PATHOLOGICAL OBSERVATIONS OF *Ficus tsiela* (Rox b) TOXICITY IN RATS

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DECLARATION

I hereby declare that this thesis entitled "**PATHOLOGICAL OBSERVATIONS OF *Ficus tsiela* (Rox b) TOXICITY IN RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **"PATHOLOGICAL OBSERVATIONS OF *Ficus tsiela* (Rox b) TOXICITY IN RATS"** is a record of research work done independently by **Dr. Litty Mathew** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

20	Kidney – Fresh juice from 10 g leaves – Degeneration of the tubular epithelium and haemorrhage - H&E x 400
21	Kidney – Extract 1500 mg/kg body weight - Degeneration of the tubular epithelium and haemorrhage - H&E x 400
22	Liver - Fresh juice from 10 g leaves - focal necrosis H&E x 400
23	Liver - Extract 1500 mg/kg body weight - focal necrosis - H&E x 400
24	Liver - Fresh juice from 10 g leaves- sinusoidal congestion- H&E x 400
25	Liver - Extract 1500 mg/kg body weight - sinusoidal congestion –H&E x 400
26	Thyroid – Control - H&E x 100
27	Thyroid - Fresh juice from 10 g leaves - Depletion of colloid H&E x 100
28	Thyroid - Fresh juice from 10 g leaves - Depletion of colloid H&E x 400
29	Thyroid - Extract 1500 mg/kg body weight - Depletion of colloid H&E x 400
30	Heart - Fresh juice from 10 g leaves- intermuscular haemorrhage and attenuation of muscle fibres- H&E x 400
31	Heart - Extract 1500 mg/kg body weight - intermuscular haemorrhage and attenuation of muscle fibres- H&E x 400
32	Adrenal - Fresh juice from 10 g leaves – Cystic dilatation of medulla - H&E x 100
33	Spleen - Fresh juice from 10 g leaves – Reactive spleen with multiple cortical follicles - H&E x 100
34	Lung- Fresh juice from 10 g leaves - Peribronchial lymphoid hyperplasia H&E x100
35	Intestine - Fresh juice from 10 g leaves - Goblet cell hyperplasia and fragmentation of villi - H&E x100
36	Cerebrum - Fresh juice from 10 g leaves – No histological lesions- H&E x 400

37	Cerebellum - Fresh juice from 10 g leaves - No histological lesions H&E x 400
38	Pituitary - Fresh juice from 10 g leaves - No histological lesions H&E x100
39	Spinal cord - Fresh juice from 10 g leaves - No histological lesions H&E x100

Introduction

INTRODUCTION

Ficus tsiela Rox b, commonly known as Chela belongs to the family Moraceae and genus Ficus. Ficus is a large and varied genus and comprises about 800 species. It is distributed widely throughout the warmer parts of Asia, Africa, America and Australia. In India, it is common in the Western Ghats. It is a large deciduous tree up to 20 m tall with spreading branches, usually without aerial roots, often epiphytic and known for the presence of profuse white latex.

This is one of the known toxic plants in Kerala. A plant is called toxic when through contact or ingestion it hinders or destroys normal processes leading to distressing symptoms, pathology or mortality. The toxic (active) principles present in the plant are called phytotoxins. These are secondary plant metabolites and most of these do not have any apparent function in the plant except for defense mechanisms or survival adaptations. On the basis of the chemical nature, the toxic plant principles may be categorized as alkaloids, terpenes, glycosides, proteinaceous compounds, organic acids, resins and resinoids.

Poisoning of livestock by toxic plants is one of the serious causes of economic loss to the livestock industry. Losses can be classified as direct or indirect. Direct losses include deaths, abortions, lengthened calving intervals and decreased efficiency of the animals. In addition to these, there are indirect losses such as supplement feeding, medicinal costs and managemental alterations which are associated with efforts to prevent or minimize the poisoning of livestock by plants. Besides causing adverse effect in animals, the phytotoxins present in plants may enter the human food chain through animal products such as eggs, meat and milk.

The fruits and foliages of most Ficus species are well accepted by livestock, but *Ficus elastica* and *F. tsiela* are known to be toxic, particularly to cattle and buffaloes, where ingestion of large amounts of foliage can lead to animal mortality (Paterson and Clinch, 1994).

The leaves of *Ficus tsiela* Rox b are used as manure. When the leaves are cut from the tree and stocked for use, cattle accidentally feed upon the leaves and show symptoms of toxicity. Besides this, farmers in certain part of the state, ignorant of the toxicity of the plant leaves, feed their cattle with these leaves. Spontaneous cases of toxicity in cattle have been reported by many field veterinarians with symptoms similar to Rabies. Experiments have been conducted in calves and found that the plant is neurotoxic with formation of symptoms from the changes of brain. Paterson and Clinch (1994) reported that the leaves contained substances which predominantly affected the capillaries of the central nervous system.

The members of the genus Ficus are known for the presence of different compounds. These include ficusin (also known as psoralene), ficin, tylophorine, septicine, autofine and ficuseptine etc. Paterson and Clinch (1994) stated that although precise identification of the toxic principles are yet to be made , it is likely that one or more of the groups noted above could be involved in the reported animal mortality resulting from the ingestion of *F. tsiela* and *F. elastica.*

For every experiment conducted, the susceptible animal is the most suitable model. Often it is not possible to carry out experiments in suitable animals because of the stringent restrictions imposed by animal ethics committee. Therefore the accepted suitable alternative is to assess the effect in the laboratory animals following O.E.C.D (Organization for Economic Co-operation and Development) guidelines. According to them, for oral toxicity experiments, rat can be used as a

suitable model. But no sufficient data is available regarding the toxicity of *Ficus tsiela* in laboratory animals. So in view of the above facts, a study was designed utilizing rat as a model with the following objectives

(1) Study the toxic effects of *Ficus tsiela* fresh juice and alcoholic extracts in rats and assessing the suitability of rat as a laboratory animal model for expressing all the established toxicities.

(2) Study the pathomorphological and haemato-biochemical alterations caused by the active principles.

(3) Qualitative analysis of the extract for the detection of active principles.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 INCIDENCE OF PLANT POISONING IN GRAZING ANIMALS

Newsholme *et al.* (1985) reported an outbreak of paralytic condition in sheep associated with the ingestion of the plant *Trachyandra divaricata* (Jacq.) kunth.

Singh *et al.* (1987) induced bracken fern toxicity in calves by feeding each calf with dried powder of bracken fern at a dose rate of 500g/day to study the changes in blood and urine. Changes were pronounced after 30 days of bracken feeding.

In spontaneous cases of potato-plant induced dermatitis in buffaloes, the skin was thickened, oedematous, cracked and dried. Circular cracks were common with peeled off epidermis from dependent parts simulating boiled potato skin (Somvanshi *et al.*, 1992).

Eventhough the fruits and foliage of most *Ficus* species were well accepted by the livestock, *Ficus elastica* and *Ficus tsiela* were toxic, particularly to cattle and buffaloes, where ingestion of large amounts of foliage led to animal mortality (Paterson and Clinch, 1994).

Myburgh *et al.* (1994) reported about two outbreaks of neurotoxicosis in cattle browsing on the leaves of *Ficus ingens* var. ingens and *Ficus cordata* subsp. Salicifolia.

Ali *et al.* (1994) produced Lantana toxicity experimentally in goats by administering the leaves at a dose rate of 25 g/kg body weight on wet basis and studied the biochemical changes in detail.

Flaoyen *et al.* (1997) studied nephrotoxicity in goats caused by dosing with a water extract from the stems of *Narthecium ossifragum* plants by giving a single dose of an aqueous extract derived from 30g (wet weight) /kg live weight.

Ipomoea carnea flowers throughout the year, thus supplying food to cattle, sheep and goats at the time of food scarcity. Hence it was responsible for several outbreaks of livestock poisoning mainly in goats (De Balogh *et al.*, 1999).

Senna occidentalis (formerly *Cassia occidentalis*) is one of the most important toxic plants of veterinary interest with regard to the contamination of animal rations. Skeletal muscle degeneration was the preponderant lesion found in the majority of animals intoxicated (Tasaka *et al.*, 2000).

Eupatorium adenophorum was toxic to horse on prolonged exposure (Kaushal *et al.*, 2001).

Misri *et al.* (2003) summarized that the mature dry pods of *Prosopsis juliflora* especially in the month of May-June, when ingested by goats led to apparent impairment in digestion, nervous manifestations, deleterious effects on visceral organs and at times death. It also led to cyanide poisoning in cattle when mixed with sugarcane.

In an outbreak of *Lantana camera* toxicity in a sheep herd, the major clinical manifestations were mild to moderate salivation, yellowish white lacrimation, constipation, icteric mucous membrane, partial to complete anorexia, dry skin lesions, dry muzzle and grinding of teeth. Lesions of photosensitization were also present (Singh *et al.*, 2003).

Oleander (*Nerium oleander*) had long been known to be poisonous to animals and human beings. All parts of the plant are toxic. Dry leaves are as toxic as

green ones. Water in which leaves of oleander were floating was reported to be toxic and had caused death in cattle and dogs (Aslani *et al.,* 2004).

2.2 EXPERIMENTAL STUDIES ON PLANT TOXICITY

2.2.1 **Experimental Studies on Plant Toxicity in Grazing Animals**

Tripathy *et al.* (1984) produced experimental tannic acid toxicity in goats by feeding tannic acid at two per cent level of the average dry matter intake for a period of 20 days. In the treatment group, only one goat developed bloat and the rest exhibited skin lesions throughout the body.

Keeler *et al.* (1985) studied toxicity of *Thermopsis montana* (commonly called as poison bean) in cattle. Cattle had severe signs of toxicity with a prolonged recumbency lasting up to nine days when gavaged dried ground poison bean at doses of 0.6-2.8 g/kg/day in a water suspension.

Singh *et al.* (1988) fed pure sun-dried water hyacinth *ad lib* to sheep and studied liver pathology. Sheep, fed sun-dried water hyacinth alone deteriorated gradually in weight showing anaemia and liver dysfunction.

Calves which were fed with aqueous extract of *Parthenium hysterophorus* at a dose rate of 10 g/kg body weight for eight weeks showed symptoms of acute toxicity and two calves died during the course of experiment. Calves which were fed with either fresh chaffed weed or sun-dried and powdered weed at a dose of 10 g/kg body weight showed no symptoms of acute toxicity. But the carcasses in all cases were highly emaciated with alopecia, depigmentation and erythematous eruptions of the skin (Ahmed *et al.,* 1988).

Tripathy *et al.* (1989) experimentally produced bracken-fern induced haematuria in calves by feeding bracken-fern powder at a dose of 500 g/kg up to 285 days.

On prolonged *Leucaena leucocephala* diet in lambs, blood plasma and wool zinc level showed a declining trend and was significantly lower on the tenth day (Prasad *et al.*, 1989).

Rao *et al.* (1990) fed two groups of calves with powdered leaves and whole plant of Bracken-fern at a dose rate of 800 g/day. Microhaematuria was observed on days 310 and 325.

In chronic *Lantana camara* intoxication in sheep produced by feeding powdered shade-dried *Lantana camara* leaves at a dose rate of 200 mg/kg body weight for 110 days, endochondral bone growth was affected (Ganai and Jha , 1990).

Srilatha *et al.* (1997) produced *Ipomoea carnea* toxicity in goats by feeding the leaves daily at a dose rate of 50 g and 5 g/kg body weight and observed Purkinji cells damage of the cerebellum resulting in incoordination and ataxia.

Flaoyen *et al.* (1997) studied about the presence, qualities and effects of the toxic principles of *Narthecium ossifragum* in calves by giving aqueous extract, insoluble plant residue, flower stem and leaves. They could differentiate the nephrotoxic and hepatotoxic principles as water-soluble and water-insoluble compounds respectively. Both the toxic principles were present in the flower stem rather than in leaves.

Hepatotoxicity and ECG deviations were observed in a sheep dosed with 5g/kg dried *Nierembergia hippomanica* Miers var. violacea plant material on four consecutive

days. A calf dosed with 2.5 g/kg dried plant material on two consecutive days, did not show any overt clinical symptoms. Voluntary ingestion of approximately 30 g/kg fresh flowering plants resulted in nervous signs in calves (Botha *et al.*, 1999).

Dawra *et al.* (2001) conducted a preliminary study on the carcinogenicity of the common fern *Onychium contiguum*. This fern was present on the pastures in areas where grazing bovines suffered from urinary bladder cancer.

The daily use of 0.25 g/kg of *Citrullus colocynthis* fruits for 42 days was not fatal to sheep. Single oral use of *Nerium oleander* leaves at a dose of 0.25 g/kg was lethal to sheep within 18-24 hours. Rapid death was also observed in sheep receiving single dose of the mixture of the two plants (Adam *et al.*, 2001).

Henrique *et al.* (2003) reported that the signs of toxicity of *Ipomoea carnea* in growing goats reflected damage to the central nervous system and manifested as depression, staggering gait, muscle tremors, ataxia and nervousness, mainly when the animals were stressed.

The feeding of hill heifer calves with bracken fern and dryopteris fern with 30 per cent w/w of their ration for 24 months resulted in hypoproteinaemia, suppressed humoral immunity, tissue damage and possible initiation of tumour formation (Bhure *et al.*, 2006).

Shridhar and Narayana (2007) studied about the toxicity of *Cassia spectalis* in crossbred male calves and there were no observable signs in 20 g/kg group. In the 40 g/kg group all the calves died within 15 days, while in the 60 g/kg group, all calves died within ten days.

Sastry and Singh (2008) studied the toxic effects of Subabul on the thyroid and reproduction in goats by feeding green subabul leaves and found that it caused

hypothyroidism and reproductive failure in female goats and congenital goitre in the progeny.

2.2.2 **Experimental Studies on Plant Toxicity in Laboratory Animals**

Rao *et al.* (1986 and 1990) produced bracken fern induced microhaematuria in albino rats by feeding leaves, shoots and roots of the plant in the ratio 1:3 with crushed boiled maize. Roots appeared to be more toxic in rats.

Feeding of dried and pulverized *Leucaena leucocephala* leaves at a dose rate of 25 per cent in diets caused toxicity in rats (Rahman, 1995).

Gounalan *et al.* (1999) induced bracken fern (*Pteridium aquilinum)* toxicity in laboratory rats by mixing the rat concentrate ration with ground bracken fern at 25 per cent level and studied haematological, biochemical and pathological alterations in rats for three months.

Adedapo *et al.* (2004) studied the toxic effects *Euphorbia balsamifera, E. heterophylla, E. hirta, E. hyssopifolia* and *E. lateriflora* in albino rats by feeding for fourteen days. Dosage of the extract was 1g/kg body mass of rats. The extract of all the plants caused dullness, anorexia, stairy hair coat, and 20 per cent mortality rate in *E. hirta and E. hyssopifolia.*

Jayasree *et al.* (2007) conducted an experiment to observe the toxic effects of mimosa and the effect of ensiling *Mimosa invisa* on the expression of mimosine toxicity in rabbits. Rapid weight reduction, alopecia, and sluggishness were the effects of mimosine toxicity and admixture of pasture with *Mimosa invisa* up to 50 per cent was safe for feeding after cutting and ensiling for 60 days.

2.2 TOXIC PLANT POISONING AND HAEMATOLOGICAL CHANGES

2.2.1 **Haematological Changes in Grazing Animals**

In bracken fern toxicity, all the calves revealed progressive decrease in Haemoglobin (Hb), Packed Cell Volume (PCV), and Total Erythrocyte Count (TEC) values with lymphocytosis and neutropenia. However, the values of erythrocyte indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were within the normal range (Singh *et al.*, 1987).

Singh *et al.* (1988) fed pure sun-dried water hyacinth *ad lib* to sheep and studied liver pathology. There was significant decrease in TEC, Hb and PCV. The Erythrocyte Sedimentation Rate (ESR) was increased significantly.

Haematological evaluation in a natural case of toxicity of Oee (*Albizzia stipulata*) in cows revealed normal Hb and haematocrit value. Differential leucocytes count (DLC) showed neutrophilia and lymphocytopenia. Van den Bergh test was positive indicating hepatic involvement (Wadhwa *et al.*, 1993).

Immature Ohi (*Albizia stipulate*) leaves feeding in calves caused marginal increase in Hb and TEC. But relative neutrophilia and lymphopenia were observed (Manuja and Prasad, 1999).

Wadhwa *et al.* (2001) conducted haemato-biochemical studies in animals affected with enzootic bovine haematuria caused by the ingestion of bracken fern [*Pteridium aquilinum*]. Haematological evaluation revealed significantly low Hb, PCV, TEC, MCV and MCH.

Values of Hb, MCV, WBC and neutrophils were lower and those of lymphocytes were higher in *Citrullus colocynthis* fruits toxicity in sheep. Increased

WBC, lymphocytosis and neutropenia were observed in *Nerium oleander* toxicity. Combination of these two plants produced increase in Hb, TEC, PCV, WBC and lymphocytes and decrease in neutrophils. No significant differences were observed in eosinophil, basophil and monocyte values in all the groups (Adam *et al.,* 2001).

In experimental *Tribulus terrestris* poisoning in sheep, there were no significant differences in the PCV, neutrophils, lymphocytes, monocyte or eosinophil counts. Total Leucocyte Count (TLC) was significantly increased (Aslani *et al.,* 2003).

