)	15.33 $\pm$ 1.31	15.67 $\pm$ 0.58
7	MCHC(%)	33.21 $\pm$ 1.93	28.21 $\pm$ 0.79**
8	Neutrophils (%)	16.90 $\pm$ 4.08	20.82 $\pm$ 1.40
9	Lymphocytes (%)	76.09 $\pm$ 4.04	71.90 $\pm$ 1.52
10	Monocyte (%)	2.80 $\pm$ 0.69	4.27 $\pm$ 0.44
11	Eosinophil (%)	3.50 $\pm$ 0.56	2.70 $\pm$ 0.39
12	Blood clotting time (min)	11.12 $\pm$ 1.71	8.30 $\pm$ 0.52**

*Significant at 5% level ($P < 0.05$)** Significant at 1% level ($P < 0.01$)

4.2.8.1.3 Post-Treatment blood biochemical profile

The mean values of various biochemical parameters recorded before and after the treatment are given in Table 4.18 and Figs. 4.20 & 4.21.

There was significant ($P < 0.05$) decrease in total protein and globulin level. The level of total protein and globulin decreased to 6.97 ± 0.19 g/dl and 3.36 ± 0.14 g/dl from pre-treatment value of 8.80 ± 0.40 g/dl and 5.06 ± 0.41 g/dl respectively. There was significant ($P < 0.01$) increase in A/G ratio (1.0 ± 0.01)

from the pre-treatment ratio of 0.81 ± 0.09 and it became close to normal A/G ratio.

Table 4.18 Post- Treatment biochemical parameters in lantana toxicity cases (n=12, Mean $\pm$ S.E.)

S. No.	Parameters	Pre-Treatment values	Post- Treatment values
1	Blood glucose (mg/ dl)	42.62 ± 2.27	43.48 ± 1.90
2	Total protein (g / dl)	8.80 ± 0.40	$6.97 \pm 0.19^*$
3	Albumin (g/dl)	3.80 ± 0.17	3.64 ± 0.11
4	Globulin (g/dl)	5.06 ± 0.41	$3.36 \pm 0.14^{**}$
5	A/G ratio	0.81 ± 0.09	$1.00 \pm 0.01^*$
6	ALT (IU/l)	39.72 ± 1.32	28.20 ± 1.48
7	AST (IU/l)	153.45 ± 20.98	$117.0 \pm 9.46^{**}$
8	BUN (mg /dl)	32.00 ± 5.59	$22.88 \pm 2.26^*$
9	Alkaline phosphatase (IU/l)	162.30 ± 10.85	$125.23 \pm 5.05^{**}$
10	Acid phosphatase (KA units)	5.90 ± 0.44	$2.68 \pm 0.31^{**}$
11	Total bilirubin (mg /dl)	2.37 ± 0.61	$0.64 \pm 0.09^{**}$

* Significant at 5% level ($P < 0.05$) ** Significant at 1% level ($P < 0.01$)

After the treatment, level of AST and ALT showed improvement. AST activity decreased significantly ($P < 0.01$) to the level of 117 ± 9.46 IU/l from pre- treatment activity of 153.45 ± 20.98 IU/l, although the level was still higher than the control group. Similarly, ALT

Fig. 4.20 Post-treatment total protein Albumin and Globulin levels

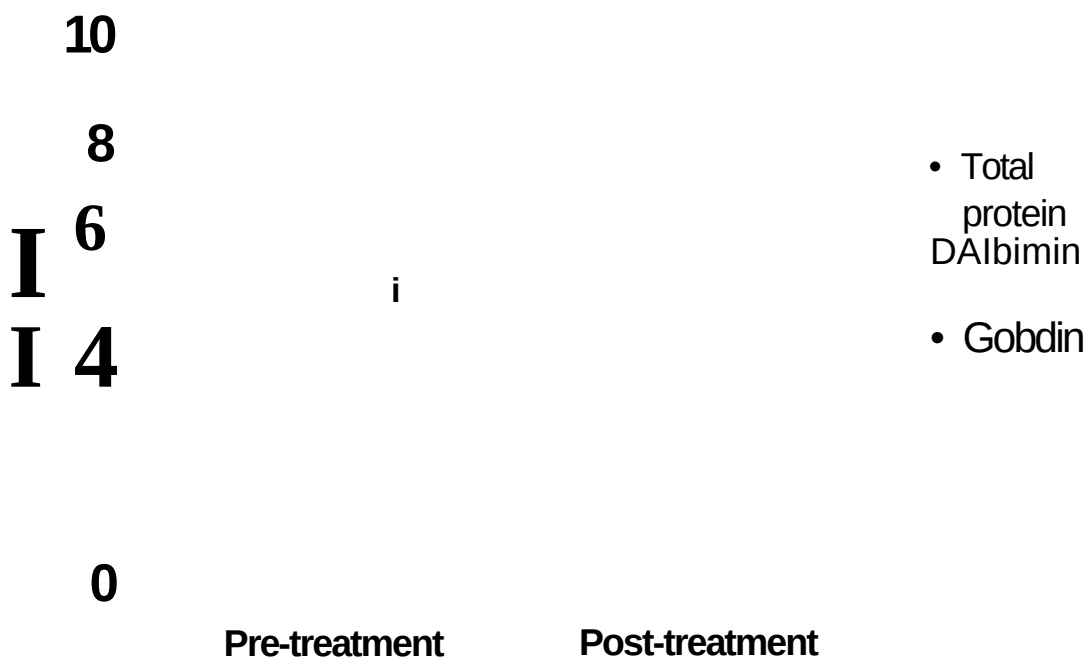
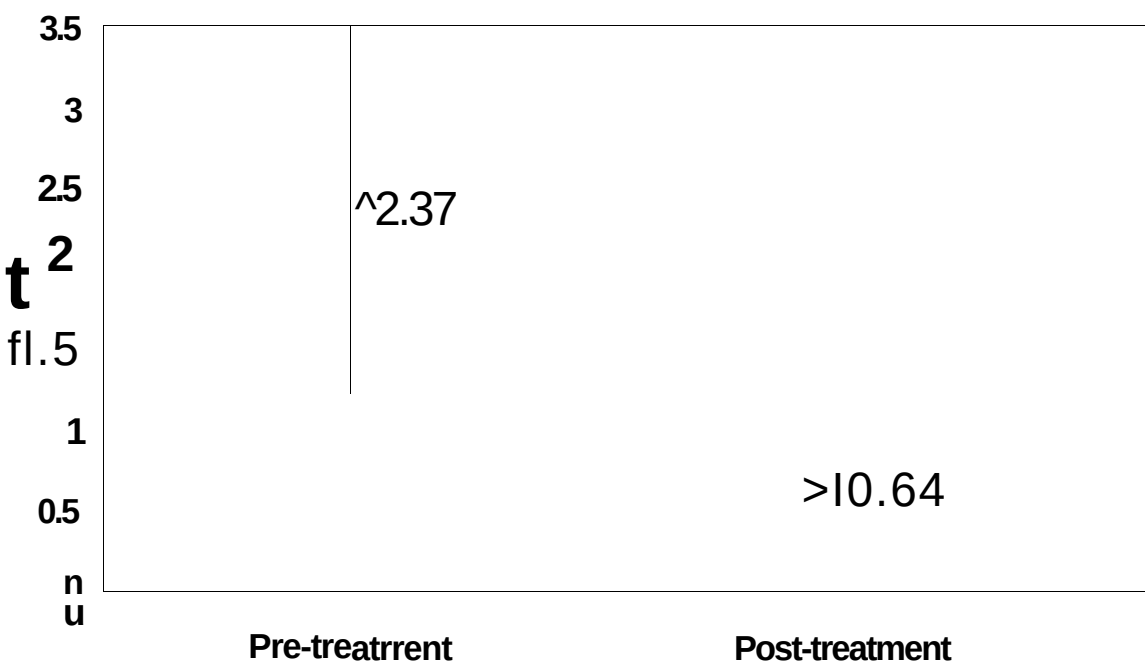