Singh *et al*. (2003) observed a significant decrease in Hb and TEC whereas significant increase in TLC and PCV in *Lantana camera* poisoning in a sheep herd.

Henrique *et al.* (2003) studied the clinical, biochemical, haematological and pathological effects of long-term administration of *Ipomoea carnea* to growing goats. A significant decrease was observed in body weight gain, erythrocyte numbers and PCV. The MCV, MCH, MCHC, TLC and DLC were similar in all groups.

Decreased concentrations of Hb and serum immunoglobin were observed in bracken fern and dryopteris fern toxicity in hill heifer calves (Bhure *et al.*, 2006).

There were no changes in haematological parameters like Hb, PCV, TLC, and DLC in *Cassia spectalis* toxicity in cross bred male calves (Shridhar and Narayana, 2007).

No haematological alterations were found in sheep which received only *Panicum miliaceum* grass as ration (Badiei *et al.,* 2009).

2.2.2 **Haematological Changes in Laboratory Animals**

Gounalan *et al.* (1999) studied the haematological effects of bracken-fern toxicity in rats and found an increase in clotting time and ESR and a concurrent decrease in erythrocyte count, PCV and Hb. A distinct leucopenia, lymphopenia, and relative neutrophilia were also present.

The crude extract of *Euphorbia balsamifera, E. heterophylla E. hirta, E. hyssopifolia* and *E. lateriflora* when administered orally produced anaemia in rats. While *E. heterophylla , E. lateriflora and E. hyssopifolia* caused leucopenia; *E. balsamifera* and *E. hirta* caused leucocytosis (Adedapo *et al.,* 2004).

2.3 TOXIC PLANT POISONING AND BIOCHEMICAL CHANGES

2.3.1 **Biochemical Changes in Grazing Animals**

Keeler *et al.* (1985) observed increase in blood levels of aspartate amino transferase (AST), creatine phosphokinase (CK), and lactate dehydrogenase (LDH) in animals poisoned by *Thermopsis montana* which ultimately led to myopathy.

In bracken fern toxicity all the calves revealed progressive increase in Blood urea nitrogen (BUN), uric acid, creatinine and total acid phosphatase activity (Singh *et al.,* 1987).

Tirkey *et al.* (1987) observed no effect on alkaline phosphatase (ALP), alanine amino transferase (ALT), AST, LDH, blood cholesterol, BUN, blood sugar, serum sodium, potassium and calcium levels and total serum protein, albumin, globulin levels and albumin: globulin ratio in administration of the aqueous extract of *Ipomoea carnea* in goats.

Singh *et al.* (1988) fed pure sun-dried water hyacinth *ad lib* in sheep and studied liver pathology. There were marked hypoglycemia and increased AST concentrations.

Bracken-fern fed calves revealed progressive increase in total protein, potassium, copper and magnesium levels. Calcium, phosphorus, and thiamine values were significantly low (Tripathy *et al.,* 1989).

Ganai and Jha (1990) studied the effect of *Lantana camara* toxicity on bone growth in sheep and found that there was no significant difference in the serum calcium level at any stage of the experiment. The level of serum inorganic phosphorus decreased significantly on 45^{th} day. The level of serum ALP increased significantly on 30^{th} day.

In experimental toxicity of tansy ragwort (*Senecio jacobaea*) in calves, serum glutamate dehydrogenase (GDH) was the first enzyme to increase, with a short-term increase to peak values followed by a rapid return to normal. This was followed by increase in ALP and gamma glutamyl transferase (GGT) (Craig *et al.*, 1991).

In *Lantana camara* toxicity in goats, the mean values of ALP, AST and icteric index increased significantly. The mean values of total protein, albumin and albumin: globulin ratio decreased significantly (Ali *et al.,* 1994).

Aqueous extract of *Narthecium ossifragum* caused an increase in creatinine, urea, and magnesium concentrations but decrease in calcium concentration. The phosphorus concentration and the activities of AST, LDH, and GGT were increased in calves dosed with the insoluble plant residue. When the calves dosed with flower stem, serum GDH, AST, GGT, creatinine and urea concentrations were increased significantly, whereas in calves dosed with leaves, increase in creatinine concentration was only temporary (Flaoyen *et al.*, 1997).

Botha *et al.* (1999) observed increased AST, LDH and CK levels and serum urea and creatinine concentrations in *Nierembergia hippomanica* Miers var. violacea induced neurotoxicity in calves and sheep. But the serum GGT and GDH activities fluctuated within or near the pre-dosing ranges and the total calcium and inorganic phosphate levels were decreased.

Mandal and Randhawa (1999) studied the liver function test profile in lantana-induced hepatitis in buffalo calves and found that there was progressive and significant increase in values of plasma GGT, ALP, arginase, AST and ALT activity. There was also 4.2 fold increase in direct plasma bilirubin and 1.77 fold increase in indirect bilirubin.

Blood biochemical investigation showed a marginal increase in the levels of blood glucose, total protein, total bilirubin and ALT in immature Ohi (*Albizia stipulate*) leaves feeding. An increase in the plasma AST level was observed at 24hr after Ohi feeding which started decreasing afterwards (Manuja and Prasad, 1999).

Flaoyen *et al.* (2001) observed an increase in both creatinine and urea concentrations in sheep due to the nephrotoxic component of *Narthecium ossifragum*.

Wadhwa *et al.* (2001) conducted haemato-biochemical studies in animals affected with enzootic bovine haematuria caused by the ingestion of bracken fern (*Pteridium aquilinum*) toxin. Biochemically, affected animals had hypoglycemia, hypocalemia, hypophosphataemia with markedly elevated BUN and creatinine levels.

LDH and AST activities and urea concentrations were increased whereas cholesterol, total protein, albumin, globulin and bilirubin levels were unaltered in sheep with *Citrullus colocynthis* toxicity. LDH, AST, cholesterol, bilirubin and urea were increased in *Nerium oleander* toxicity and same result was obtained when both

C. colocynthis and *N. oleander* were given to goats. Total protein and albumin were decreased in *N. oleander* toxicity (Adam *et al.,* 2001).

There were significant decrease in blood glucose and total protein values and significant increase in total bilirubin, creatinine, BUN, AST, ALP, and LDH values observed in Lantana toxicity in a sheep herd (Singh *et al.,* 2003).

In experimental *Tribulus terrestris* poisoning in sheep, the serum AST, ALT, total and direct bilirubin concentrations, serum creatinine, and BUN showed a marked increase. The concentrations of Calcium, Phosphorus, Sodium, and Potassium in the serum showed no significant differences (Aslani *et al.,* 2003).

Henrique *et al.* (2003) studied the clinical, biochemical, haematological and pathological effects of long-term administration of *Ipomoea carnea* to growing goats. ALT was significantly increased in the experimental animals. AST, BUN and creatinine levels were fluctuated. Blood cholesterol, GGT and glucose levels were similar in the control and treated groups.

There was significant decline in blood pH but increase in BUN together with increased serum amylase and AST activity in toxicity of *Prosopis juliflora* in goats (Misri *et al.*, 2003).

In goats with *Cassia spectalis* toxicity Shridhar and Narayana (2007) observed an increase in plasma levels of AST and ALT. Toxicity with the same plant in calves caused no change in the blood serum creatinine, BUN, and total protein concentrations.

Badiei *et al.* (2009) studied *Panicum miliaceum* toxicity in sheep experimentally. Clinically, affected sheep had significant elevation of serum GGT, ALP and AST activities and total bilirubin, conjugated bilirubin, BUN, and creatinine

concentrations. Serum inorganic phosphorus concentration was significantly increased.

2.3.2 **Biochemical Changes in Laboratory Animals**

In bracken fern fed rats, the bilirubin, serum creatinine, uric acid, BUN and serum LDH were gradually increased. A progressive non-significant increase in the activity of AST and ALT was also observed (Rao *et al.,* 1986).

Gounalan *et al.* (1999) studied the biochemical effects of bracken-fern toxicity in rats and found a decrease in the blood glucose level while an increase in the values of ALT, AST, ALP, urea and creatinine.

In toxicity testing of *Senna occidentalis* seed in rabbits, Tasaka *et al.* (2000) found that there was a statistically significant diminution in total protein in the animals receiving ration containing one per cent of ground seed. A decrease in ALT activity, total protein and albumin concentrations was present in the animals receiving ration containing three per cent of ground seed.

Administraton of freeze-dried leaf powder of *Eupatorium adenophorum* to mice led to significant increase in the total and conjugated bilirubin concentration in the plasma. The creatinine, urea and protein contents in the plasma were comparable in the test and control groups. There were significant increase in ALP, 5'nucleotidase, AST, ALT and LDH activities, but no significant change was observed in leucine aminopeptidase or acid phosphatase activity (Kaushal *et al.,* 2001).

Euphoria hirta, E. Hyssopifolia and *E. laterifolia* caused a significantly increased level of protein whereas *E. balsamifera* and *E. heterophylla* caused an insignificant increase. All the five plants caused an increase in level of albumin and

decrease in the level of globulin. All the plants caused significant increase in levels of AST and ALT (Adedapo *et al.*, 2004).

2.4 GROSS PATHOLOGICAL OBSERVATIONS OF PLANT POISONING

2.4.1 **Gross Pathological Observation in Grazing Animals**

Bapat and Abhyankar (1984) reported cyanide poisoning in cattle due to feeding of sorghum. In this case, lungs were edematous, congested and emphysematous. Hydropericardium was present. The liver was enlarged and congested with a distended gall-bladder. Abomasum and small intestine showed congestion.

Newsholme *et al.* (1985) reported a paralytic condition in goat associated with ingestion of the plant *Trachyandra divaricata* (Jacq.) kunth. At necropsy, yellowish-brown discolouration of the grey-matter of the brain and spinal cord, liver, renal cortex and lymph nodes were noticed.

Singh *et al.* (1988) fed pure sun-dried water hyacinth *ad lib* in sheep and studied liver pathology. The liver was congested and grossly enlarged.

In experimental induction of acute toxicity in buffalo calves by feeding *Parthenium hysterophorus*, the calves developed patchy erythematous eruptions. They were found on the head, around the ears, eyes, shoulders, neck, and abdomen. There were ulcers on the tongue, mouth cavity and in the abomasum. Catarrhal enteritis was also observed. Kidneys revealed gelatinous fat on section (Ahmed *et al.,* 1988).

In bracken-fern fed calves, carcasses were debilitated and anaemic. Excess fluid was present in the abdominal cavity and the wall of the urinary bladder was thickened (Tripathy *et al.,* 1989).

Rao *et al.* (1990) reported that in bracken-fern toxicity in calves, animals were anaemic and debilitated. Liver was congested, enlarged and had small areas of focal degeneration. Kidneys were pale and anaemic. Urinary bladder was hyperaemic and distended and had multiple haemorrhages.

In experimental *Ipomoea carnea* toxicity in goats, the lesions were hydrothorax, hydropericardium, enlargement of mesenteric lymph nodes and congestion of meninges and brain (Srilatha *et al.,* 1997).

Botha *et al.* (1999) induced neurotoxicity in calves and sheep by the plant *Nierembergia hippomanica* Miers var. violacea and the gross lesions noted were mild cerebral oedema, mild interstitial pneumonia, slight swelling of liver with more distinct lobulation, and congestion of the wall of the gall bladder and mucosa of the small and large intestine.

Congestion of intestine, liver, spleen, heart and hepatorenal fatty changes were the lesions observed in *Citrullus colocynthis* toxicity. Widespread congestion and haemorrhage on the heart, lungs, liver, intestine, abomasum and spleen were the lesions observed in *Nerium oleander* toxicity (Adam *et al.,* 2001).

Flaoyen *et al.* (2001) studied the tolerance to the nephrotoxic component of *Narthecium ossifragum* in sheep by feeding the plant *ad libitum*. At necropsy they found intussusception of the small intestine. Proximal to the intussusception, there was distension of the jejunum with accumulation of fluid and gas. The kidneys had a soft texture and there was perirenal oedema.

In toxicity study of *Prosopis juliflora* in goats, liver was enlarged and kidneys were pale, enlarged, and oedematous. Lungs were pale and emphysematous. Thyroid, pancreas, and lymph nodes were congested and revealed degenerative changes (Misri *et al.,* 2003).

Aslani *et al.* (2003) reported about experimental *Tribulus terrestris* poisoning in sheep. At necropsy, all the animals showed various degrees of generalized icterus. Liver was swollen and discoloured by bile pigments. The gall bladder was enlarged and filled with concentrated bile. The kidneys were swollen and yellow to green in colour.

Gross lesions in experimental Oleander (*Nerium oleander*) toxicosis in sheep were congestion and extensive haemorrhages of the subdermal tissues, mild hydrothorax, hydropericardium and ascites, petechial haemorrhages on the epicardium and endocardium, especially on the left ventricle and congestion of kidneys and liver. Pulmonary congestion and gastroenteritis were present. Meninges and brain parenchyma showed marked congestion and petechial haemorrhages (Aslani *et al.*, 2004).

Barbosaa *et al.* (2008) evaluated the pathological effects of oleander (*Nerium oleander*) in goats and found that there were no gross lesions.

Badiei *et al.* (2009) studied *Panicum miliaceum* poisoning in sheep by experimentally producing the toxicity. Moderate to severe yellow discolouration of all tissues was evident. Gall bladder was distended with thick dark bile. Liver and kidney were swollen with yellowish appearance in cut surfaces.

2.4.2 **Gross Pathological Observation in Laboratory Animals**

Rao *et al.* (1990) reported that in bracken-fern toxicity in rats, the animals were anaemic and debilitated. Urinary bladder was hyperaemic and distended and had multiple haemorrhages. Urine had prominence of RBC, amorphous phosphates, and epithelial cells.

Rahman (1995) observed that in *Leucaena leucocephala* toxicity in rats, no gross lesions were detected except in ovary which was smaller. The horns and body of the uterus was atrophic. The vagina was thin and transparent. The testes appeared inflamed and doughy in consistency.

Greyish white streaks of necrosis in kidney, hepatosis with pale nodularity in the parenchyma and catarrhal enteritis were the lesions found in the experimental toxicity of *Mimosa invisa* in rabbits (Jayasree *et al.,* 2007).

2.5 HISTOPATHOLOGICAL OBSERVATIONS IN PLANT POISONING

2.5.1 **Histopathological Observations in Grazing Animals**

In experimental tannic acid toxicity in goats, the liver showed cloudy swelling and fatty changes. Renal tubular epithelium showed necrosis. The islet cells of pancreas underwent coagulation necrosis. Desquamation of the tips of the villi and mononuclear cell infiltration in the wall of the intestine were present. Heart and spleen showed congestion. The skin showed patchy degeneration with complete absence of epidermis and focal epidermal hyper-keratinization (Tripathy *et al.,* 1984).

Bapat *et al.* (1984) studied cyanide poisoning in cattle due to feeding of sorghum. In this case, kidney and lungs showed congestion. Liver showed hepatitis. Congestion and necrosis of mucous membrane was present in the intestine. Cerebrum showed gliosis and satellitosis.

Post-mortem examination of goats fed with water-hyacinth (*Eichornea cracipes* Solms) *ad libitum* revealed right ventricular hypertrophy and hepatic necrosis. In kidney, chronic interstitial nephritis was observed. In the heart,

myocardium revealed hyalinization in the sub-endocardial region. Proliferative enteritis along with severe congestion was also observed (Dutta *et al.,* 1984).

Newsholme *et al.* (1985) observed the histopathological lesions in goat following ingestion of the plant *Trachyandra divaricata* (Jacq.) kunth. There was accumulation of lipofuscin pigment in large neurons of brain, spinal cord, hepatic Kupffer cells, macrophages in the medulla of lymph nodes, splenic red pulp and pulmonary alveolar walls. In the white matter of the spinal cord, loss of occasional axons with swelling of the surrounding myelin sheath was observed.

In *Ipomoea carnea* toxicity in goats, nephrosis was observed. Heart showed fragmentation of the myofibrils along with infiltration of mononuclear cells. Liver showed vacuolar degeneration, Kupffer cell hypertrophy and bile-duct hyperplasia. Peri-neuronal vacuolation, satellitosis, neuronophagia and wallerian degeneration were observed in the spinal cord (Tirkey *et al.,* 1987).

Singh *et al.* (1988) fed pure sun-dried water hyacinth *ad lib* to sheep and studied liver pathology. Histopathological examination revealed areas of congestion, haemorrhage and fatty changes in centrilobular and periportal locations.

In an experimental study of acute toxicity in buffalo calves by feeding *Parthenium hysterophorus*, calves developed haemorrhages on the epicardium, endocardium, diaphragmatic lobes of the lung, brain and in abomasum. Necrotic enteritis was recorded. Pancreas revealed vacuolation in the acinar cells. Hyaline cast was present in the kidney. Bronchiectasis was seen. Brain showed neuronal degeneration, satellitosis and neuronophagia. The follicles in the thyroid were irregular with pale and scanty colloid. Pituitary gland revealed haemorrhages and mild fibrosis. Adrenal gland showed congestion and haemorrhages in the cortex. (Ahmed *et al.,* 1988).