Fig. 4.21 Post- treatment bilirubin level in lantana toxicity



activity decreased considerably from 39.72 ± 1.32 IU/l to 28.2 ± 1.48 IU/l to 28.20 ± 1.48 IU/l which were quite close to normal value.

A definite improvement was found in the BUN level after the treatment. It decreased significantly ($P < 0.01$) to the level of 22.88 ± 2.26 mg/dl from pre-treatment value of 32.0 ± 5.59 mg/dl and was close to the control group value.

Marked improvement was seen in the levels of total bilirubin (fig.4.21). Bilirubin was significantly ($P < 0.01$) decreased from pre-treatment value of 2.37 ± 0.631 mg/dl to 0.64 ± 0.09 mg/dl, which showed about 4 fold decrease and the level was close to control value of 0.43 ± 0.02 mg /dl. Similarly, significant ($P < 0.01$) improvement in level of Alkaline phosphatase and Acid phosphatase was observed. Both were decreased considerably but still higher than control group values.

4.2.8.1.4 Post- Treatment rumen liquor evaluation:

Post treatment physical examination of rumen liquor revealed improvement in colour, consistency and odour. The rumen liquor started becoming greenish in colour with normal aromatic odour. The pH of rumen liquor which was significantly increased before treatment (8.29 ± 0.99) showed marked ($P < 0.01$) decrease (6.92 ± 0.05) and was comparable to the healthy control values (table 4.19, Fig. 4.22). Protozoal motility was increased from nil to ++ after the treatment. There was significant ($P < 0.01$) improvement in the rumen motility. It increased from pre treatment value of 1.00 ± 0.31 to 1.45 ± 0.15 per 2 min. The microbial evaluation showed that there was improvement in SAT. It was 12.71 ± 0.11 min after lantana ingestion and decreased significantly ($P < 0.01$) to $7.18 \pm 0.7X$ min which was quite comparable to control value of 6.39 ± 0.63 min (Table 4.19 and Fig. 4.22).

Table 4.19 Post- treatment rumen liquor parameters in lantana toxicity
(n=12, Mean $\pm$ S.E.)

S. No.	Parameters	Pre- Treatment values	Post- Treatment values
1	Rumen motility (per 2 min)	1.00 $\pm$ 0.31	1.45 $\pm$ 0.15**
2	pH	8.29 $\pm$ 0.99	6.92 $\pm$ 0.05*
3	Color	Greenish brown to straw color	Straw to green color
4	Consistency	Viscous to watery	Slightly watery
5	Odour	Putrid	Normal
6	Protozoal motility	Nil	+ +
7	SAT (min)	12.71 $\pm$0.11	7.18 $\pm$ 0.72**
8	GFT (ml Per hr)	0.88 $\pm$ 0.07	1.81 $\pm$ 1.32**
9	Total VFA (mEq/l)	43.08 $\pm$ 4.37	92.25 $\pm$ 2.08**

* Significant at 5% level (P< 0.05) ** Significant at 1% level (P< 0.01)

There was significant (P< 0.01) increase in the value of GFT to 1.81 $\pm$ 1.32 ml/hr after the treatment from pre-treatment value of 0.88 $\pm$ 0.07 ml/hr showing marked improvement in the fermentation activity. The post-treatment value was quit close to the GFT value in healthy control group (2.14 $\pm$ 0.21 ml/hr). Similarly, marked improvement was seen in the levels of TVFA. The level increased significantly (P< 0.01) from pre treatment value of 43.08 $\pm$ 4.47 mEq/l to 92.25 $\pm$ 2.08 mEq/l which was very close to the

Fig. 4.22 Post-treatment levels of rumen liquor pH and SAT(min)