Ganai and Jha (1990) produced *Lantana camara* toxicity in sheep and on histopathological examination of costo-chondral junction there were slight reductions in the sizes of proliferating, maturing and degenerating cartilage cells. The trabeculae in the metaphysis appeared thinner and smaller than normal.

Rao *et al.* (1990) reported that in bracken-fern toxicity, degenerative changes were present in cortex and medulla of the kidney in calves. Hyperplasia of transitional epithelium was prominent in the urinary bladder. Hepatic cells showed hydropic degeneration, necrosis and mild periportal fibrosis along with a tendency for neoplasia of the bile ducts. Congestion and desquamation of superficial mucosa was present in the intestine. Emphysema, septal thickening, and patchy interstitial pneumonia were present in the lungs. In spleen, lymphoproliferative changes with accumulation of large number of plasma cells were present. Heart revealed muscular degeneration.

Biliary hyperplasia, fibrosis and vacuolation of hepatocytes were the lesions observed in tansy ragwort (*Senecio jacobaea*) toxicity in calves (Craig *et al.*, 1991).

Somvanshi *et al.* (1992) induced potato plant toxicity in both young and adult rabbits as two separate groups. Histopathological lesions were similar in both groups. Marked congestion was present in the lungs, heart, small intestine and liver. Spleen was atrophied. Small intestine was dilated with congested mesentery. Kidneys were pale. Brain was hyperaemic, oedematous and showed marked gliosis. Lungs showed oedema. Liver revealed engorged sinusoids and focal proliferation of mononuclear cells.

In experimental *Ipomoea carnea* toxicity in goats, prominent lesions in cerebrum were congestion, haemorrhage, satellitosis, neuronophagia, gliosis and perineuronal oedema. In cerebellum, focal loss and grouping of the Purkinji cells with thinned out appearance of molecular and granular layer was noticed. Hepato-

nephrotoxic changes were also observed. The changes were not prominent in lungs, spleen, heart and lymph nodes except severe congestion and haemorrhages. Mild depletion in germinal centers of lymph nodes was also observed (Srilatha *et al.*, 1997).

Degeneration and necrosis of the tubular epithelial cells along with multifocal mononuclear cell infiltration was present in the kidney in *Narthecium ossifragum* toxicity in goats. Lungs showed fibrinous bronchopneumonia and some animals showed broncho-interstitial pneumonia. Liver, heart and intestine showed no specific pathological changes (Flaoyen *et al.,* 1997).

Botha *et al.* (1999) induced neurotoxicity in calves and sheep by the plant *Nierembergia hippomanica* Miers var. violacea and the microscopical lesions in the liver included degeneration and necrosis of hepatocytes along with infiltration of neutrophils. Mild cerebral oedema of the white matter in periventricular areas was also evident.

Cytoplasmic fatty vacuolation of the centrilobular hepatocytes and mild degeneration of the cells of the PCT and mild catarrhal enteritis were observed in *Nerium oleander* toxicity in sheep. When sheep was fed with a combination of *N. oleander* leaves and *Citrullus colocynthis* fruits, the lesions observed were emphysema, congestion and haemorrhage of alveoli of lungs. Fatty vacuolation and necrosis were present in the hepatocytes. Necrosis of the renal tubular cells and catarrhal enteritis were also observed (Adam *et al.*, 2001).

Narthecium ossifragum was implicated in a severe outbreak of nephrotoxicity in cattle. Flaoyen *et al.* (2001) studied the nephrotoxic effect of *Narthecium ossifragum* in sheep by feeding the plant *ad libitum*. On histopathological examination, kidney revealed degeneration and necrosis of the tubular epithelial cells.

There was interstitial fibroblastic proliferation in the cortex. Large amounts of proteinaceous exudates were found in the alveoli of the lungs.

Degenerative vacuolar changes in the neurons of the cerebellum, liver, pancreas, thyroid and kidney cells were reported by Henrique *et al.* (2003) in long-term administration of *Ipomoea carnea* to growing goats.

Coagulative necrosis with cellular infiltration in liver, hyperplastic bile ducts, golden yellow inspissated bile pigment in rumen, early degenerative changes in kidneys and lymph nodes were the lesions observed in *Prosopis juliflora* poisoning in goats (Misri *et al.*, 2003).

Aslani *et al.* (2003) reported the histological findings of experimental *Tribulus terrestris* poisoning in sheep. Liver revealed varying amounts of crystalloid materials in the bile ducts and hepatocellular degenerations. Biliary fibrosis, bile duct proliferation and epithelial necrosis of the gall bladder were also present. Tubular necrosis of kidney and focal necrosis of cardiac muscle were also observed. There was pustule formation, fibrino-purulent exudates, cell debris and infiltration of inflammatory cells in the dermis.

Histopathological examination of tissues in experimental Oleander (*Nerium oleander*) toxicosis in sheep revealed myocardial degeneration, necrosis, haemorrhage and infiltration of inflammatory cells. Lesions in the liver were vacuolar degeneration, fatty change, focal necrosis and infiltration of inflammatory cells. In the kidney, congestion and widespread tubular epithelial necrosis were present. Congestion and oedema were observed in the lungs. Gastroenteritis was evident in the fore-stomachs, abomasum and intestines. Perivascular and perineuronal oedema and ischaemic cell changes were observed in the cerebrum (Aslani *et al.*, 2004).

In *Cassia spectalis* toxicity in crossbred cattle, massive epicardial haemorrhage and sarcoplasmic vacuolation in the myocardium were present. Liver showed distension of sinusoidal spaces with hyperaemic changes (Shridhar and Narayana 2007).

Barbosaa *et al.* (2008) evaluated the pathological effects of oleander (*Nerium oleander*) in goats. Microscopic examination showed degeneration of renal tubular cells and necrosis of PCT and DCT. Cardiomyocytes showed different staining with dark and pale myocytes. Brain and other CNS structures did not show any microscopic lesion.

In subabul poisoning in goats, thyroid revealed small, medium or abnormally large sized follicles containing pale to light pink and watery colloid. The lining follicle cells were squamous type and in some hyperplastic changes with desquamation were present. In kids with congenital goitre, spherical to irregular shape follicles were present with hyperplastic lining epithelium forming projections into the lumen. Interfollicular areas showed congestion (Sastry and Singh, 2008).

Badiei *et al.* (2009) studied *Panicum miliaceum* poisoning in sheep. Liver showed degeneration and necrosis of periportal hepatocytes associated with scattered apoptotic cell. Fatty change of hepatocytes and peribiliary oedema were also observed. In the kidneys, swelling and degenerative changes were evident in the epithelium of PCT.

2.5.1 **Histopathological Alterations in Laboratory Animals**

Rao *et al.* (1990) reported nephrosis, hyperplasia of transitional epithelium and congestion of muscular layer in urinary bladder in bracken-fern induced toxicity in rats. Atrophic changes and vascular congestion were present in the liver. Congestion and desquamation of the superficial mucosa was present in the intestine.

Emphysema, septal thickening and patchy interstitial pneumonia were present in the lungs. In spleen, lymphoproliferative changes with accumulation of large number of plasma cells were present. Heart revealed muscular degeneration and myocardial infarction.

Rahman (1995) observed that in *Leucaena leucocephala* toxicity in rats, oocytes in graafian follicles and corpus luteum was absent in ovary. In the uterine horn, the myometrium lost their muscles and blood vessels were few. Uterine glands were scanty. The vaginal surface devoid of cornified layer and was smooth. In the testes, epithelium of seminiferous tubules was degenerated, desquamated and devoid of any spermatozoa. Liver showed mild hepatitis and had the evidence of apoptosis. There was interstitial nephritis with haemorrhage. Desquamation of epithelial lining of the descending loop of Henle and collecting tubules were seen.

Rats fed with bracken fern at 25 per cent level showed vacuolar degenerative changes in the hepatocytes. Spleen revealed passive congestion, hyperplasia of reticuloendothelial cells, thickened trabeculae and presence of haemosiderin laden macrophages. Other changes were oedema in brain, subepicardial haemorrhages, emphysema, hypersecretary activity in the intestine and presence of eosinophilic homogenous contents and degenerative changes in the testes (Gounalan *et al.,* 1999).

The histopathological study of rabbits receiving concentrates containing four per cent ground *Senna occidentalis* seed for 30 consecutive days revealed severe lesions in the heart tissue in the form of intense vacuolation of myocardial fibres and a characteristic inflammatory process and necrosis. These animals showed vacuolar degeneration of hepatocytes in the centrilobular regions. Electron-microscopic study of the liver revealed dilated mitochondria with destruction of the internal cristae (Tasaka *et al.,* 2000).

In *Onychium contiguum* toxicity, intestinal tumours were found in the ileal region of four guinea-pigs. There were thickening of the urinary bladder wall, oedema, ulceration and desquamation of epithelium, haemorrhages, nodular and papillary hyperplasia and proliferation of the venules in the exposed animals. Microhaematuria and mammary tumour were also present (Dawra *et al.*, 2001)

Administraton of freeze-dried leaf powder of *Eupatorium adenophorum* to mice resulted in loss of body weight. Post-mortem examination revealed yellowish subcutaneous tissue and musculature. The gastric mucosa and intestine were severely congested and haemorrhagic with desquamation of the lining cells. Focal areas of necrosis and biliary proliferation were observed in the liver parenchyma. Hepatocytes showed megalocytosis. The bile ducts were dilated and epithelium showed degenerative to necrotic changes (Kaushal *et al.,* 2001).

Renal tubular degeneration, necrosis, cystic dilation and glomerular congestion in kidneys, congestion, haemorrhage, and oedema of the lungs, congestion and haemorrhage along with necrosis and desquamation of epithelial cells of intestine, sinusoidal congestion and bile duct hyperplasia in liver were the lesions encountered in *Mimosa invisa* toxicity in rabbits (Jayasree *et al.,* 2007).

2.6 OXIDATIVE EFFECT OF VARIOUS TOXIC PLANTS

The activity of lipid peroxidase and catalase was significantly reduced in rats treated with diazepam at a dose of 1.2 mg/kg and datura extract at a dose of 1.2 mg/kg. The datura treated group showed higher activity of lipid peroxidase and catalase than the diazepam group (Khan, 1987).

Purified alcoholic extract of seeds of *Strychnos nuxvomica* inhibited the process of lipid peroxidation. The extract reduced the rate of decline of glutathione in a dose- and time-dependent manner (Tripathy *et al.*, 1996).

Sarathchandra and Balakrishnamurthy (1997) reported that in acute oral toxicosis of *Cleistanthus collinus* produced by administration of leaf extract at LD50 dose orally to rats (8 g/Kg.), glutathione was depleted significantly in liver, kidney, heart, brain and skeletal muscles.

In liver, heart and skeletal muscles of hypothyroid rats, the lipid peroxidation was not modified, whereas in hyperthyroid rats, lipid peroxidation increased in liver and heart but not in skeletal muscles. The glutathione peroxidase activity increased significantly in the heart and muscle of hypothyroid rats and in the muscle of hyperthyroid rats (Venditti *et al.*, 1997).

Exposure to the fern *Onychium contiguum* caused significant increase in the preformed lipid peroxides in the urinary bladder of guinea-pigs. The concentrations of glutathione and alpha- tocopherol and the activities of glutathione reductase and catalase were elevated. No effect was observed in the concentration of ascorbic acid and the activities of glutathione peroxidase, glutathione –S-transferase and superoxide dismutase (Sood *et al.*, 2003).

In bracken fern fed hill heifer calves, no effect on lipid peroxidation of erythrocytes and increased catalase activity was observed. A significant increase in reduced glutathione level of erythrocytes was also observed (Bhure *et al.,* 2006).

2.7 PHYTO-CHEMICAL CONSTITUENTS OF VARIOUS PLANTS

Preliminary phytochemical screening of the methanolic extract of *Ficus platyphylla* stem bark gave positive test for flavanoids, tannins, and saponins (Amos *et al.,* 2001, Wakeel *et al.,* 2004).

Wiam *et al.* (2005) reported that in ethanolic extract of *Cassia siamea* alkaloids, glycosides and steroids were present in high concentrations. Tannins and

anthraquinones were present in moderate concentration while saponins in low concentration.

2.8 TOXICITY OF *Ficus tsiela* (Rox b)

Nair *et al.* (1985) studied toxicity of *Ficus tsiela* in calves by feeding fresh tender and mature leaves along with soft stem. The leaves were found to be highly neurotoxic

Rajan *et al*. (1986) reported that there was a nervous disorder in cattle caused by the toxicity of the leaves of the tree *Ficus tsiela* and there was a dose related development of toxicity. The onset of symptoms within 48 hours after the consumption of leaves appeared to be a sign of acute toxicity.

The leaves of *Ficus tsiela* (Rox b) contained substances which predominantly affected the capillaries of central nervous system (Paterson and Clinch, 1994).

Mamckam *et al.* (2009) reported that perivascular space dilatation progressing to cavitation and crowding of gemistocytes in *Ficus tsiela* poisoning in calves was similar to *Trypanosoma brucei rhodesiense* infection in cattle and in PEM.

2.8.1 **Haemato-biochemical Changes in *Ficus tsiela* Toxicity**

Nair *et al*. (1987) studied the clinico- pathological features of *Ficus tsiela* in calves in experimental toxicosis and observed transient hypoglycemia and increase in AST activity.

2.8.2 **Gross and Histopathological Alterations in *Ficus tsiela* Toxicity**

Nair *et al.* (1985) produced toxicity in calves by feeding fresh leaves of *Ficus tsiela*. In this case the brain was moderately enlarged, gyri flattened and the

meninges congested. The liver was enlarged with scattered areas of necrosis. The gall bladder was distended. The kidneys and lungs were congested. Histologically the sinusoids of the liver were congested with centrilobular and midzonal necrosis. The tubular epithelial cells of the kidney underwent coagulative necrosis. The medullary zones showed congestion and minute foci of haemorrhage. Focal areas of necrosis were seen in the myocardium. Mild catarrhal enteritis was seen. In the cerebrum, there was perivascular oedema and demyelination in white matter. Neurons showed pyknosis, gliosis and satellitosis.

In natural cases of *Ficus tsiela* toxicity in cattle, the liver was enlarged with scattered patches of necrosis. The cortex of kidney was congested and the cut surface showed scattered greyish white areas. The heart was enlarged and the spleen was congested. The abomasum was hyperaemic and showed ulcers. The brain was enlarged with hyperaemic meninges and flattened gyri. The lungs showed patchy areas of congestion. Histologically in brain, neurons showed degenerative changes, neuronophagia, satellitosis and diffused gliosis. The sinusoids of the liver were engorged and there was centrilobular necrosis. The renal tubular epithelial lining was swollen, necrotic and some of the tubules contained hyaline casts (Rajan *et al.,* 1986).

Nair *et al*. (1995) studied the ultra structural changes of brain in *Ficus tsiela* poisoning. The cytoplasm of neurons and glial cells had an expanded volume. Many neurons of the cerebral cortex and Purkinje cells showed partial degranulation of ribosome and fragmentation of the rough endoplasmic reticulum. The neurotubules showed slight dissolution and the neurofilaments were clumped.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Adult female Sprague Dawley rats weighing approximately 150-200 g procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for the study. Rats were maintained on identical feeding and management practices in the laboratory for one week before the commencement of studies. The experiment was conducted for a period of 21 days.

3.2 PLANT MATERIALS

Leaves and tender stem of the plant *Ficus tsiela* (Rox b*)* were collected from the campus of College of Veterinary and Animal Sciences, Mannuthy and identified (Fig. 1). Fresh leaves along with its latex were collected for the preparation of the fresh juice.

3.2.1 **Preparation of Alcoholic Extract of Leaves of *Ficus tsiela***

The leaves and tender stem of *Ficus tsiela* (Rox b) were air-dried at room temperature and coarsely pulverized using an electrical pulverizer. The powder obtained was extracted using a soxhlet apparatus with 95 per cent ethanol. The ethanolic extract were then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55ºC) and kept under refrigeration for the complete evaporation of the solvent. The yield of the extract was 4.1 per cent on dry matter basis (Fig. 3).

The dried extract was suspended in two per cent gum acacia and administered orally to the experimental animals using an orogastric tube.