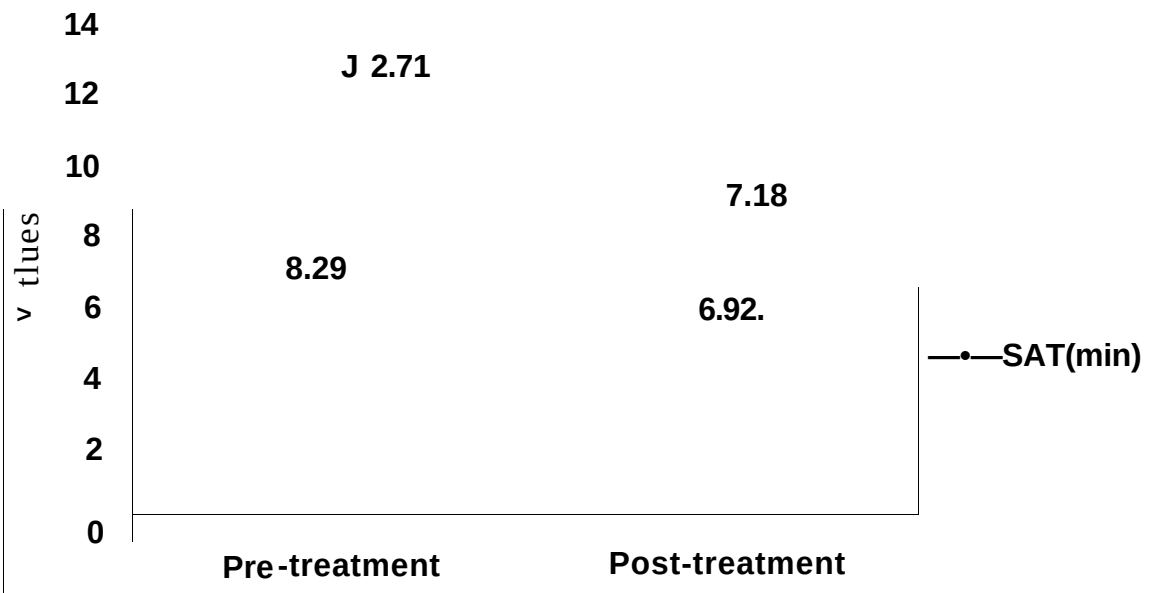
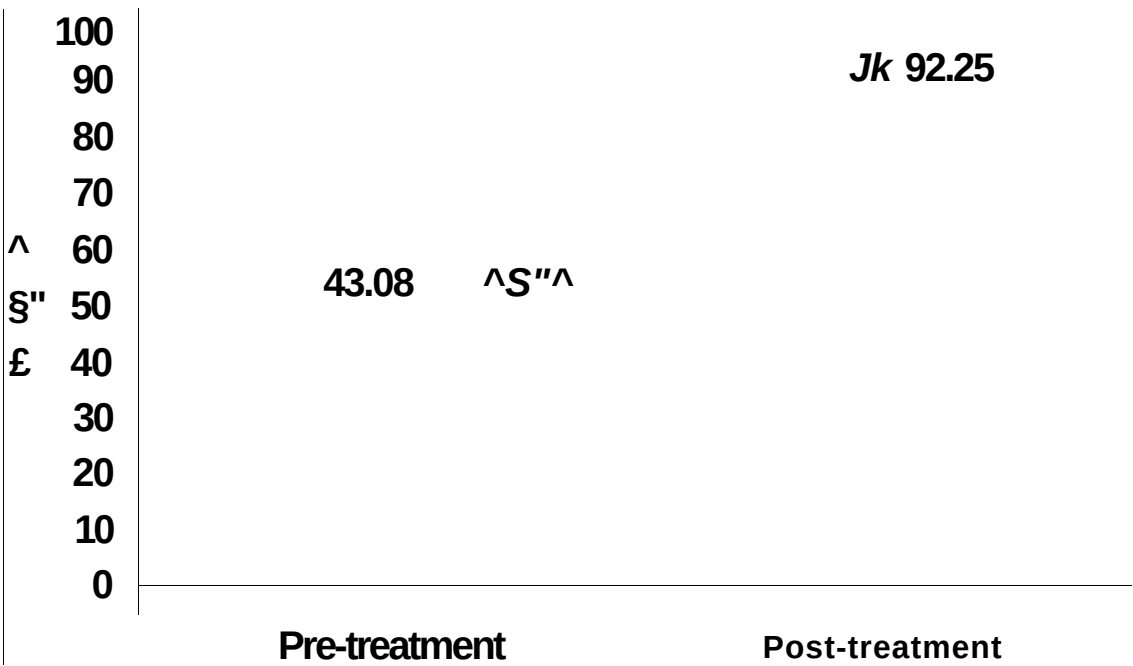


Fig.4.23 Post-treatment changes in the levels of TVFA



TVFA level observed in the healthy cattle (87.08 ± 4.73 mEq/l, Table 4.19 and Fig. 4.23).

4.2.8.2 Therapeutic efficacy

Combined treatment regimen comprising of activated charcoal, liver extract, purgative, antihistaminic, dextrose saline and rumenotorics (see section 3.5 for detail) was followed for treatment of both early and late cases of lantana toxicity. Therapeutic efficacy is summarized in Table 4.20. Following this combined therapy, out of 13 early presented cases, 10 animals (76.92 per cent) were treated successfully and 3 (23.70 per cent) died. Maximum morality was observed in late presented cases (4th day onward). Out of 17 late presented cases, only 2 animals (11.76 per cent) were recovered (Table 4.20). The recovered animals took variable time for their recovery. In the early cases, out of 10 recovered animals 4 animals took 3 days and 6 animals took 4 days for clinical recovery. Where as 2 cattle recovered from late group took 7 days.

Table 4.20 Therapeutic efficacy in lantana toxicity cases

S.No.	Result obtained	Early Cases	Late cases	Total cases
1	No. of animals recovered	10(76.92)	2(11.76)	12(40.00)
2	No. of animals died	3 (23.07)	15(88.23)	18(60.00)
3	Total animals	13	17	30

Figures in parenthesis indicate per cent

These observations indicated that combined therapy followed in the present study gave encouraging results provided the lantana ingested cases are presented at early stage.



DISCUSSION

CHAPTER V

DISCUSSION

Disease problem due to ingestion of poisonous plant is one of the important bottlenecks affecting livestock population. In the present study on clinical cases of lantana toxicosis, various paramotors including gonoral clinical appraisal, hematological, biochemical and rumen liquor examination was done and evaluated. A combined therapy was followed to assess its efficacy in clinically effected cases.

5.1 OBSERVATIONS ON CONTROL GROUP ANIMALS

5.1.1 Clinical observations

The respective mean values of rectal temperature, heart rate and respiration rate were $100.68 \pm 0.14^{\circ}\text{F}$, 67.50 ± 2.05 /min and $20.9 + 1.23$ / min. All these values were within the normal physiological range (Prasad, 1992)

5.1.2 Haematological parameters and erythrocytic fragility

The respective mean values of haemoglobin, packed cell volume, total erythrocyte count and total leukocyte count were 10.26 ± 0.20 g/dl, 37.30 ± 0.35 per cent, $7.99 \pm 0.32 \times 10^6$ /pi and $7.50 \pm 0.77 \times 10^3$ /pi, which are comparable to the values given by Jain (1986); Benjamin (1998) and Brar *et al.* (2000). The values of various erythrocytic indices observed in the present study (Table 4.2) were also in normal physiological range (Brar *et al.*, 2000) indicating normal haemopoiesis in control group animals. The blood clotting time (6.67 ± 0.55 min)

was also comparable to the observation of Dhillon *et al.* (1970) and was in normal range (Benjamin, 1998).

The result of erythrocytic fragility test showed that initial haemolysis was at 0.75 per cent saline solution and complete haemolysis occurred at 0.25 per cent saline solution (Table 4.3). These findings simulated with the observations of Wadhwa and Prasad (2000), they also reported initial haemolysis, complete haemolysis and mean corpuscular fragility at 0.75, 0.25 and 0.48 per cent saline solution respectively. Sarkar *et al.* (1999) observed initial and complete haemolysis of erythrocytes of cattle of age group of 2-3 years at 0.70 per cent and 0.30 per cent saline solution. Jain (1986) reported several extrinsic (pH, temperature and oxygenation etc.) and intrinsic (age, breed, species, age of erythrocyte and cell membrane) factors influencing erythrocytic fragility.

5.1.3 Blood biochemical profile

Mean blood glucose, total protein, albumin, globulin and A/G ratio were 50.96 ± 1.76 mg/dl, 6.64 ± 0.35 g/dl, 3.08 ± 0.08 g/dl, 3.56 ± 0.15 g/dl and 0.86 ± 0.02 respectively. These levels were within the normal physiological range (Benjamin, 1998 and Brar *et al.*, 2000). Blood glucose level was in concurrence with earlier reports of More *et al.* (1982) and Wadhwa *et al.* (2001).

Blood urea nitrogen level (15.84 ± 1.25 mg/dl) was simulating the level reported by Thirunavukkarasu *et al.* (2001a). The concentration of total bilirubin in the present study (0.42 ± 0.02 mg/dl) simulated to the findings of Bahadure *et al.* (1992). However, higher level (0.96 ± 0.23 mg/dl) has been reported by Thirunavukkarasu *et al.* (2001a). The level of bilirubin was well within the range of

0.01 - 0.47 mg/dl given by Benjamin (1998) and 0.01 to 0.50 mg/dl by Kaneko *et al.* (1999).

The mean plasma AST, ALT and AP activities were 60.40 ± 4.76 IU/L, 35.70 ± 2.10 IU/L and 44.95 ± 4.00 IU/L respectively (Table 4.4). The level of AST was simulating to the observation of Bahadure *et al.* (1992). However, activity of ALT was higher than recorded by Bahadure *et al.* (1992) and Thirunavukkarasu *et al.* (2001a). Much lower activity of AP (97.20 ± 0.89) was reported by Thirunavukkarasu *et al.* (2001a) than the present study. However the activities of AST, ALT and AP were within the normal range (Kaneko *et al.*, 1999).

5.1.4 Rumen liquor evaluation

The color, odour, consistency, pH (7.08 ± 0.11) and protozoal motility (++ to +++) observed in the present study simulate with the findings of Misra and Singh (1974) and Chakrabarti (2002).

The mean values of SAT, GFT and TVFA were 639 ± 0.63 min, %AH $\pm 0. \text{£}1$ ml/hr and 87.18 ± 4.73 mEq/l respectively. All these values were in accordance with the findings of Ahuja *et al.* (1989) and Kaushal (1994).

5.2 OBSERVATIONS ON DISEASED GROUP

5.2.1 Occurrence

.In this study, various aspects of lantana toxicosis were studied in cattle only. Natural incidence of lantana toxicity has been observed in sheep, goat, buffalo and cattle (Yadava and Verma, 1978; Sharma and Makkar, 1981).

Age wise, animals up to one year of age were found more affected. As many as 53.33 per cent cases were below 1 year of age. This was probably

because the young animal consumes this plant due to unawareness of its toxicity. Maximum cases were recorded in Jersey cross (15 cattle, 50 per cent) followed by local hill cattle (13 cattle, 43.3 per cent) and least in Holstein Friesian (2 cattle, 6.6 per cent). No breed wise susceptibility has been reported. Frisch *et al.* (1984) recorded higher incidence in *Bos Taurus* than *Bos Indicus* cattle and their crosses in Australia. But no difference has been found in incidence amongst different breeds of cattle.

Female cattle were distinctly more affected (83.3 per cent) than males (16.67 per cent) probably because of their higher population.

5.2.2 Clinical observations and manifestations

It was found that feed intake reduced from the day of lantana ingestion and anorexia developed after second day. Constipation was seen in all animals. Constipation in animals results due to effect of lantana toxins on liver. Radostits *et al.* (1994) suggested that in hepatitis and obstruction or stasis of biliary system, the partial or complete absence of bile salts from alimentary tract deprives it of laxative effect resulting in constipation. Ruminal motility was decreased to nil on the second day of toxicity. Water intake was also reduced in all lantana toxicity cases. Oligouria was seen in all the cases after third day to seventh day. Conjunctival mucous membrane was icteric in most of the animals presented on 3rd day onward. Most of the affected animals showed mild signs of photosensitization like swelling of eye lids and base of ear pinna, cracks of skin over ears and drooping of ears.

However severe signs of photosensitization were not seen in present clinical cases which might be due to the reason that animals were of dark colour or

might not have consumed very large quantity of lantana and were not exposed to sunlight after ingestion of lantana plant. Photosensitization has been reported in experimental lantana toxicity (Lin *et al.*, 1985; Mandial, 1996) as well as in natural cases of lantana intoxication (Yadav and Verma, 1978, Aloni, 1979; Mandial *et al.*, 2000 and Singh *et al.*, 2003).

In the present study, 66.67 per cent case showed jaundice on clinical examination. Pass (1986), Thirunavukkarasu *et al.* (2000b & 2001a) and Singh *et al.* (2003) also observed jaundice in natural or experimental lantana poisoning as a consequence of cholestasis.

Rumen stasis observed in lantana toxicated cattle was due to decreased passage of ingesta from rumen to small intestine and stopping of feed intake. The other factor inhibiting rumen contraction could be inhibitory reflex initiated from the damaged liver which led to cholestasis (McSweeney and Pass, 1983a). In corroborating to the present observation, depressed rumen motility has also been recorded by Dhillon and Paul (1971); Pass (1986); Hussain and Roychoudhury (1992b); Ali *et al.* (1995); Mandial *et al.* (2000) and Thirunavukkarasu *et al.* (2000b). Decreased rumen motility causes toxic material to release in the rumen and continuous absorption of toxin from the rumen maintained the disease (Sharma *et al.*, 1988c).

Most of the lantana intoxicated animals showed different degree of dehydration manifested in the form of ruffled skin coat, sunken eyes, dried muzzle and increased packed cell volume was perhaps due to low intake of water (McKenzie, 1991) and electrolyte imbalance (McSweeney and Pass, 1983b).

Rectal temperature was found increased only in early stages of disease and in late stages of disease, decrease in temperature was recorded. Similar observations were made by Sastry and Singh (1979). They recorded rectal temperature as high as 105° F in the peak stages of toxicity and sub normal temperature in terminal stages of toxicosis. Heart rate was found increased in all the cases of lantana toxicosis with cardiac arrhythmia in advance stages of disease. Simulating to the present observation, increase in heart rate was also found by Kaushal (1994) between 24 hr and 48 hr of lantana feeding. Variation in heart rate was also in agreement with finding of Thirunavukkarasu *et al.* (2000c), who suggested that myocardial hemorrhages with decreased conduction of heart rate and bio-electrical activity of myocardium is disrupted leading to death of animals.