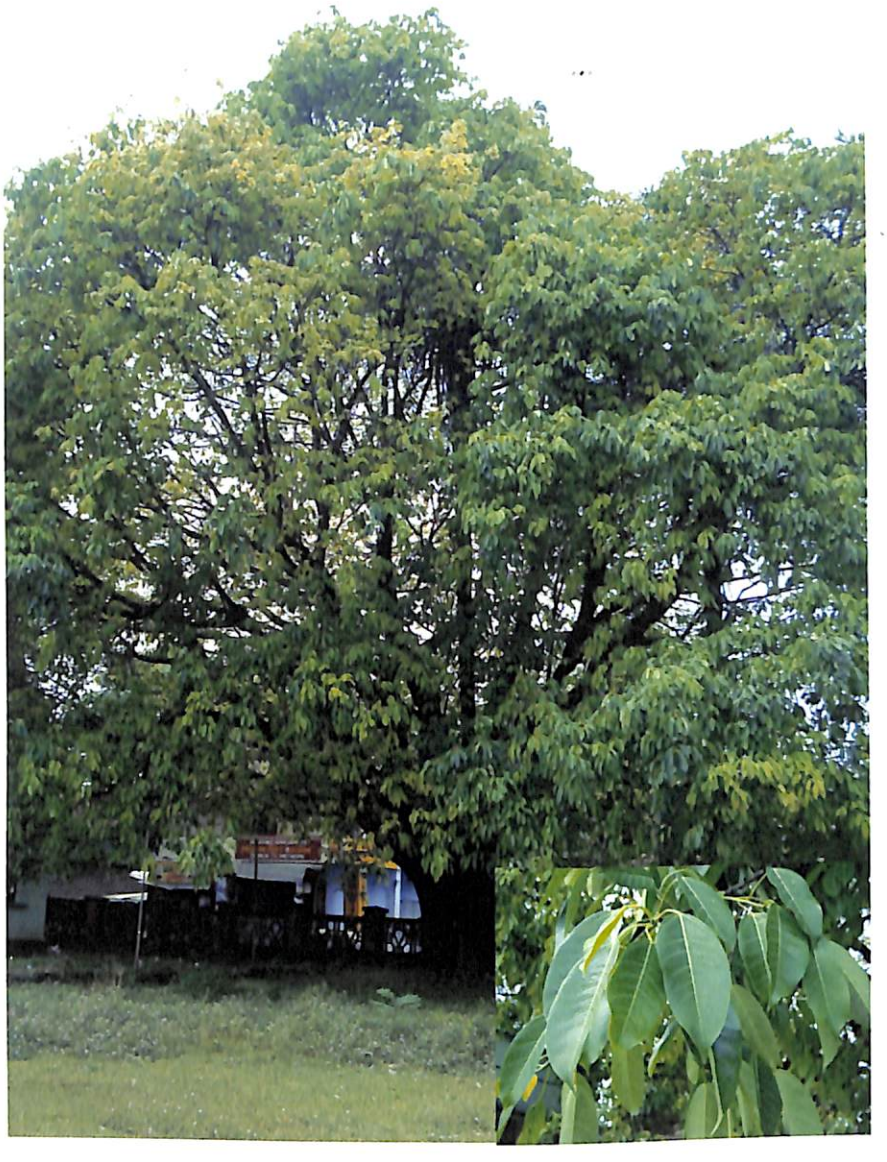

Fig. 1

Fig. 2

Fig. 3

Fig. 1 *Ficus tsiela* Rox b tree with leaves (insst)

Fig.2 Ficus tsiela Rox b leaves while drying

Fig. 3 *Ficus tsiela* Rox b leaves extract

3.2.2 **Preparation of Fresh Juice of Leaves of *Ficus tsiela* (Rox b)**

The leaves of the plants were always harvested freshly for the preparation of the fresh juice. The leaves were weighed and macerated using mortar and pestle. A small quantity of water was added to ensure proper maceration. Thereafter, the solution was filtered and the filtrate was administered to the rats using an orogastric tube.

3.3 PHYTOCHEMICAL SCREENING

The ethanolic extract and fresh juice obtained from the leaves of *Ficus tsiela* (Rox b) was tested for the presence of various active principles namely steroids, alkaloids, tannins, phenolic compounds, flavonoides, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3.3.1 **Test for Steroids**

Salkowski test

About 5 mg of the dried extract was mixed with 3 ml of chloroform and then shaken with 3 ml of concentrated sulphuric acid. Development of red colour indicates the presence of steroids.

Liberman Burchardt test

About 5 mg of the dried extract was mixed with 3 ml of chloroform in a test tube. Then 5 drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it through the sides of the test tubes. Development of a reddish ring at the junction of the two layers indicates the presence of steroids.

3.3.2 **Test for Alkaloids**

About 0.5 g of the extract was mixed with 5 ml of ammonia and then extracted with equal volume of chloroform. To this, 5 ml of dilute hydrochloric acid was added. The acid layer obtained was used for the following chemical tests for alkaloids.

Mayer's test

To 1 ml of acid layer, a few drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g potassium iodide in 10 ml of water and then made up the volume to 100 ml with distilled water) were added. Development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

A few drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 ml of distilled water) were added to 1 ml of the acid layer. Development of reddish brown precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of acid layer, a few drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were mixed. Development of yellow precipitate indicates the presence of alkaloids.

Dragendroff's test

A few drops of Dragendroff's reagent [stock solution (1) 0.6 g of Bismuth subnitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of

water was added. stock solution (2) 6 g of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 40 ml] were mixed with 1 ml of acid layer. Development of reddish brown precipitate indicates the presence of alkaloids.

3.3.3 **Test for Phenolic Compounds**

About 5 mg of the extract was dissolved in 1ml of water and 5 drops of 10 per cent ferric chloride was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

3.3.4 **Test for Tannins**

Ferric chloride test

About 2 mg of the extract was mixed with 3 ml of one per cent ferric chloride solution. Development of a blue, green or brownish colour indicates the presence of tannins.

Gelatin test

About 0.5 g of extract was mixed with a few drops of one per cent solution of gelatin containing 10 per cent sodium chloride. Development of a white precipitate indicates the presence of tannins.

3.3.5 **Test for Flavonoids**

Ferric chloride test

To 2 ml of alcoholic solution of the extract (0.5 g of extract in 10 ml of methanol), a few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavonoids.

Lead acetate test

To 2 ml of alcoholic solution of the extract (0.5 g of extract in 10 ml of methanol), a few drops of neutral 10 per cent lead acetate solution was mixed. Development of yellow precipitate indicates the presence of flavonoids.

3.3.6 **Test for Glycosides**

Sodium hydroxide test

A small amount of extract (about 5 mg) was mixed with 1 ml of water and 5-6 drops of Sodium hydroxide (10 per cent) solution were added. Development of a yellow colour indicates the presence of glycosides.

Benedict's test

To about 1 ml of the extract (0.5 g extract in 1 ml of water), 5 ml of Benedict's reagent was added. The mixture was boiled for two minutes and cooled. Development of brown to red colour indicates the presence of glycosides.

3.3.7 **Test for Diterpenes**

About 5 mg of the extract was mixed with 3 ml of copper acetate solution (5 per cent). Development of green colour indicates the presence of diterpenes.

3.3.8 **Test for Triterpens**

Salkowski test

About 3 mg of the extract was mixed with 3 ml of chloroform and then it was shaken with 3 ml of concentrated Sulphuric acid. Development of yellow colour indicates the presence of triterpenes.

Lieberman Burchardt test

A few drops of acetic acid and 1 ml of concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (about 3 mg extract in 3 ml chloroform). Development of deep red ring at the junction of two layers indicates the presence of triterpenes.

3.3.9 **Test for Saponins**

Foam test

A small amount of the extract (about 5 mg) was shaken with 3 ml of water. Development of the foam that persists for 10 minutes indicates the presence of saponins.

3.4 EXPERIMENTAL DESIGN

Group	Treatment
Group -1	Healthy control was administered with vehicle (2 per cent gum acacia at the rate of 5 ml/Kg body weight/day) orally.
Group -2	Fresh juice obtained from 5 g leaves of *Ficus tsiela* was administered.

Group -3	Fresh juice obtained from 10 g leaves of *Ficus tsiela* was administered.
Group -4	Ethanolic extract of *Ficus tsiela* leaves at a dose rate of 750 mg /Kg body wt in 2 per cent gum acacia.
Group -5	Ethanolic extract of *Ficus tsiela* leaves at a dose rate of 1000mg /Kg body wt in 2 per cent gum acacia.
Group -6	Ethanolic extract of *Ficus tsiela* leaves at a dose rate of 1500mg /Kg body wt in 2 per cent gum acacia.

In all the experimental groups, the oral administration was continued up to a period of 21 days and observed for symptoms. Animals were weighed and blood was collected on day zero and at weekly intervals. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Leukocyte count (TLC) and Differential Leucocyte count (DLC) were estimated. Serum was used for the estimation of Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Creatine kinase (CK), creatinine and blood glucose. At the end of experiment, all the animals were sacrificed and detailed postmortem examination was conducted and lesions were recorded. Weighed quantity of liver was collected in chilled normal saline for estimation of lipid peroxides and reduced glutathione from all the animals. Tissues for histopathological examination were collected and preserved in buffered formalin.

3.5 PARAMETERS

3.5.1 **Body Weight**

The body weight of individual rats was recorded at days 0, 7, 14 and 21. From this data mean body weight was noted. The animals were routinely observed for the clinical signs exhibited.

3.5.2 **Haematological Parameters**

Blood was collected from the retro-orbital plexus under mild ether anaesthesia with capillary tubes into fresh vials. EDTA was used as the anticoagulant at the rate of 2 mg/ml.

Total Leukocyte count (TLC), Packed Cell Volume (PCV) and Differential Leucocyte count (DLC) were estimated by the method suggested by Thrall *et al*. (2004) on days 0, 7, 14 and 21. Concentration of Haemoglobin (Hb) was estimated by acid haematin method as described by Feldman *et al.* (2000)

3.5.3 **Biochemical Studies**

Blood was collected from the retro-orbital plexus under mild ether anaesthesia with capillary tubes, into clean vials (non-heparinised) and allowed to clot. The serum was separated from the clot and then it was centrifuged at 2000 rpm for 20 minutes. The clear serum was aspirated into another vial and used for biochemical analysis. AST and ALP were estimated by using Ecoline® kit (M/s. E. Merck India Limited, Mumbai). CPK estimation was done by using Creatine kinase (S.L) kit (M/s. Agappe diagnostics, Ernakulam). Creatinine was estimated by using Mekotest® kit (M/s. E. Merck India Limited, Mumbai). Blood glucose was estimated by using NICE diagnostic kit (Nice chemicals Pvt. Ltd., Cochin).

3.5.4 **Lipid Peroxides and Reduced Glutathione**

The animals were sacrificed and dissected upon and the liver was collected. It was washed in running tap water to remove the blood clots and weighed amount of tissue was kept in chilled normal saline.

3.5.4.1 ***Estimation of Tissue Reduced Glutathione***

Levels of reduced Glutathione in liver homogenate were estimated by the method of Moron *et al.* (1979).

a. Principle

Reduced glutathione was measured by its reaction with 5, 5'-dithiobis-2- nitrobenzoic acid (DTNB) to give a yellow coloured complex with absorption maximum at 412 nm.

b. Reagents

Phosphate buffer – 0.2 mol, pH 8.0

Trichloroacetic acid (TCA)- 5 per cent

Trichloroacetic acid (TCA)- 25 per cent

DTNB – 0.6 mMol.

c. Procedure

1. Preparation of tissue homogenate:

Homogenate of liver was prepared in the ratio of 0.5 g of wet tissue to 4 ml of phosphate buffer. It was then centrifuged at 5000 rpm and the supernatant was used for the estimation of reduced glutathione.

2. 125 µl of 25 per cent Trichloroacetic acid was added to 500 µl of supernatant from the tissue homogenate taken in a test tube, for the precipitation of proteins and mixed well.

3. The tubes were then cooled in ice bath for 5 minutes.

4. The mixture was again diluted with 575 µl of 5 per cent TCA and centrifuged for 5 minutes at 5000 rpm.

300 µl of the supernatant was transferred into another test tube and 700 µl of phosphate buffer was added to it.

To the above mixture, 2 ml of freshly prepared DTNB was added, mixed well and the yellow colour formed was read at 412 nm.

d. Preparation of standard curve

Standard curve of glutathione was prepared by using concentrations varying from 1-10 µg of glutathione standard which was dissolved in 5 per cent TCA. The volume of standard solution was made up to 1 ml with 0.2 mol phosphate buffer. Added 2 ml of freshly prepared 0.6 mMol DTNB to the tubes and the intensity of yellow color formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of the reduced glutathione was read directly from the calibration curve and expressed as µg/g wet tissue.

3.5.4.2 ***Estimation of Lipid Peroxides***

The levels of lipid peroxides in liver tissues were estimated by the method of Fraga *et al.* (1988).

a. Principle:

Thiobarbituric acid (TBA) reacts with malondialdehyde, an end product of fatty acid peroxidation to form a red colored pigment, which has maximum absorbance at 532 nm. 1,1,3,3 tetra methoxy propane was used as standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

b. Reagents:

1. Trichloro acetic acid (TCA) - 15 per cent

2. Thiobarbituric acid (TBA) - 0.38 per cent in hot distilled water

3. Hydrochloric acid (HCl) - 0.25 N

4. TCA-TBA-HCl reagent solution - 1, 2 and 3 were mixed freshly in the ratio of 1:1:1

5. Tris – HCl buffer (pH 7.5) - Dissolved 6.85 g Tris in 40 ml distilled water (A) and 1 ml of 12 N HCl was made up to 100 ml (B). Mix solution A and B and adjust the pH to 7.5.

6. Standard solution - 1,1,3,3 tetra methoxy propane (4.8 nMol).

c. Procedure:

1. 500 mg of freshly excised liver tissue was homogenized with 5 ml Tris – HCl buffer (pH 7.5)

2. From the tissue homogenate, 1.0 ml was transferred into a clean test tube and mixed thoroughly with 2.0 ml of TBA- TCA- HCl reagent.

3. The mixture was placed in boiling water bath for 15 minutes, cooled and then centrifuged at 3200 rpm for 10 minutes. Finally, the supernatant was taken for measurement.

4. The absorbance of the chromophore was read at 532 nm against the TBA- TCA- HCl reagent blank using genesis spectrophotometer.

d. Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 nMol to 5 nMol of 1,1,3,3 tetra methoxy propane in double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nMol of malondialdehyde/g of wet tissue.

3.6 PATHOANATOMICAL STUDIES

At the end of experiment, animals were sacrificed. Detailed postmortem examination was conducted and gross lesions were noted. Cerebrum, cerebellum, spinal cord, pituitary, liver, kidney, adrenal, urinary bladder, stomach, intestine, pancreas, spleen, heart, trachea, lungs and thyroid were collected for histopathology in neutral buffered formalin. Sections were cut at 5 µ thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Cook, 1995).

3.7 STATISTICAL ANALYSIS

Data collected from various parameters were analysed as per the method of Snedector and Cochran (1994) by using one way analysis of variance (ANOVA) and followed by Duncans multiple range test for grouping means having significance.

RESULTS

4. RESULTS

4.1 PHYTOCHEMICAL SCREENING OF LEAVES OF *Ficus tsiela*

Ethanolic extract and fresh juice from the leaves of *Ficus tsiela* Rox b was tested for the presence active principles like steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins. The results obtained are summarized in Table 1.

4.1.1 **Steroids**

In both the ethanolic extract and fresh juice of *Ficus tsiela* Rox b steroids were absent as indicated by the absence of formation of red colour and red ring in the Salkowsky test and Lieberman Burchadt test respectively.

4.1.2 **Alkaloids**

Creamy white precipitate was obtained in Mayer's test with the fresh juice but not with the ethanolic extract of *Ficus tsiela*. Characteristic yellow coloured precipitate was obtained with Hager's reagent in the case of fresh juice. But this reaction was absent in the case of ethanolic extract. Wagner's reagent on reaction with fresh juice, gave a characteristic reddish brown precipitate. No such reaction was obtained with ethanolic extract. Dragendroff's test also gave a positive result with the fresh juice and a negative result with the extract. The above results indicated the presence of alkaloids in the fresh juice and absence of it in the ethanolic extract of *Ficus tsiela* Rox b.

4.1.3 **Phenolic compounds**

The fresh juice and extract from the leaves of the *Ficus tsiela* Rox b when mixed with 10 per cent ferric chloride produced dark blue colour which indicated the presence of phenolic compounds.

4.1.4 **Tannins**

Brownish green colour in ferric chloride test and white precipitate in gelatin test was obtained when the reagents were mixed with either the extract or the fresh juice. It revealed the presence of tannins both in the extract and in the fresh juice of *Ficus tsiela* Rox b.

4.1.5 **Flavonoids**

Both the ferric chloride test and lead acetate test gave positive reaction with fresh juice whereas the extract gave negative results indicating the presence of flavonoids in the fresh juice and absence in the ethanolic extract of *Ficus tsiela*.

4.1.6 **Glycosides**

A red colour was obtained in the Benedict's test which indicated the presence of glycosides in the fresh juice. When the fresh juice was mixed with sodium hydroxide, a yellow colour was obtained which also indicated the presence of glycosides. The ethanolic extract gave negative results which indicated the absence of glycosides

4.1.7 **Diterpenes**

Diterpenes were detected in alcoholic extract and in the fresh juice of *Ficus tsiela* as indicated by the green colour when mixed with copper acetate solution.

4.1.8 **Triterpenes**

Salkowsky test and Leibermann Burchardt's test gave positive results with both fresh juice and alcoholic extract.

4.1.9 **Saponins**

No saponins were present in the fresh juice and in the extract as the foam were not persisted for 10 minutes in the foam test.

4.2 PHYSIOLOGICAL PARAMETERS

4.2.1 **Body Weight**

The individual and mean body weights of rats of Group I, II, III, IV, V, and VI were recorded on day 0, 7, 14 and 21 of the experiment and are presented in the table 2. Body weight of rats of all groups showed a gradual increase throughout the experimental period. The mean body weight of treatment groups did not differ significantly with that of control group ($p>0.05$).

4.2.2 BIOCHEMICAL PARAMETERS

4.2.1 **Aspartate amino transferase (AST)**

The mean AST levels of group I, II, III, IV, V and VI on day 0, 7, 14 and 21 are shown in Table 3. A significant increase ($p<0.01$) in the AST levels was observed between treatment groups on day 7, 14 and 21. On day seven, significant increase was observed in rats which were fed with extract at the dose rate of 1000 and 1500 mg/kg body weight (group V and VI) when compared to the control group (144 ± 2.46 IU/L). Also, the mean values of group VI were increased significantly at one per cent level than group V (Fig.4).

On day 14, group V (178.00 ± 2.49 IU/L) and VI (183.63 ± 3.53 IU/L) showed significant increase at one per cent level and group III (153.38 ± 1.89 IU/L) showed significant increase at five per cent level when compared with control (143.13± 3.00 IU/L). Mean values of group V and VI revealed significant increase at one percent level when compared with group II (146.88 ± 3.91 IU/L). When compared to group III, group V and VI values increased at five percent level. These changes are depicted in Fig. 5.

On day 21, group III (167.38 ± 4.11 IU/L), V (221.75 ± 5.63 IU/L) and VI (243.5 ± 9.63 IU/L) showed significant ($p<0.01$) increase in the mean AST values when compared to control (143.75 ± 1.96 IU/L). When compared to group II (148.38 ± 3.83 IU/L) and IV (149.88 ± 4.25 IU/L), group III values increased significantly. The mean values of rats fed with 1000 mg/kg body weight of extract increased at one per cent level when compared with those fed with fresh juice from 10 g leaves. The mean values further increased significantly at one per cent level in rats fed with 1500 mg/kg body weight of extract (Fig. 6).

4.2.2 **Alkaline phosphatase (ALP)**

The mean values of serum alkaline phosphatase level on day 0, 7, 14 and 21 are listed in Table 4. On day zero and seven no significant variations was observed in the levels of alkaline phosphatase.

On day 14, group III (fresh juice from 10 g leaves), V (extract - 1000 mg/kg body weight) and VI (1500 mg/kg body weight of extract) showed significant ($p< 0.01$) increase as compared to control. When compared to rats fed with fresh juice from 5g leaves (637.25 ± 7.76 IU/L) and extract at a dose of 750 mg/kg body weight (635.5 ± 9.69 IU/L), mean values of rats fed with fresh juice from 10g leaves increased significantly. The mean values of group V (1000 mg/kg body weight extract) showed increase at one per cent level when compared to group III (fresh

juice from 10 g leaves). Group VI (1500 mg/kg body weight of extract) values revealed significant increase at one per cent level when compared to group V (Fig.7).

Group III (668.0 ± 22.52 IU/L), V (723.0 ± 27.13 IU/L) and VI (737.38 ± 40.38 IU/L) values increased at one per cent level when compared to control group (634.25 ± 5.73 IU/L) on day 21. Group III values increased significantly when compared to group II and group V and VI showed significant increase when compared to group IV (Fig. 8).

4.2.3 **Creatine kinase**

The mean values of serum creatine kinase level on day 0, 7, 14 and 21 are listed in Table 5. Significant variations were not observed in the levels of creatine kinase on day 0, 7 and 14. But on 21st day, the mean value of the sixth group (369.25 ± 14.51 IU/L) was increased significantly ($P>0.05$) when compared to control group (326.88 ± 8.70 IU/L) (Fig. 9).

4.2.4 **Creatinine**

The results obtained are presented in the Table 6. The mean creatinine value showed significant increase ($P<0.01$) on day 7, 14 and 21 day when compared between groups.

On day seven, statistically significant increase in mean creatinine values was observed in group V (0.650 ± 0.001 mg/dl) and VI (0.688 ± 0.001 mg/dl) when compared to control group (0.500 ± 0.001 mg/dl) at one per cent level. But group VI showed an increase which was statistically non-significant when compared to group V (Fig. 10).

In rats fed with high doses of extract (1000 and 1500 mg/kg body weight), statistically significant increase in creatinine values were observed on day 14 when compared to control group at one per cent level. Group II (fresh juice from 5 g leaves) and III (fresh juice from 10 g leaves) showed significant difference in values with group V (1000 mg/kg body weight of extract) at five per cent level and with group VI (1500 mg/kg body weight of extract) at one per cent level. Group VI showed significant difference at five per cent level when compared to group V (Fig. 11).

Statistically significant increase was observed in III (0.563 ± 0.002 mg/dl), IV (0.612 ± 0.002 mg/dl), V (0.612 ± 0.002 mg/dl) and VI (0.612 ± 0.002 mg/dl) groups on 21st day when compared to control group (0.488 ± 0.002 mg/dl) (Fig. 12).

4.2.5 **Blood Glucose**

The mean values of blood glucose are presented in the table 7. No significant difference in the blood glucose level was observed between the treatment groups.

4.3 HAEMATOLOGICAL PARAMETERS

4.3.1 **Haemoglobin (Hb)**

The mean haemoglobin values of all experimental groups showed no significant difference when compared with the control group (Table 8).

4.3.2 **Packed Cell Volume (PCV)**

The results are shown in Table 9. The values of PCV in all the groups were comparable with the control group on day 0, 7, 14 and 21.

4.3.4 **Total Leukocyte Count (TLC)**

The mean TLC values are listed in the table 10. Results indicated that there were no variations in the TLC value between the six groups.

4.3.5 **Differential leukocyte Count (DLC)**

Table 11 represents the mean DLC values. All the values of DLC were within the normal range.

4.4 CLINICAL SIGNS AND MORTALITY PATTERN

None of the animals in all experimental groups revealed any signs of toxicity throughout the experimental period even at the highest dose. No mortality could be observed during the observation period.

4.5 OXIDATIVE EFFECT ON LIVER

4.5.1 **Lipid Peroxides**

The mean values of lipid peroxides in liver are presented in Table 12 and Figure 13. Group III (388.13 ± 17.16 μg/g) and VI (416.88 ± 22.77 μg/g) revealed significant increase ($P<0.01$) in mean values when compared with control group (314.38 ± 15.48 μg/g). Group III showed significant increase ($P<0.01$) compared to group IV (319.63 ± 13.67 μg/g). Group V (346.88 ± 8.81 μg/g) values increased significantly ($P<0.05$) compared to group IV.

4.5.2 **Reduced Glutathione**

The mean values of reduced glutathione are listed in table 13 and Figure 14. Group III (482.50 ± 9.96 nMol /g), V (441.25 ± 15.86 nMol /g) and VI (381.25 ± 20.39 nMol /g) showed significant decrease at one per cent level when compared

with control group. Group III (fresh juice from 10 g leaves) revealed significant decrease in mean values at five per cent level and group V (1000 mg/kg body weight extract) and VI (1500 mg/kg body weight extract) showed significant decrease at one per cent level when compared to group II (fresh juice from 5 g leaves). The comparison between groups also revealed that group V (at five per cent level) and group VI (at one per cent level) showed significant decrease when compared with group III.

4.6 GROSS AND HISTOPATHOLOGICAL LESIONS

Gross and histopathological lesions of all six groups of experimental animals were recorded. Gross lesions were seen predominantly in liver and kidney of rats. Liver was moderately enlarged and was congested in rats fed with 1.5 g/kg body weight of extract and fresh juice from 10 g of leaves (Fig.15). Blood oozed out from the cut surfaces. Focal necrotic spots were also present (Fig.16). In all the above mentioned animals, kidney appeared to be darker in colour due to severe congestion (Fig. 17).

Histopathological lesions of varying intensity were seen in various organs of rats in the experimental groups.

4.6.1 Group 1- Control Group

Control group animals were administered with two per cent gum acacia at a dose rate of 5 ml/kg body weight. Kidney, liver, cerebrum, cerebellum, pituitary, spinal cord, stomach, intestine, adrenal, thyroid, parathyroid, trachea, lungs, urinary bladder, pancreas and spleen were examined. All the organs were histologically normal.

4.6.2 Group II- Rats Fed with Fresh Juice from 5 g of *Ficus tsiela* Rox b Leaves

Liver of rats fed with fresh juice of *Ficus tsiela* leaves showed mild degeneration of hepatocytes. Goblet cell hyperplasia was present in the intestine. Spleen was reactive. In this group of animals the lungs revealed focal peribronchial lymphoid hyperplasia.

Kidney, cerebrum, cerebellum, pituitary, spinal cord, stomach, adrenal, thyroid, parathyroid, trachea, urinary bladder and pancreas showed no detectable changes from that of control animals.

4.6.3 Group III- Rats Fed with Fresh Juice from 10 g of *Ficus tsiela* Rox b Leaves

In rats fed with high doses of fresh juices moderate to severe lesions were observed in the kidney, liver, heart and thyroid glands. Kidney revealed congestion, haemorrhage, degeneration and necrosis of both tubular epithelium and glomeruli (Figs.18 & 20). Occasional glomerular shrinkage and hyalinization was also observed.

In the liver, diffuse and multifocal necrosis (Fig. 22) and sinusoidal congestion (Fig. 24) were observed. Kupffer cells appeared prominent in certain lobules. Most of the hepatocytes revealed pyknotic nuclei and granular cytoplasm. Necrosis was predominant in the subcapsular area.

Consistent changes were observed with the thyroid gland in rats fed with high doses of extract and fresh juice. The thyroid follicles appeared devoid of colloid and some follicles with scanty colloid (Figs. 27 & 28). Intermuscular haemorrhage and attenuaton of muscle fibres were the lesions present in the heart (Fig. 30). Adrenal of the experimental animals showed dilatation of sinusoids and cystic dilatation of the

medulla (Fig. 32). Spleen appeared to be reactive with well formed secondary follicles with prominent germinal centres (Fig. 33).

Congestion and focal peribronchial lymphoid hyperplasia was present in the lungs (Fig. 34). In stomach, hyperkeratinisation of non-glandular area was present. Intestine showed goblet cell hyperplasia (Fig. 35) and intense accumulation of mononuclear cells.

Eventhough the plant was proved to be neurotoxic in calves, it did not produce any lesions in the nervous system of rats. Cerebrum, cerebellum, pituitary and spinal cord, revealed no detectable changes (Fig. 36, 37, 38 & 39) Sections of urinary bladder, pancreas, trachea and parathyroid also did not show any lesions.

4.6. 4 Group IV- Rats Fed with 750 mg/kg body weight Extract of *Ficus tsiela* Rox b Leaves

Rats of group IV (750 mg/kg body weight) showed necrotic changes in liver. Lungs revealed pulmonary collapse at focal areas. Spleen was reactive in this case also.

4.6.5 Group V- Rats Fed with 1000 mg/kg body weight Extract of *Ficus tsiela* Rox b Leaves

Rats fed with 1000 mg/kg body weight of extract, histological lesions in liver were diffuse, multifocal necrosis and central venous congestion. Mild degenerative changes were present in kidney and intestine. Spleen was reactive with prominent germinal centres. Urinary bladder revealed collapsed mucosa in two animals. Other organs like cerebrum, cerebellum, pituitary, spinal cord, stomach, adrenal, thyroid, parathyroid, trachea, lungs and pancreas were apparently normal.

4.6.6 Group VI- Rats Fed with 1500 mg/kg body weight Extract of *Ficus tsiela* Rox b Leaves

Rats fed with high doses of extract, lesions were more severe and comparable with those fed with high doses of fresh juice. In such rats, renal lesions observed included congestion, haemorrhage, diffuse tubular degeneration and necrosis in the cortical and medullary areas (Figs. 19 & 21). Congestion, haemorrhage and occasional hyalinization of the glomeruli were also observed.

Liver showed diffuse and multifocal necrosis and central venous congestion (Figs. 23 & 25). Many lobules were seen affected.

The thyroid follicles appeared devoid of colloid and some follicles with scanty colloid (Fig. 29). There was variation in the size of thyroid follicles and varying degree of depletion of colloid. Heart revealed intermuscular haemorrhage and attenuaton of muscle fibres (Fig. 31).

Adrenal, spleen, lungs and intestine showed lesions similar to those present in group III. Adrenal showed dilatation of sinusoids, congestion and cystic dilatation of the medulla (Fig. 32). Spleen was normal but reactive with well formed primary and secondary follicles with germinal centres (Fig. 33). Lungs showed congestion and diffuse pulmonary collapse along with focal peribronchial lymphoid hyperplasia (Fig. 34). Intestine showed goblet cell hyperplasia, villus damage and accumulation of mononuclear cells (Fig. 35).

Group VI also showed no lesions in central nervous system. All layers of the cerebrum were intact and apparently normal (Fig. 36). Cerebellum (Fig 37) and pituitary (Fig. 38) were intact structurally and showed no lesions. All the layers of spinal cord were intact and showed no characteristic histopathological lesions (Fig. 39). Pancreas was normal in all animals with well preserved acinar and islet cells.

Table 1: Results of phytochemical screening of ethanolic extract and fresh juice of *Ficus tsiela* Rox b.

No.	Active principle	*Ficus tsiela* ethanolic extract	*Ficus tsiela* fresh juice
1.	Steroids	Absent	Absent
2.	Alkaloids	Absent	Present
3.	Phenolic compounds	Present	Present
4.	Tannins	Present	Present
5.	Flavonoids	Absent	Present
6.	Glycosides	Absent	Present
7.	Diterpenes	Present	Present
8.	Triterpenes	Present	Present
9.	Saponins	Absent	Absent

Table 2: Mean body weight (g) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Groups	Day 0	Day 7	Day 14	Day 21
I	$150^{a} \pm 1.34$	$159.38^{a} \pm 1.48$	$165.63^{a} \pm 2.40$	$169.38^{a} \pm 1.48$
II	$153.75^{a} \pm 2.06$	$157.50^{a} \pm 2.31$	$161.88^{a} \pm 2.30$	$166.88^{a} \pm 2.30$
III	$141.88^{a} \pm 6.19$	$147.50^{a} \pm 6.20$	$158.25^{a} \pm 3.18$	$156.88^{a} \pm 5.59$
IV	$148.75^{a} \pm 1.57$	$155.38^{a} \pm 1.39$	$159.38^{a} \pm 1.58$	$165^{a} \pm 2.31$
V	$145.00^{a} \pm 2.5$	$151.88^{a} \pm 1.62$	$158.5^{a} \pm 1.64$	$163.13^{a} \pm 1.88$
VI	$151.25^{a} \pm 1.25$	$156.00^{a} \pm 1.36$	$160.63^{a} \pm 1.19$	$165.00^{a} \pm 1.10$

(Means bearing same superscript in the same column does not differ significantly)

Table 3: Mean values of aspartate aminotransferase (IU/L) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	144.88^{a} ± 2.34	144 ^{Aa} ± 2.46	143.13^{Aa} ± 3.00	143.75 ^{Aa} ± 1.96
II	145.88^{a} ± 3.19	145.13^{Aa} ± 3.06	146.88 ^{Aa} ± 3.91	148.38 ^{Aa} ± 3.83
III	144.13 ^{a} ± 2.21	146.50^{Aa} ± 2.62	153.38 ^{Ab}± 1.89	167.38^{C} ± 4.11
IV	144.63 ^{a} ± 3.59	147.0 ^{Aa} ± 3.19	146.50^{Aa} ± 3.65	149.88 ^{Aa} ± 4.25
V	145.63 ^{a} ± 3.60	155.38^{Ba} ± 1.50	178.00^{Bc} ± 2.49	221.75^{D} ± 5.63
VI	145.63 ^{a} ± 1.93	180.63^{Ca} ± 3.59	183.63^{Bc} ± 3.53	243.5^{E} ± 9.63

(Means bearing same superscript in the same column does not differ significantly)

A, B, C, D, E- Showing significance at one per cent level

a, b, c - Showing significance at five per cent level)

Table 4: Mean values of alkaline phosphatase (IU/L) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	635.88 ^{a} ± 8.98	632.38 ^{a} ± 8.76	633.88 ^{Aa} ± 9.28	634.25 ^{a} ± 5.73
II	635.63 ^{a} ± 9.23	637.63 ^{a} ± 9.40	637.25 ^{Aa} ± 7.76	639.63 ^{a} 7.03
III	633.88 ^{a} ± 8.75	632.63 ^{a} ± 8.54	656.63^{B} ± 17.63	668.0^{B} ± 22.52
IV	633.13 ^{a} ± 8.11	633.63 ^{a} ±8.46	635.5 ^{Aa} ± 9.69	631.5 ^{a} ± 7.65
V	639.0 ^{a} ± 10.47	639.88 ^{a} ± 8.85	674.0^{C} ± 14.5	723.0 ^{C}± 27.13
VI	640.63 ^{a} ± 8.43	643.75 ^{a} ± 8.95	675.75 ^{D}± 13.91	737.38 ^{D}± 40.38

(Means bearing same superscript in the same column does not differ significantly)

A, B, C, D- Showing significance at one per cent level

a, b, c- Showing significance at five per cent level)