Respiration rate was also found slightly increased with respiratory distress after 6 to 7th day of lantana ingestion. These findings simulate with the observation of Kaushal (1994) in experimental lantana toxicosis in calves.

5.2.3 Haematological evaluation

The respective mean value of haemoglobin in early and late cases of lantana toxicity were 11.31 ± 0.47 g per cent and 11.96 ± 0.53 g per cent and the values were significantly higher than control group value (Table 4.11). This may be because of haemoconcentration, because of decreased water and feed intake. These simulate the observation of Thirunavukkarasu *et al.* (2001a). The hematocrit of lantana affected cases were significantly increased than the healthy cattle which corroborate to the finding of Dhillon *et al.* (1970) in experimentally induced lantana poisoning in buffalo calves and of Singh *et al.* (2003) in lantana

toxicated sheep. The increased hematocrit was the result of haemoconcentration and could also be attributed to the liver damage as it decreases the synthesis of fibrinogen and other proteins (Dhillon *et al.*, 1970). However decreased packed cell volume was observed by Thirunavukkarasu *et al.* (2001a) in experimental lantana toxicosis in calves.

TEC was non-significantly increased in both early and late presented cases simulating to the finding of Alt *et al.* (1995) and Singh *et al.* (2003). The increased TEC might be due to haemoconcentration. Total leukocytes count of 7.88 ± 0.45 and $9.74 \pm 0.78 \times 10^3/\mu\text{l}$ was observed in early and late cases respectively. The leukocyte count of late cases was significantly ($P < 0.05$) higher than control as well as early cases (Table 4.11). In corroboration to the present observation, leukocytosis has been reported by Kaushal (1994), Ali *et al.* (1995) in experimental lantana toxicosis in calves and goats respectively. Leukocytosis in lantana toxicosis might be due to normal body reaction to foreign object (Thirunavukkarasu *et al.*, 2001a).

The mean values of erythrocyte indices of all the diseased groups (early, late and all cases) were comparable to their respective control values (Table 4.11). However, some cases (10) showed decreased MCV (32.68 to 36.66 fl) and increased MCV (62-76 fl). This alteration in MCV could be due to deficiency of hemopoietic factors (Benjamin, 1998). MCHC was also found under the normal range but only in nine cases, it was found below the normal range (20 to 24.23 g/dl) which showed that in true hypochromic anemia the reduction in hemoglobin is relatively greater than the average decrease in the erythrocyte volume. The normal MCHC indicates that in many type of anemia, an increase or decrease in

the average size of the cell is accompanied by a corresponding increase or decrease in the average haemoglobin content, so that the MCHC remains within the normal range (Benjamin, 1998).

No significant change was observed in differential leukocyte count. In contradiction to this, many workers reported neutrophilia and lymphopenia with leukocytosis (Dwivedi *et al.*, 1971; Seawright, 1963 and Kaushal, 1994).

Mean blood clotting time observed in early (10.14 ± 1.19 min) and late (15.82 ± 1.89 min) presented cases were significantly ($P < 0.01$) higher than control values. In cases reported on 4th and 6th day it was found between 15 to 18.50 min and in terminal stages in one case reported on 13th day it was 27 min. But depending upon the severity of toxicity even on 4th day, it was found more than 30 min. These observations corroborated well with the finding of Dhillon *et al.* (1970), Uppal and Paul (1978), Bahadure *et al.* (1992); Hussain and Roychoudury, (1992a) and Ali *et al.* (1995), Progressive increase in clotting time after ingestion of lantana was probably due to progressive liver damage as a consequence of which prothrombin and fibrinogen synthesis were interfered (Ali *et al.*, 1995). Similarly O' Neil *et al.* (1998) reported increased blood clotting time by giving triterpene of lantana leaves due to prothrombin inhibitory activity.

5.2.4 Osmotic fragility of erythrocyte

The results of erythrocytic fragility showed that hemolysis of erythrocyte of lantana intoxicated animals was significantly higher at each concentration of saline solution (Table 4.12). The mean corpuscular fragility was found to be 0.51 and 0.53 per cent NaCl solution in early and late cases which was significantly higher than control group value of 0.47 per cent. In corroboration to the present

observation, Sharma *et al* (1981b) also reported increased fragility of erythrocyte of calves and reported complete hemolysis at 0.44 per cent NaCl. The present study showed that erythrocytes of lantana intoxicated animals were much more fragile than normal cattle. This increased fragility might be the result of some alteration in the erythrocytic membrane making them more susceptible to hemolysis. Jain and Subramanyam (1976) observed that phenyl hydrazine administration to rats caused higher osmotic fragility of erythrocyte which could be attributed to alteration in cholesterol, phospholipids ratio of the erythrocyte membrane. Several extensive factors (pH, temperature and oxygenation) and intrinsic factors (age of animal, species, breed and age of RBC) influence the fragility (Jain, 1986). Any derangement in the permeability of red cells to electrolytes (Na^+ and K^+) lead to osmotic swell and lysis.

5.2.5 Blood biochemical profile

Vanden bergh test on the freshly collected plasma was done to assess the functioning status of liver. Three cases presented on first and second day with the history of very small amount of lantana ingestion was found negative. In early cases 61.63 per cent cases showed biphasic reaction where as in late cases group, cent per cent animal showed biphasic reaction indicating hepatocellular jaundice (toxic jaundice). The present results simulate with the finding of Sharma (1998) that there is first hepatic damage and then post hepatic obstruction occur later. Jaundice is a result of accumulation of bilirubin, but more pronounced in direct bilirubin then indirect bilirubin. So icterus is more intense in obstructive (direct positive) and hepatocellular jaundice than in haemolytic jaundice. This was

reflected clinically as the most of the late presented cases (13 out of 17) showed icteric mucous membrane.

Blood glucose levels of 47.06 ± 2.37 and 43.25 ± 1.91 mg /dl were observed in early and late cases of lantana poisoning respectively. The level was significantly ($P < 0.05$) decreased in late cases than the healthy control (Table 4.14). It corroborate with the finding of Bahadure *et al.* (1992) and Thirunavukkarasu *et al* (2001a). Hypoglycemia observed in the late presented cases may be due to anorexia and complicated by liver disorder. Gopinath and Ford (1969) reported marked loss of glycogen in parenchymal cells of periportal region in lantana poisoning in sheep. Hoe and Wilkinson (1973) also observed that hypoglycemia was associated with liver disorder.