Table 5: Mean values of creatine kinase (IU/L) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	324 ^{a} ± 7.38	324.75 ^{a} ± 8.75	322.88 ^{a} ± 9.58	326.88 ^{Aab} ± 8.70
II	324.75 ^{a} ± 8.58	324.63 ^{a} ± 8.93	327.25 ^{a} ± 5.90	326.13 ^{Aab} ± 8.78
III	326.13 ^{a} ± 5.96	326.38 ^{a} ± 6.32	332.63 ^{a} ± 6.63	336.75 ^{Aab} ± 8.36
IV	324.88 ^{a} ± 8.01	325.25 ^{a} ± 7.72	326.25 ^{a} ± 6.71	326.63 ^{Aab} ± 8.01
V	327.50 ^{a} ± 7.95	331.25 ^{a} ± 7.21	335.00 ^{a} ± 8.55	341.63 ^{Aab} ± 9.37
VI	323.25 ^{a} ± 6.52	327.13 ^{a} ± 5.79	339.75 ^{a} ± 7.07	369.25 ^{Bc} ± 14.51

(Means bearing the same superscript in the same column does not differ significantly)

A,B- Showing significance at one per cent level

a, b,c- Showing significance at five per cent level)

Table 6: Mean values of creatinine (mg/dl) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	0.475 ^{a} ± 0.001	0.500 ^{a} ± 0.000	0.488 ^{Aa} ± 0.001	0.488 ^{Aa} ± 0.002
II	0.488 ^{a} ± 0.001	0.488 ^{a} ± 0.001	0.500 ^{Aa,} ± 0.000	0.513 ^{Aa} ± 0.002
III	0.475 ^{a} ± 0.001	0.513 ^{a} ± 0.002	0.500 ^{Aa} ± 0.000	0.563 ^{B} ± 0.002
IV	0.500 ^{a} ± 0.000	0.500 ^{a} ± 0.001	0.500 ^{Aa} ± 0.000	0.612 ^{B} ± 0.002
V	0.462 ^{a} ± 0.001	0.650 ^{B} ± 0.001	0.550 ^{B,b} ± 0.001	0.612 ^{B} ± 0.002
VI	0.500 ^{a} ± 0.000	0.688 ^{B} ± 0.001	0.600 ^{B,c} ± 0.002	0.650 ^{C} ± 0.001

(Means bearing same superscript in the same column does not differ significantly

A,B,C- Showing significance at one per cent level

a, b- Showing significance at five per cent level)

Table 7: Mean blood glucose (mg/dl) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	82.1788 [a] ± 2.29	80.7887 [a] ± 1.53	81.223 [a] ± 2.33	83.0950 [a] ± 1.51
II	82.8475 [a] ± 2.09	83.0138 [a] ± 1.45	82.084 [a] ± 2.99	82.9600 [a] ± 2.85
III	81.2088 [a] ± 2.04	79.0813 [a] ± 2.18	78.720 [a] ± 1.89	77.0863 [a] ± 1.62
IV	81.4263 [a] ± 2.13	81.5788 [a] ± 1.91	81.451 [a] ± 2.25	78.3125 [a] ± 1.98
V	83.01 [a] ± 2.45	80.9838 [a] ± 2.01	80.145 [a] ± 2.49	81.49 [a] ± 2.58
VI	82.99 [a] ± 2.28	81.30 [a] ± 1.98	80.401 [a] ± 1.596	79.1313 [a] ± 1.47

(Means bearing same superscript in the same column does not differ significantly)

Table 8: Mean haemoglobin concentration (g/dl) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	14.85 [a] ± 0.16	14.61 [a] ± 0.96	14.50 [a] ± 0.01	14.1 [a] ± 0.01
II	14.48 [a] ± 0.12	14.38 [a] ±0.94	14.30 [a] ± 0.08	14.32 [a] ± 0.08
III	14.92 [a] ± 0.18	14.542 [a] ± 0.01	14.38 [a] ± 0.08	14.69 [a] ± 0.07
IV	14.69 [a] ± 0.22	14.521 [a] ± 0.94	14.54 [a] ± 0.08	14.55 [a] ± 0.08
V	14.51 [a] ± 0.22	14.41 [a] ± 0.12	14.42 [a] ± 0.08	14.32 [a] ± 0.04
VI	14.35 [a] ± 0.28	14.133 [a] ± 0.95	14.34 [a] ± 0.08	14.33 [a] ± 0.28

(Means bearing same superscript in the same column does not differ significantly)

Table 9: Mean packed cell volume (%) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	42.50 [a] ± 0.27	42.00 [a] ± 0.27	42.38 [a] ± 0.18	41.63 [a] ± 0.42
II	40.63 [a] ± 0.56	40.63 [a] ±0.37	40.63 [a] ± 0.42	40.63 [a] ± 0.37
III	42.13 [a] ± 0.64	41.63 [a] ± 0.53	40.88 [a] ± 0.72	40.63 [a] ± 0.63
IV	40.50 [a] ± 0.98	40.25 [a] ± 0.70	40.13 [a] ± 0.64	40.00 [a] ± 0.57
V	40.63 [a] ± 0.73	40.13 [a] ± 0.87	40.50 [a] ± 0.71	40.25 [a] ± 0.75
VI	41.63 [a] ± 0.98	40.50 [a] ± 0.76	40.88 [a] ± 0.87	39.63 [a] ± 0.68

(Means bearing same superscript in the same column does not differ significantly)

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Table 10: Mean total leukocyte count (thousands per mm^3) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0	Day 7	Day 14	Day 21
I	7.51 [a] ± 1.89	7.45 [a] ± 1.13	7.45 [a] ± 1.1219	7.48 [a] ± 1.165
II	7.43 [a] ± 1.59	7.44 [a] ± 1.02	7.38 [a] ± 1.1213	7.52 [a] ± 1.19
III	7.46 [a] ± 1.19	7.48 [a] ± 1.817	7.53 [a] ± 1.174	7.51 [a] ± 1.107
IV	7.42 [a] ± 1.62	7.41 [a] ± 1.335	7.43 [a] ± 1.114	7.48 [a] ± 1.592
V	7.44 [a] ± 1.59	7.56 [a] ± 1.76	7.56 [a] ± 1.758	7.50 [a] ± 1.190
VI	7.49 [a] ±1.12	7.44 [a] ± 1.1005	7.39 [a] ± 1.1166	7.39 [a] ± 1.138

(Means bearing same superscript in the same column does not differ significantly)

Table 11: Mean differential leukocyte count (%) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Day 0		Day 7		Day 14		Day 21	
	Lymphocyte	Neutrophil	Lymphocyte	Neutrophil	Lymhocyte	Neutrophil	Lymhocyte	Neutrophil
I	80.13 [a] ± 0.52	18.13 [a] ± 0.44	80.25 [a] ± 0.53	18.25 [a] ± 0.25	80.25 [a] ± 0.45	17.88 [a] ± 044	80.38 [a] ± 0.53	17.88 [a] ± 0.44
II	80 [a] ± 0.46	17.88 [a] ± 0.44	80.13 [a] ± 0.58	17.88 [a] ± 0.52	80.50 [a] ± 0.93	17.63 [a] ± 0.32	80.75 [a] ± 0.37	17.88 [a] ± 0.44
III	80.25 [a] ± 0.53	17.88 [a] ± 0.55	80.38 [a] ± 0.46	17.63 [a] ± 0.46	80.38 [a] ± 0.37	17.75 [a] ± 0.37	80.50 [a] ± 0.33	17.50 [a] ± 0.33
IV	80.63 [a] ± 0.37	17.50 [a] ± 0.33	80.25 [a] ± 0.45	17.63 [a] ± 0.53	80.50 [a] ± 0.42	17.50 [a] ± 0.42	80.38 [a] ± 0.42	17.50 [a] ± 0.46
V	80.5 [a] ± 0.33	17.50 [a] ± 0.33	80.88 [a] ± 0.3	17.38 [a] ± 0.37	80.75 [a] ± 0.25	17.25 [a] ± 0.25	80.88 [a] ±0.55	17.13 [a] ± 0.55
VI	80.38 [a] ± 0.46	17.88 [a] ± 0.44	80.63 [a] ± 0.42	17.63 [a] ± 0.32	80.50 [a] ± 0.57	17.63 [a] ± 0.5	80.13 [a] ± 0.18	17.88 [a] ± 0.44

(Means bearing same superscript does not differ significantly)

Table 12: Mean values of lipid peroxides (µg/g) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Lipid peroxides
I	$314.38^{Aa} \pm 15.48$
II	$324.38^{Aa} \pm 14.31$
III	$388.13^{B} \pm 17.16$
IV	$319.63^{Aa} \pm 13.67$
V	$346.88^{Ab} \pm 8.81$
VI	$416.88^{B} \pm 22.77$

(Means bearing same superscript in the same column does not differ significantly)

A, B,- Showing significance at one per cent level

a, b- Showing significance at five per cent level)

Table 13: Mean values of reduced glutathione (nMol /g) of rats administered with fresh juice and ethanolic extract of *Ficus tsiela* Rox b

Group	Reduced Glutathione
I	$523.75^{A} \pm 13.75$
II	$526.88^{A} \pm 10.85$
III	$482.50^{Ba} \pm 9.96$
IV	$519.38^{AB} \pm 10.15$
V	$441.25^{Bb} \pm 15.86$
VI	$381.25^{C} \pm 20.39$

(Means bearing same superscript in the same column does not differ significantly)

A,B- Showing significance at one per cent level

a, b- Showing significance at five per cent

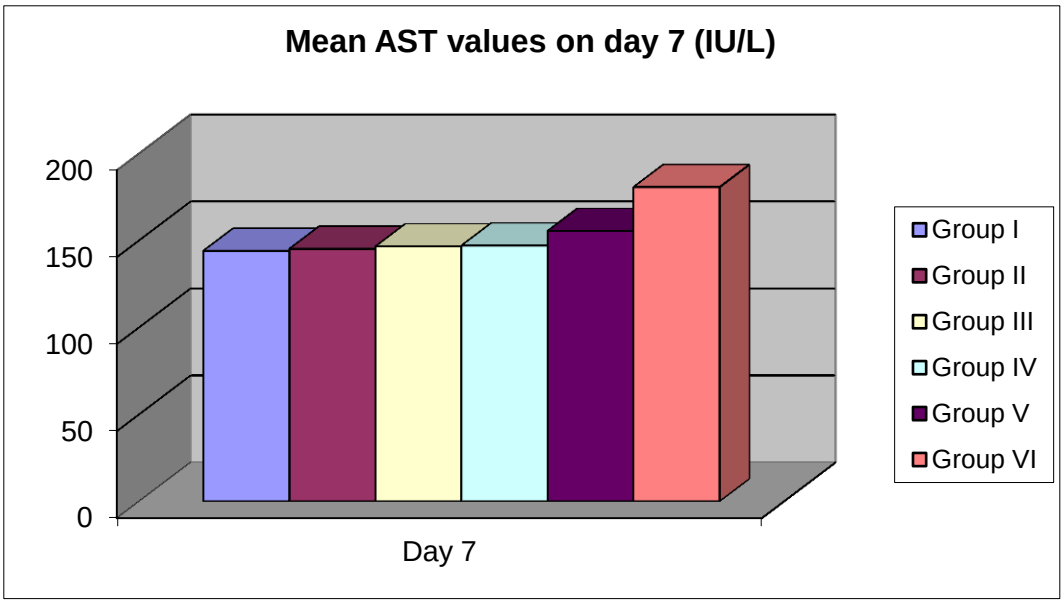

Fig. 4 Mean AST values on day 7 (IU/L)

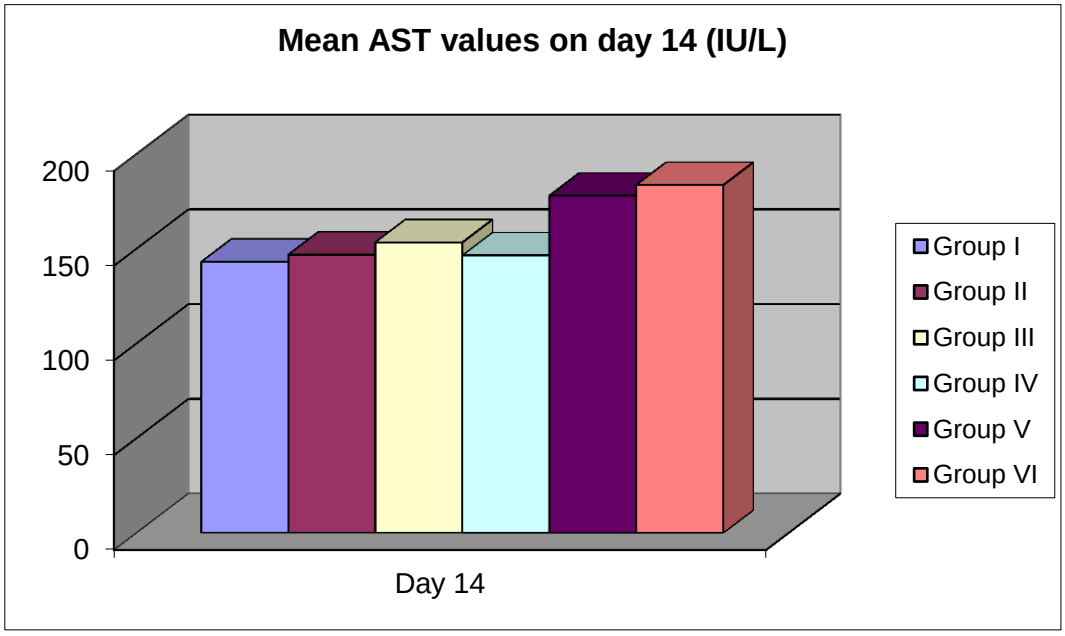

Fig. 5 Mean AST values on day 14 (IU/L)

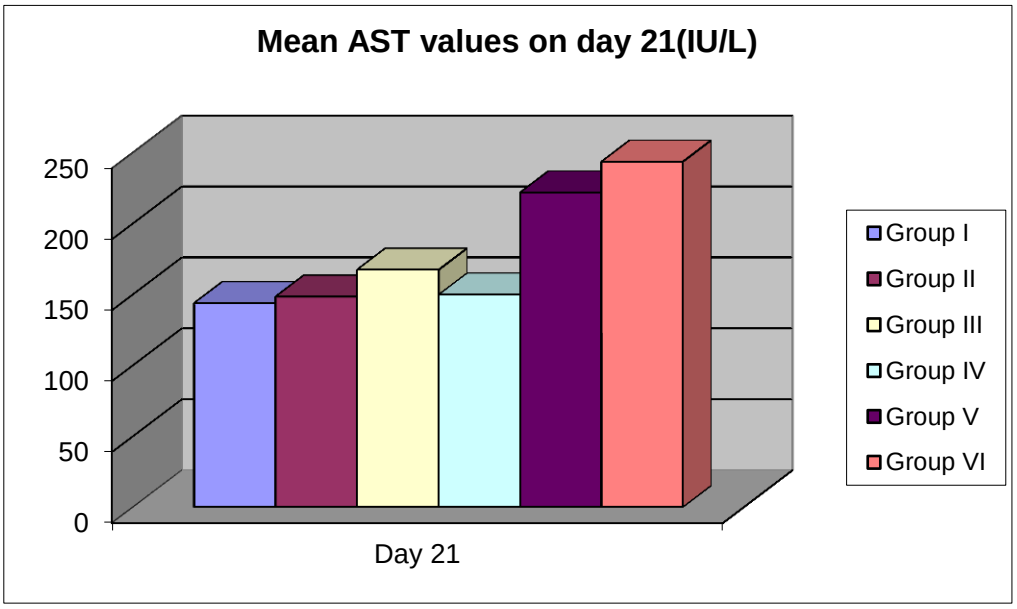

Fig. 6 Mean AST values on day 21 (IU/L)

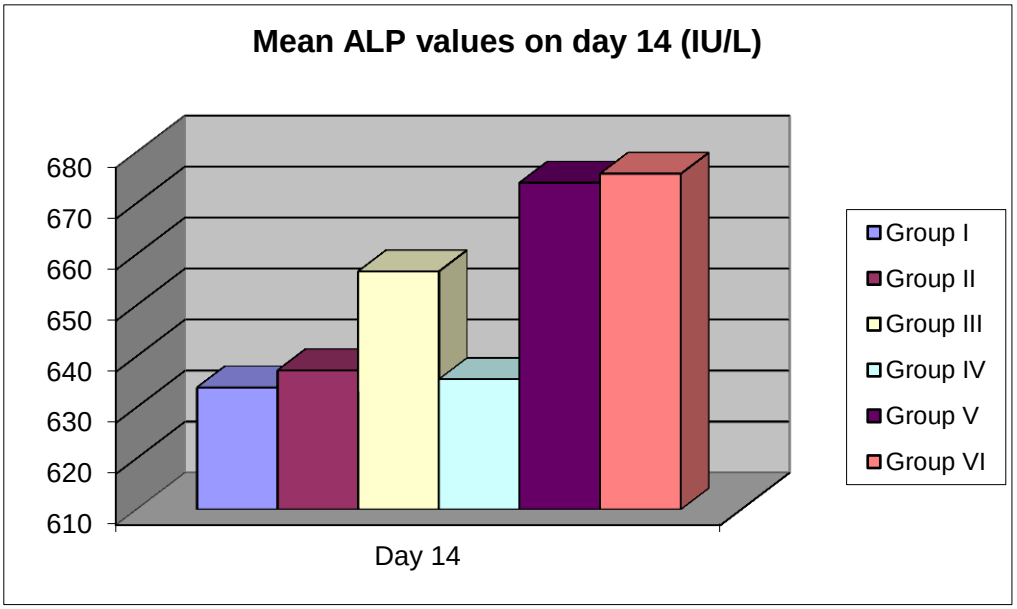

Fig. 7 Mean ALP values on day 14 (IU/L)

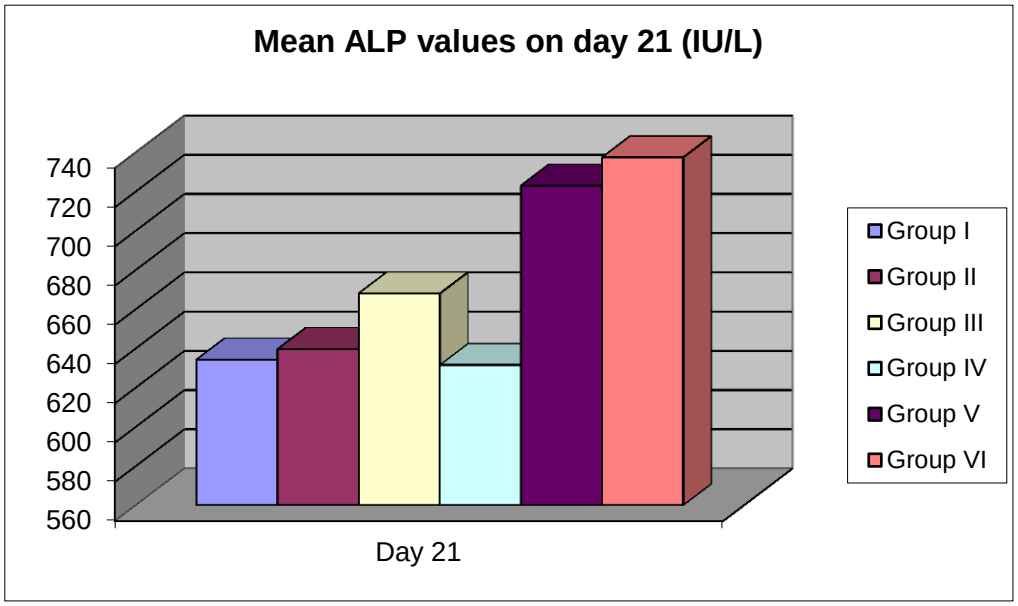

Fig. 8 Mean ALP values on day 21 (IU/L)

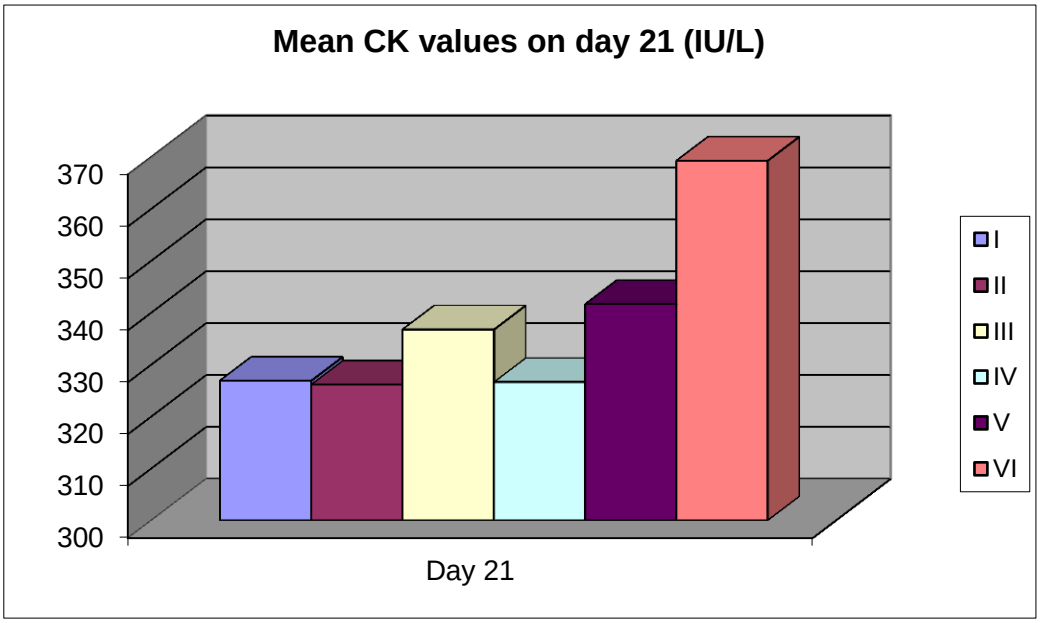

Fig. 9 Mean values of CK on day 21(IU/L)

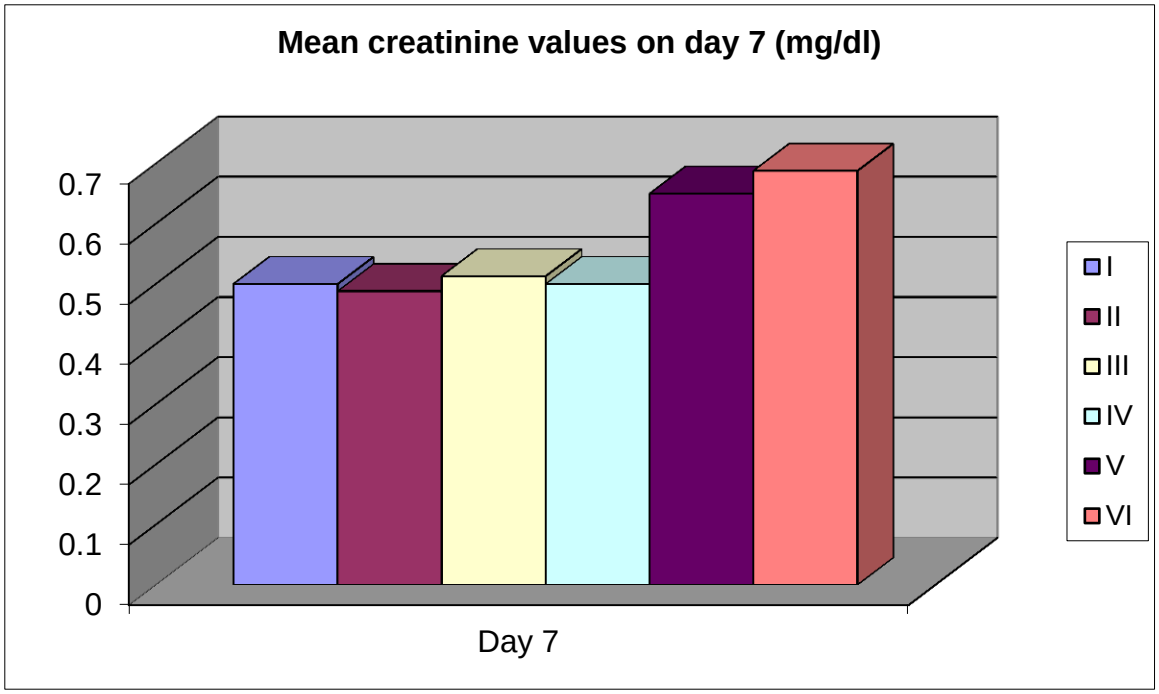

Fig. 10 Mean creatinine values on day 7 (mg/dl)

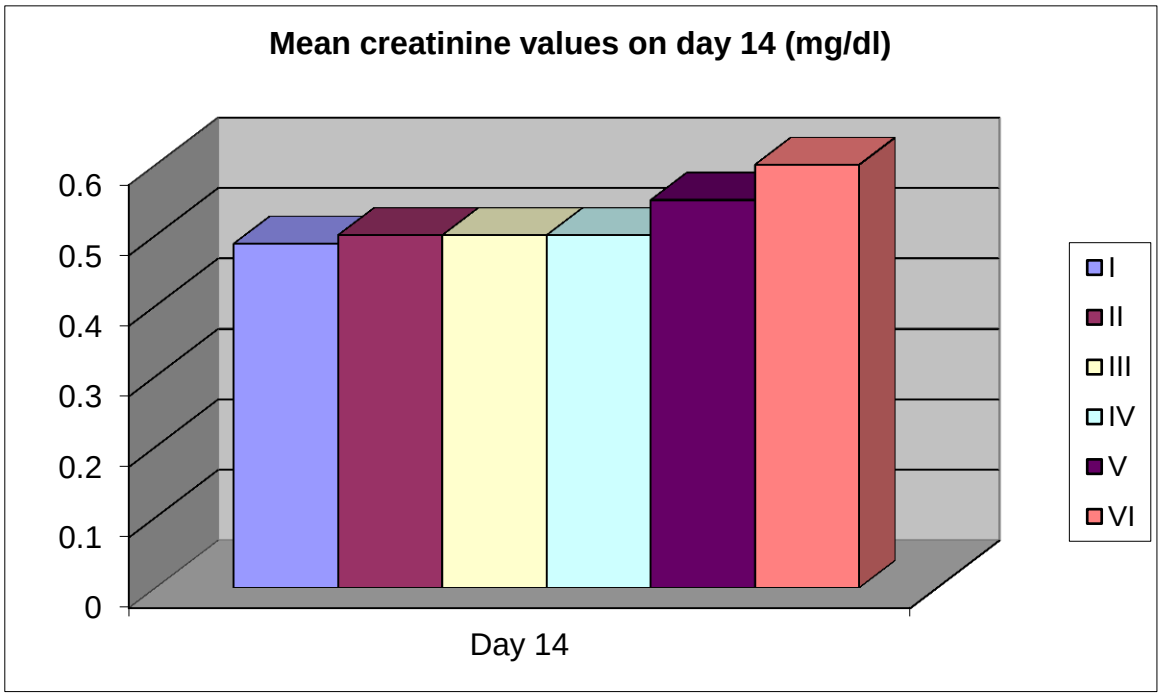

Fig. 11 Mean creatinine values on day 14 (mg/dl)

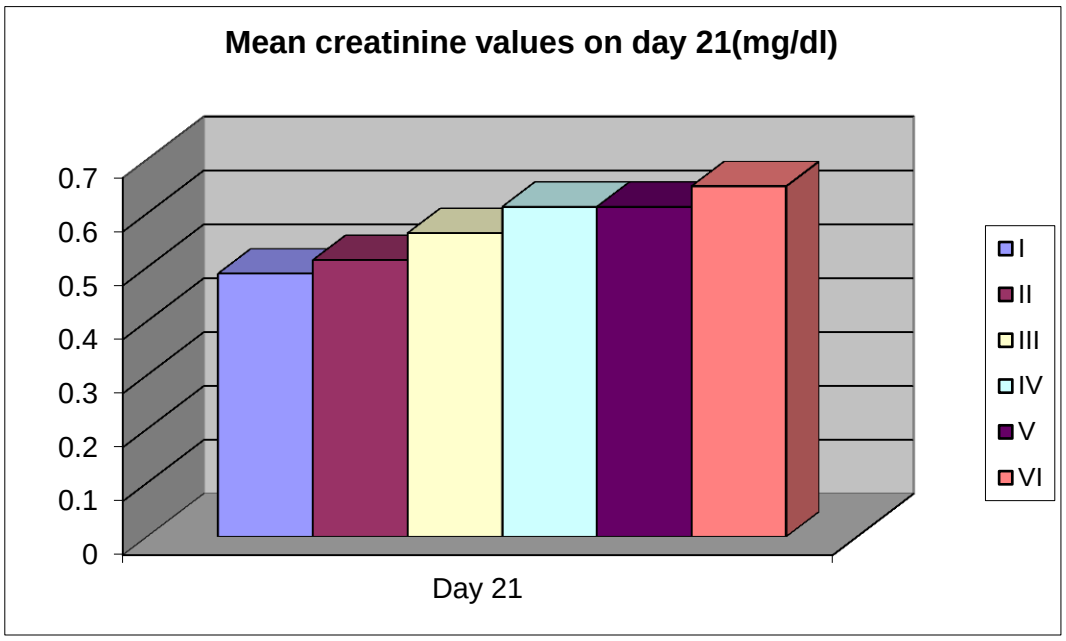

Fig. 12 Mean creatinine values on day 21 (mg/dl)

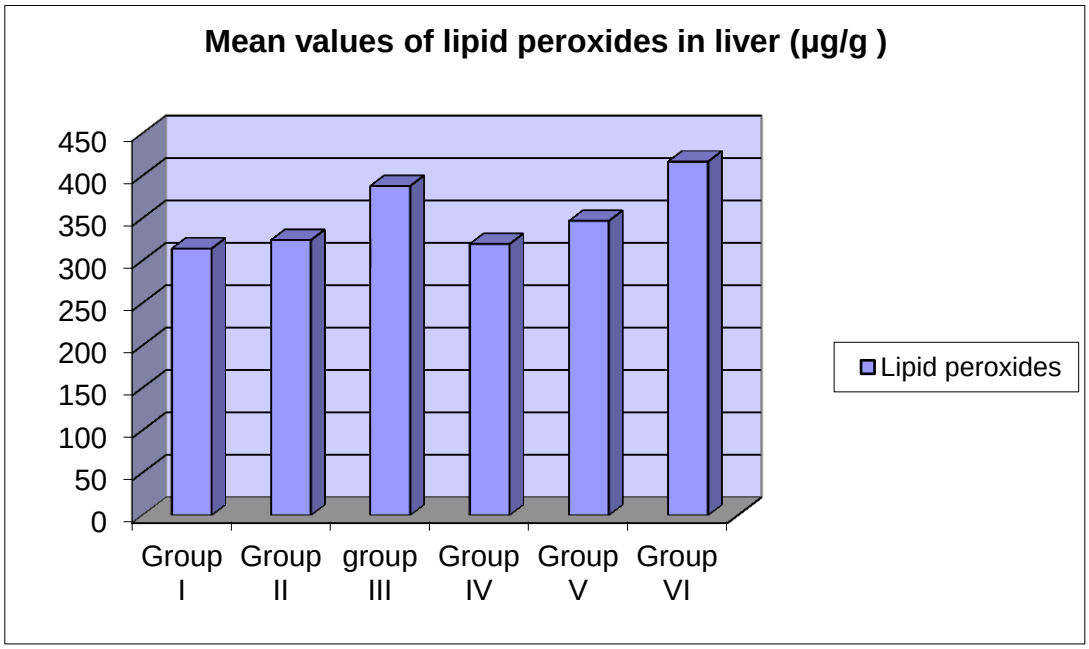

Fig. 13 Mean values of lipid peroxides in liver (µg/g)

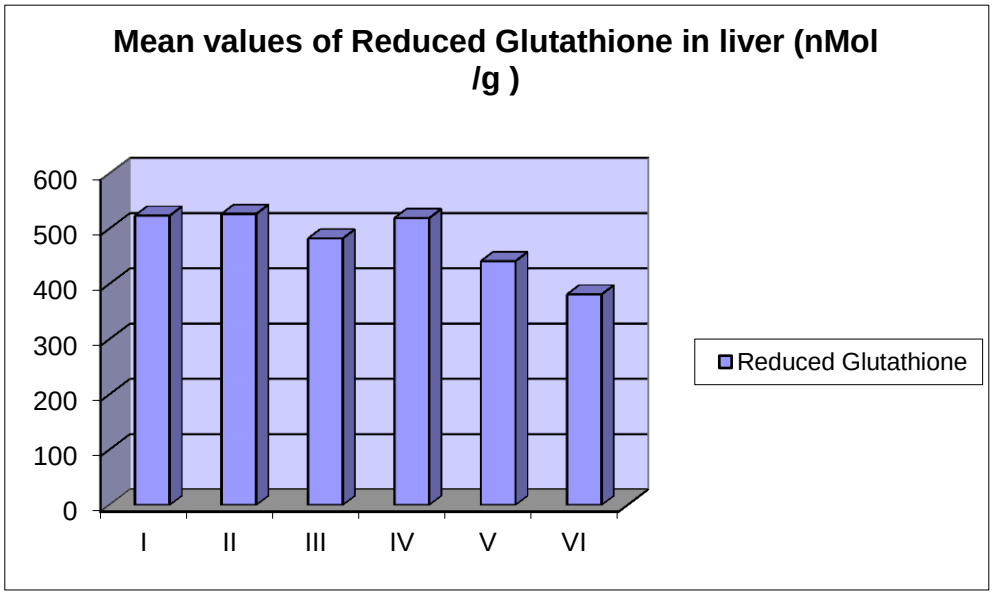

Fig. 14 Mean values of reduced glutathione in liver (nMol /g)