Total protein values of 8.44 ± 0.25 g/dl and 9.80 ± 0.58 g/dl observed in early and late cases was respectively non-significant and significantly ($P < 0.01$) higher than control value of 6.64 ± 0.35 g/dl. This was due to dehydration as the water intake was reduced leading to haemoconcentration (Seawright, 1963). However no change was found in total protein value in lantana affected animals (Bahudure *et al.*, 1992; Ali *et al.*, 1996 and Thirunavukkarasu *et al.*, 2001a). Globulin level was increased and A/G ratio was found decreased which might be due to relative increase in globulin resulting in decreased A/G ratio. Sharma *et al.* (1984) concluded from experimental toxicity in guinea pig that beta globulin was decreased and gamma globulin was increased with no change in total protein. Albumin may remain normal in hepatocellular damage and decreased A/G ratio is seen in relative or absolute increase in globulin or decrease in albumin in chronic inflammation or antigenic stimulation (Benjamin, 1998).

The activities of aspartate aminotransferase and alanine aminotransferase were found significantly ($P < 0.01$) increased in lantana toxicated animals. AST activity of 163 ± 18.54 IU/l was observed in early presented cases which further increased to 200.79 ± 18.39 IU/l in late presented cases. Level as high as 326 IU/l was recorded. ALT activity of 47.17 ± 2.15 IU/l was observed in late cases which was significantly higher than the activity in control group. These findings are in agreement with those of Dwivedi *et al.* (1971); Bahadure *et al.* (1992); Sharma (1998); Mandial and Randhawa (1996 & 1999) and Thirunavukkarasu *et al.* (2001a) in experimental lantana intoxication and Singh *et al.* (2003) in natural clinical cases of lantana toxicity. The increased activity of transferases was suggestive of degenerative changes in the liver (Kaneko *et al.* 1999). Relatively ALT level was not much increased as its activity is low in ruminants (Seawright, 1963).

Total bilirubin levels of 1.73 ± 0.30 and 4.58 ± 0.83 mg/dl were observed in early and late cases respectively which represents about 4 fold and 11 fold increase than normal control level (0.42 ± 0.02 mg/dl). Bilirubin level as high as 7.67 mg/dl showing 18 fold increase was noted. These results simulated with observations of previous workers (Seawright and Hrdlicka, 1977; Kalra *et al.*, 1984; Mandial and Randhawa, 1999 and Thirunavukkarasu *et al.*, 20001a). In corroboration to the present study, Mandial and Randhawa (1996) reported 8 fold increase in total bilirubin level in experimentally induced lantana toxicosis in buffalo calves after 12 hr of intoxication and highest level of 8.08 ± 0.92 mg/dl was recorded before the death of untreated animals. The increase in the bilirubin level indicated hepatobiliary damage and it can also occur because of decreased

activity of bile flow (Gold Fischer *et al.*, 1963 and Gopinath and Ford, 1969). Liver injury due to lantana toxicity may result in incomplete conversion of hemobilirubin to cholebilirubin and thus haemobilirubin circulate in blood (Dhillon and Paul, 1970)

Significant ($P < 0.01$) increase in alkaline phosphatase activity was observed in lantana toxicated cattle. The levels were 155.33 ± 8.85 and 202.46 ± 25.20 IU/l in early and late presented cases respectively indicating 3 to 4 fold increase. These observations corroborate to the findings of Uppal and Paul (1978), Ali *et al.* (1995); Mandial and Randhawa (1999) and Thirunavukkarasu *et al.* (2001a). Significant increase in the alkaline phosphatase activity was indicative of cholestasis (Uppal and Paul, 1978). Further, persistent constipation caused retention of bile by pressure on bile duct. As the main route of excretion of alkaline phosphatase is through bile it could not be excreted properly and thus its activity remained high.

The progressive increase in the levels of bilirubin, transferases and alkaline phosphatase activity after the ingestion of lantana weye observed as indicated by increased level in late presented cases. This progressive increase was due to continuous damage to the liver. It is well established that toxins of *Lantana camara* produce rumen stasis with most of the toxins being retained in the rumen and are then absorbed continuously to maintain the liver insult (Pass, 1986). The hepatic damage as evidenced by increased activity of AST, ALT, bilirubin, alkaline phosphatase and acid phosphatase activity have also been established histopathologically by various workers (Dhillon and Singh, 1965; Seawright and

Allen.1972; Seawright and Hrdlicka.1977; Kaushal, 1994; Mandial, 1996 and Thirunavukkarasu *et al.*, 2001b)

The mean BUN levels of 29.26 ± 4.54 and 45.49 ± 4.00 mg/dl were observed in early and late cases showing marked ($P < 0.01$) increase than the control value. Markedly increased BUN levels were suggestive of renal impairment during lantana toxicity. Renal failure associated with renal injury was reported in lantana intoxicated animals (Seawright and Allen, 1972). Secondly, reduced glomerular filtration because of decreased blood flow to kidney on account of dehydration might also have contributed to increased BUN level (Benjamin, 1998). The present observation simulate with the findings of Munyua *et al.*, (1990), Kaushal (1994), Mandial and Randhawa (1996) and Thirunavukkarasu *et al.*, (2001a) in lantana intoxication. However, McKenzie (1991) and Bahadure *et al.* (1992) did not observe any significant alteration in BUN level in lantana toxicosis.

5.2.6 Rumen liquor evaluation

Significant decrease in rumen motility ($0-10 \pm 0.1$?/ 2 min) was recorded in early cases and after 3rd day onward, it became nil. The pH of rumen liquor was found increased from the day of lantana ingestion. The pH in early stages and late stages of toxicity was 8.05 ± 0.39 and 8.82 ± 0.21 respectively (Table 4.15). Significant and progressive increase in rumen pH appears to be because of corresponding decrease in TVFA concentration and increased ammonia concentration (Tsuda *et al.*, 1991). Increased pH of rumen liquor might also be due to low water intake, poor buffering capacity of rumen liquor in ruminal stasis and increased production of saliva which could have entered the rumen. Decreased protozoal population was also due to increased alkaline pH (8.82 ± 0.21) of rumen

liquor (Hussain and Roychoudhury, 1992b) and non availability of carbohydrate substrate due to anorexia. In addition, it is highly possible that lantana toxins might have directly affected the protozoal population which needs substantiation. These findings are in agreement with those of Mcsweeny *et al.* (1983); Hussain and Roychoudhury (1992b); Kaushal (1994) and Mandial and Randhawa (1998).

The colour of rumen liquor was found toward brownish with *no* change in consistency in early stages but in late stages after 6 to 7th day, it was more like watery consistency. Odour was found ammonical in the cases up to 2 to 3 days but after that it was more like putrid. These changes in colour and odour might be because of rumen stasis, corresponding increase in rumen pH and also because of increased ammonia concentration (Mandial and Randhawa, 1998). It was observed that odour was having direct correlation with pH (Ahuja *et al.*, 1989). The present observations corroborated with the findings of Kaushal (1994) and Mandial and Randhawa (1998).

Significant increase in SAT value (1161 ± 3.35 min) was recorded in early cases and was found nil in late presented cases. Glucose fermentation test value was significantly ($P < 0.01$) decreased (0.88 ± 0.1 ml/h) than control group (2.14 ± 0.21 ml/h) in early cases and further decreased to nil in late presented cases (Table 4.15). This could be correlated to destruction of normal microflora and indicated gross inactivity of rumen microflora and marked depressed rumen fermentation. The decline in microbial activity and fermentation might be due to either direct effect of lantana toxins as caused by other plant toxins like perloine, tannins and some essential oils (Allison, 1977; Keeler *et al.*, 1978) or due to indirect effect on account of unfavorable atmosphere (Hungate, 1966;

Czerkawski, 1986). These findings simulated with the results of Kaushal (1994) and Mandial and Randhawa (1998).

Significantly ($P < 0.01$) decreased concentration of TVFA was found both in early and late presented cases of lantana toxicosis (Table 4.15). The results simulated with the findings of Mandial and Randhawa (1998). A significant decrease of more than five folds (Kaushal, 1994) and nine fold (Mandial and Randhawa, 1998) in TVFA concentration was observed by 96 h following lantana intoxication. This decrease in TVFA concentration might be attributed to significant increase in pH and ammonia level which accounted for change in the rumen microbial population resulting in marked reduction in digestibility and fermentation (Ahuja *et al.*, 1989)

5.2.7 Therapeutic Studies

Combined therapy comprising of activated charcoal, liver extract, purgative, antihistaminic, dextrose saline and rumenotorics (see section 3.5 for detail) was given to treat all the cases (early and late) of lantana toxicity. The treatment was given for 5-7 days depending upon the response in each case. Effect of the treatment on haemato-biochemical profile and rumen liquor parameters was studied in 12 cases at 7 days post treatment. Therapeutic efficacy of the combined therapy was evaluated both in early and late presented cases of lantana toxicity.

Post -treatment hemato-biochemical profile and rumen liquor parameters.

After the treatment, the animal became alert and there was improvement in appetite. Water intake and urination increased and color of the urine turned to normal and animal started rumination. Significant ($P < 0.01$) decrease in haemoglobin and non significant decrease in hematocrit and TEC values (Table 4.

17) was observed. All these parameters were close to the normal values. This was because of alleviation of haemoconcentration. Similarly blood clotting time also decreased toward control value.

Biochemically, there was significant decrease in total protein and globulin from elevated pre-treatment values showing improvement after treatment. The levels of bilirubin, AST and ALT were significantly decreased after treatment from markedly elevated pre-treatment levels (Table 4.18). After the treatment, the level of bilirubin improved significantly ($P < 0.01$) from 2.37 ± 0.61 to 0.64 ± 0.09 mg/dl. Similarly, there was significant decrease in the activity of alkaline phosphatase. It decreased from 162.30 ± 10.85 to 125.23 ± 5.05 IU/L after the treatment. Marked improvement in AST, ALT, AP and bilirubin levels were all indicative of improved hepatic function and regeneration of hepatic cells. Although there was significant decrease/ improvement in the levels of AST, ALT and AP levels after the treatment, but these levels were still higher than the control values. These findings simulated with the observations of Mandial and Randhawa (1996), who reported that in spite of initiation of treatment at 12 h of lantana intoxication, the levels were higher after 12 to 15 days and became normal up to 30 days.

After the treatment, there was improvement in pH, protozoal concentration, SAT and GFT values of rumen liquor. Significant decrease in pH to 6.92 ± 0.05 was observed from pre-treatment value of 8.29 ± 0.99 . SAT was also decreased to 7.18 ± 0.72 min which was quite close to the normal value and significant increase in TVFA from pre treatment value of 43.08 ± 4.37 to 92.25 ± 2.08 mEq/l was observed (Table 4.19). All these changes were the result of improvement in the

rumen stasis and rumen motility, increased water intake, improved rumen microfloral activity leading to improvement in fermentation.

THERAPEUTIC EFFICACY

Combined therapy consisting of activated charcoal, dextrose saline, rumenotoric, purgative and liver extract was given in the present study. The therapy was based on the findings of previous workers in experimentally induced lantana toxicosis. Kaushal (1994) conducted therapeutic efficacy of various drug combinations with or without activated charcoal and concluded that treatment with activated charcoal, rumenotorics together with 5 per cent glucose saline, liver extract and antihistaminic proved highly effective. Use of activated charcoal along with supportive therapy has been recommended by Ali *et al.* (1996), Mandial and Randhawa (1996) and Mandial *et al.* (2000).

Following the combined therapy, out of 13 early presented cases, 10 cases (76.92 per cent) were treated successfully and maximum mortality was observed in late presented cases. Out of 17 late cases, only 2 cattle (11.76 per cent) recovered. With the similar combined therapy, high success up to 100 per cent recovery has been reported in experimentally induced lantana toxicosis (Kaushal, 1994; Ali *et al.*, 1996 and Mandial and Randhawa, 1996). The difference could be due to the fact that under experimental condition, treatment started between 6 to 12 h post induction of toxicosis. But in the present study, clinical cases were presented after few days and even after 7 days of lantana ingestion, by the time hepatic and renal damage might have occurred substantially. Singh *et al.* (2003) reported that treatment of lantana intoxicated sheep (natural outbreak) with liver

tonic, antihistaminic, activated charcoal, rumenotoric and fluid therapy could save 87.75 per cent animals.

The efficacy of activated charcoal as an oral antidote in the lantana poisoning is well known. Charcoal was used together with large volume of electrolyte solution in ruminants as an absorbent in order to bind the liberated toxins to prevent further absorption from the rumen (Pass and Stewart, 1984 and Pass, 1986). Better efficacy of activated charcoal over bentonite has also been reported (McKenzie, 1991 and Sharma, 1998).

Since the lantana intake lead to rumen stasis and severe hepatic damage; rumenotorics, purgative and liver extract were included in the treatment in the present study. Dextrose saline was used to treat dehydration and also to tide over the liver dysfunction (Sastry and Singh, 1979; Radostits, *et al.*, 1994; Singh *et al.*, 2003).

Thus, it was concluded from the present study that combined therapy comprising activated charcoal, rumenotorics, purgative, antihistaminic, dextrose saline and liver tonic gave encouraging result in recovery of lantana toxicity in cattle.



SUMMARY

CHAPTER VI

SUMMARY

Lantana poisoning in ruminants is one of the important causes affecting livestock population and causing great economic losses to the farmers. In the present study, 30 clinical cases of lantana toxicity in cattle were taken up. These cases were either brought to the College Veterinary Clinic or attended at nearby veterinary hospital and dispensaries. These clinical cases of lantana ingestion were divided into two groups. The animals presented before 3rd day of lantana ingestion were considered as early cases and those presented on 4th day onward were classified as late cases. Out of 30 cases, 13 (43.33 per cent) were early cases and 17 (56.67 per cent) were late cases.

After taking the comprehensive history, the cases were examined clinically. Haematological parameters viz., Blood clotting time, Hb, PCV, TLC, TEC, DLC, erythrocytic indices and erythrocytic fragility test were studied. Biochemical estimations included Vanden bergh test, blood glucose, total protein, albumin, globulin, AG ratio, total bilirubin, BUN, AST, ALT, alkaline and acid phosphatases. Examination of rumen liquor was done in respect of physical characteristics, pH, protozoal motility, GFT, SAT and TVFA.

These lantana intoxicated animals were treated with combined therapy comprising activated charcoal, liver extract, dextrose saline, antihistaminic, purgative and rumenotronics and the efficacy of this treatment was studied in early as well as in late cases.

The study revealed that young animals upto 1 yr of age were affected more (52.33 per cent). Maximum cases were recorded in jersey cross (15 cattle, 50 per cent) followed by local hill cattle (13 cattle, 43.3 per cent) and least in HF (2 cattle, 6.6 per cent).

Clinically, affected animals were having reduced appetite on first day of lantana ingestion and anorexia after 2nd day onward, constipated faeces, decreased water intake and rumen motility was decreased to nil on 2nd day of toxicity. On clinical examination, jaundice and dehydration were observed in 66.67 and 56.66 per cent cases respectively. Most of the affected animals also showed swelling of eye lids and base of ear pinna, drooping of ears and cracks of skin over ears. However severe signs of photo sensitization were not seen.

Haematological findings revealed elevated haemoglobin, packed cell volume and total erythrocytic count indicating haemoconcentration. The leukocytic count of late cases was significantly higher than the control group. The mean values of erythrocytic indices and differential leucocyte count were comparable to their respective control values. Marked increase in blood clotting time both in early (10.14 ± 1.19 min) and late cases (15.88 ± 1.89 min) was observed than control group (6.67 ± 0.55 min).

The results of erythrocytic fragility showed that haemolysis of erythrocytes of lantana intoxicated animals was significantly higher at each concentration of saline solution. The haemolysis started at 0.80 per cent NaCl concentration in both the early and late presented cases and complete haemolysis was at 0.35 and 0.40 per cent saline solution in early and late cases respectively. The mean corpuscular fragility (50 per cent haemolysis) was found to be at 0.51 and 0.53 per cent NaCl

solution respectively in early and late cases. These concentrations were higher than control group value (0.48 per cent).

Blood biochemical profile revealed that the level of blood glucose was significantly decreased and total protein was significantly increased in late presented cases. No significant change was observed in albumin level. Globulin was increased and AG ratio was significantly decreased. The activities of AST and ALT were markedly increased in lantana intoxicated cattle. AST activity of 163.0 ± 18.54 IU/l was observed in early presented cases which further increased to 200.79 ± 18.39 IU/l in late presented cases. Significant increase in alkaline phosphatase activity was also observed and levels were 155.155 ± 8.85 and 202.46 ± 25.20 IU/l in early and late presented cases indicating 3 to 4 fold increase than control value of 44.95 ± 4.00 IU/l. These changes were suggestive of degenerative changes in the liver and cholestasis.

Total bilirubin levels of 1.73 ± 0.30 and 4.58 ± 0.83 mg/dl were observed in early and late cases respectively representing 4 fold and 11 fold increase than control value of 0.42 ± 0.02 mg/dl. Markedly increased BUN levels were observed both in early (29.26 ± 4.54 mg/dl) and late cases (45.49 ± 4.00 mg/dl) suggesting renal impairment during lantana toxicity.

Rumen liquor examination revealed that its colour was brownish with no change in consistency in early stages but in late stages, it was more like watery consistency. Odour was ammoniacal up to 2nd day but after that it was more like putrid. Rumen liquor pH was increased significantly. Significant increase in SAT value ($77.67 \pm 3-55$ min) was recorded in early cases and was found nil in late presented cases. GFT was markedly decreased (0.58 ± 0.1 ml/h) than control

group (2.14 ± 0.21 ml/h) in early cases and further decreased to nil in late presented cases. TVFA concentration was markedly decreased both in early and late presented cases of lantana toxicosis.

Post-treatment haemato-biochemical evaluation was also done. The altered haematological parameters i.e. Hb, PCV and TEC were close to the normal values after the treatment. Biochemically, significant decrease in total protein and globulin from elevated pre-treatment values were observed. The levels of bilirubin, AST, ALT and alkaline phosphatase were markedly decreased after treatment from significantly elevated pre-treatment levels indicating improved hepatic function. The level of bilirubin improved significantly from 2.37 ± 0.61 to 0.64 ± 0.09 mg/dl.

There was quite improvement in rumen liquor parameters. pH of rumen liquor was decreased to 6.92 ± 0.05 from pre treatment value of 8.29 ± 0.99 . SAT was also decreased to 7.18 ± 0.72 min which was quite close to the normal value and marked increase in TVFA concentration from pre treatment value of 43.08 ± 4.37 to 92.25 ± 2.08 mEq/l was observed.

Following the combined therapy comprising activated charcoal, dextrose saline, liver extract, purgative and rumenotronics, out of 13 early presented cases, 10 cases (76.92 per cent) were treated successfully. However out of 17 late presented cases, only 2 cattle (11.76 per cent) recovered.

CONCLUSIONS

1. Incidence of lantana toxicity in cattle was higher in young age group. However no breed susceptibility could be observed.
2. Major clinical manifestations included anorexia, severe constipation, rumen stasis and dark colored urine. Most of the affected cattle showed jaundice and dehydration.
3. Increased blood clotting time, haemoconcentration, leukocytosis and increased erythrocytic fragility were predominant haematological alterations.
4. 61.53 per cent of early presented and cent per cent of late presented cases had positive biphasic Vanden bergh reaction.
5. Markedly increased bilirubin and blood urea nitrogen were characteristic biochemical changes.
6. Activities of aspartate and alanine aminotransferases, acid phosphatase and alkaline phosphatase were markedly elevated in lantana intoxicated cattle.
7. Markedly increased rumen liquor pH, decreased protozoal motility, increased SAT and markedly decreased GFT & TVFA were noted in lantana affected cattle.
8. Combined therapy comprising activated charcoal, dextrose saline, purgative, liver tonics and rumenotronics gave encouraging result in recovery of early presented cases (up to 3 days) of lantana toxicity in cattle.



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LITERATURE CITED

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