Fig. 15

Fig. 16

Fig. 17

Fig. 15 Liver -congestion 1500mg/kg extract

Fig. 16 Liver with focal necrotic spots 1500mg/kg extract

Fig. 17 Kidney -congestion. 1500mg/kg extract

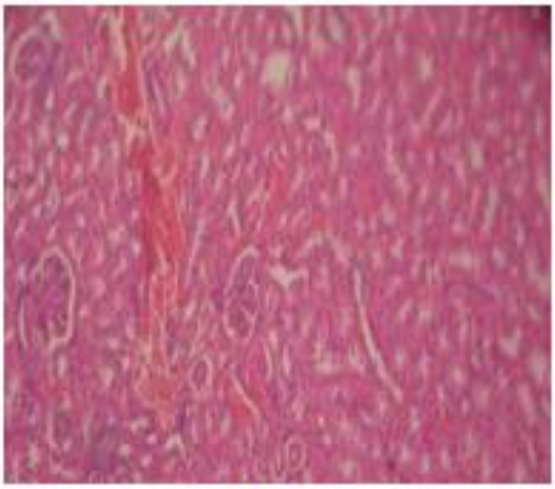

Fig. 18

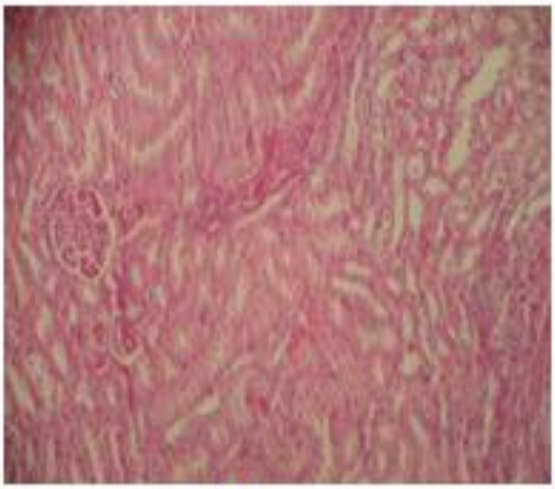

Fig. 19

Fig. 18 Kidney- Fresh juice from 10 g Leaves- Haemorrhage of the glomeruli and tubules- H&E x 100

Fig. 19 Kidney- Extract 1500 mg/kg - Haemorrhage of the glomeruli and tubules- H&E x100

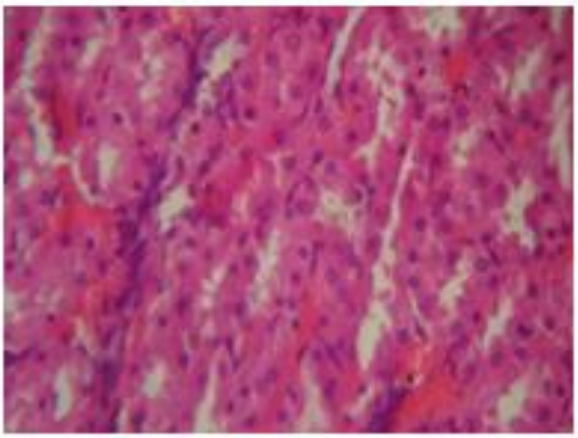

Fig. 20

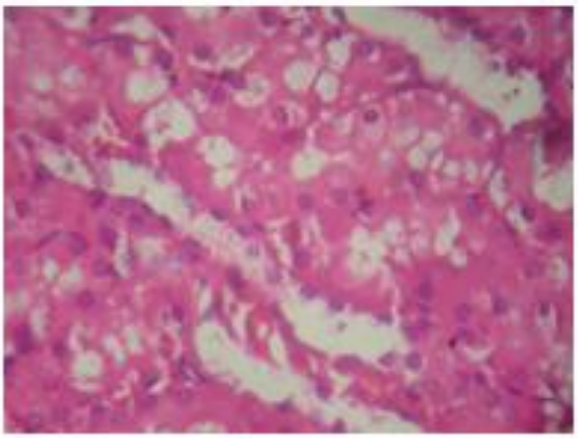

Fig. 21

Fig. 20 Kidney- Fresh juice from 10 g leaves- Degeneration of the tubular epithelium and haemorrhage- H&E x 400
Fig. 21 Kidney- Extract 1500 mg/kg- Degeneration of the tubular epithelium and haemorrhage- H&E x 400

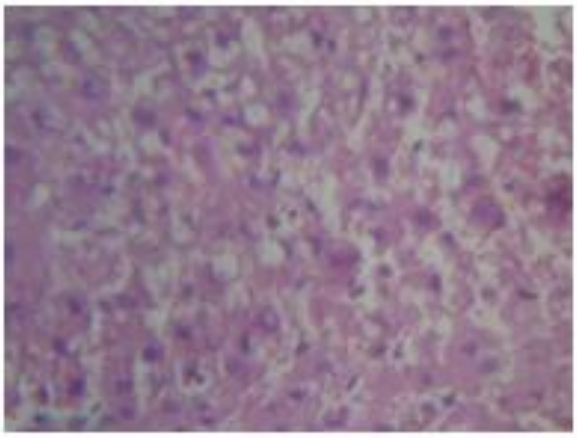

Fig. 22

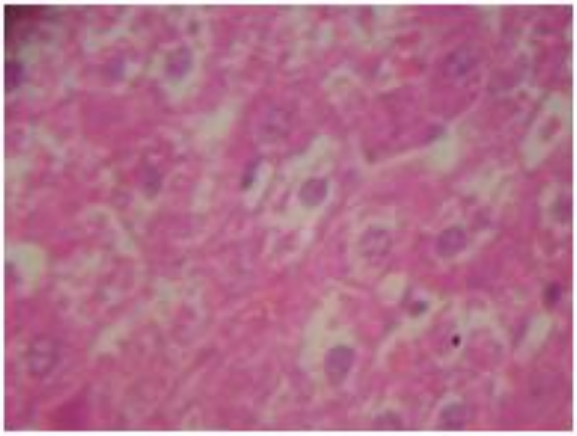

Fig. 23

Fig. 22 Liver - Fresh juice from 10 g leaves- focal necrosis- H&E x 400

Fig. 23 Liver - Extract 1500 mg/kg- focal necrosis H&E x 400

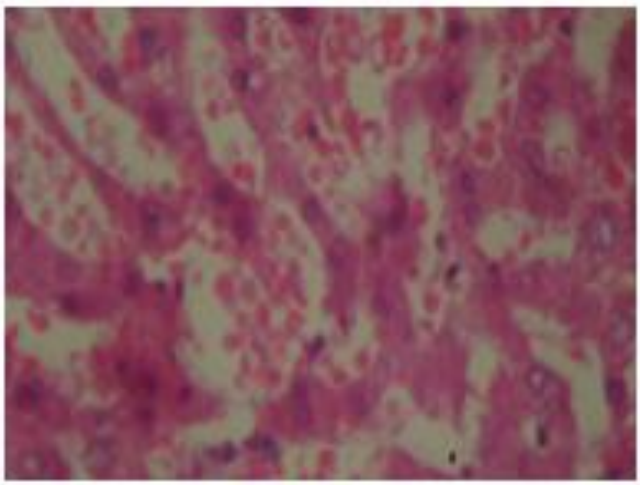

Fig. 24

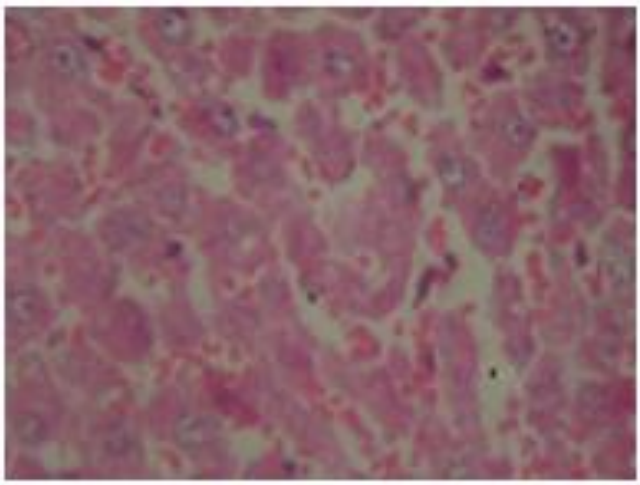

Fig. 25

Fig. 24 Liver- Fresh juice from 10 g leaves- sinusoidal congestion- H&E x 400
Fig. 25 Liver – Extract 1500 mg/kg- sinusoidal congestion H&E x 400

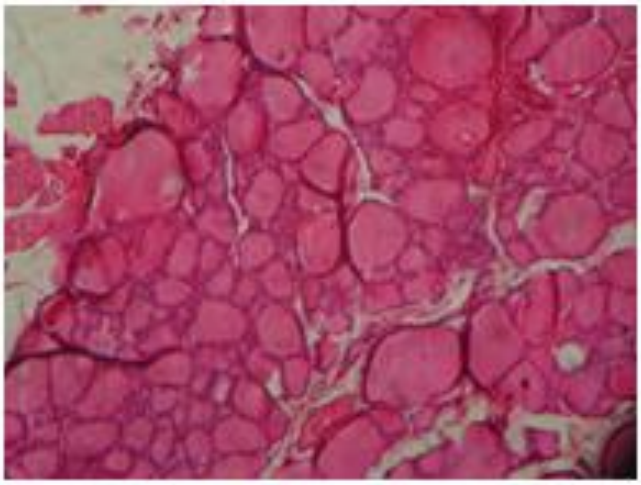

Fig. 26

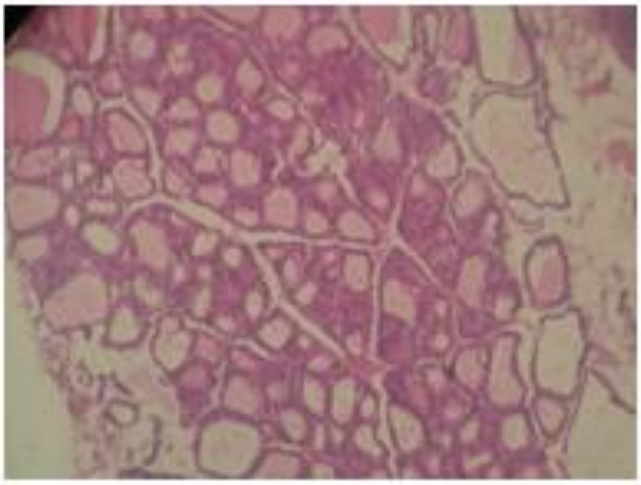

Fig. 27

Fig.26 Thyroid – control - H&E x 100

Fig. 27 Thyroid – Fresh juice from 10 g leaves - Depletion of colloid H&E x 100

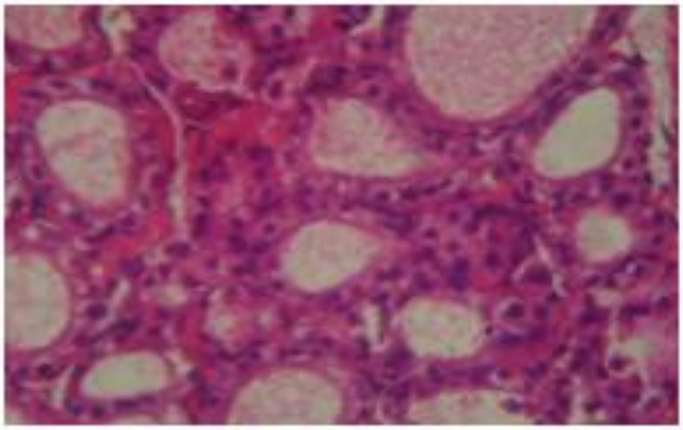

Fig. 28

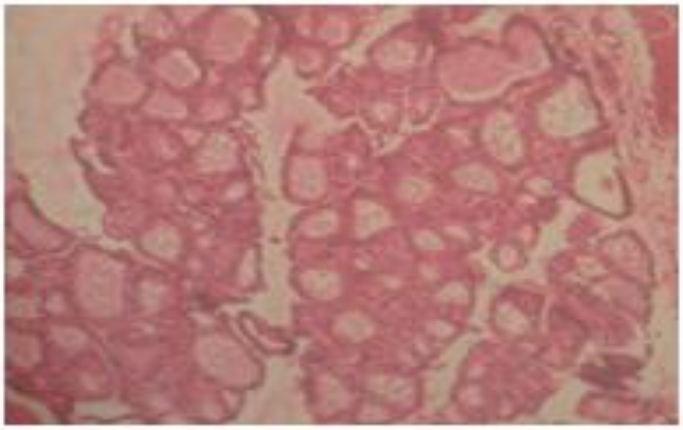

Fig. 29

Fig.28 Thyroid –Fresh juice from 10 g leaves - Depletion of colloid H&E x 400

Fig.29 Thyroid – Extract 1500 mg/kg- Depletion of colloid- H&E x 400

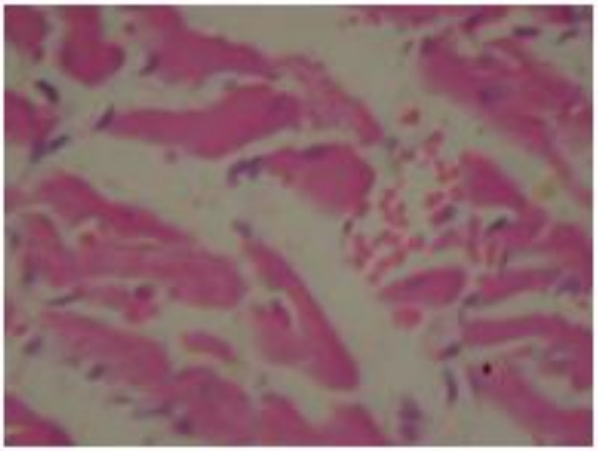

Fig. 30

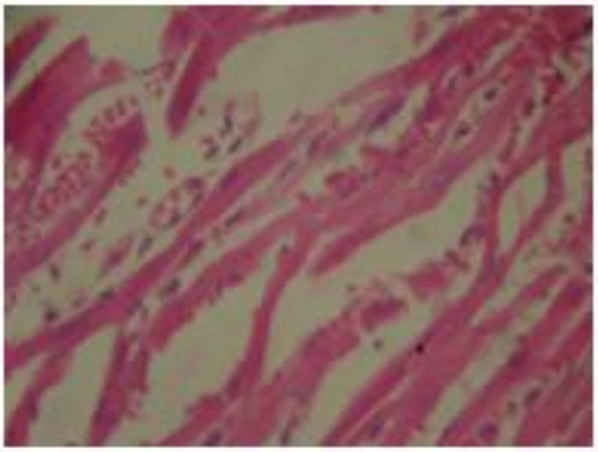

Fig. 31

Fig. 30 Heart - Fresh juice from 10 g leaves – intermuscular haemorrhage and attenuation of muscle fibres- H&E x 400
Fig. 31 Heart – Extract 1500 mg/kg - intermuscular haemorrhage and attenuation of muscle fibres- H&E x 400

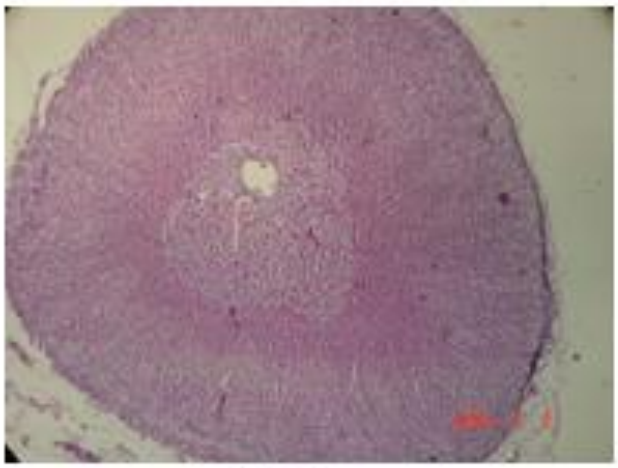

Fig. 32

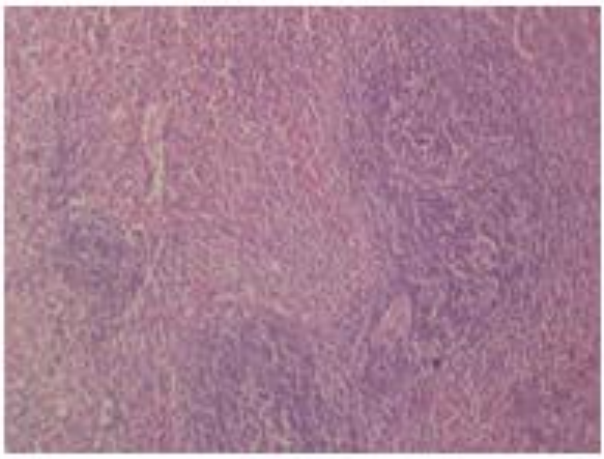

Fig. 33

Fig. 32 Adrenal – Fresh juice from 10 g leaves- Cystic dilatation of medulla- H&E x 100
Fig. 33 Spleen – Fresh juice from 10 g leaves – reactive spleen with multiple cortical follicles- H&E x100

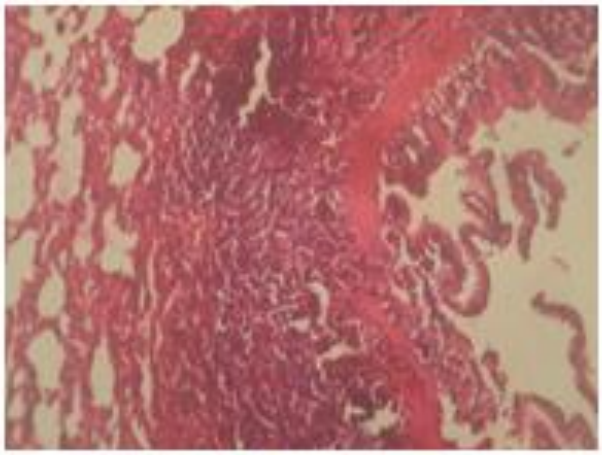

Fig. 34

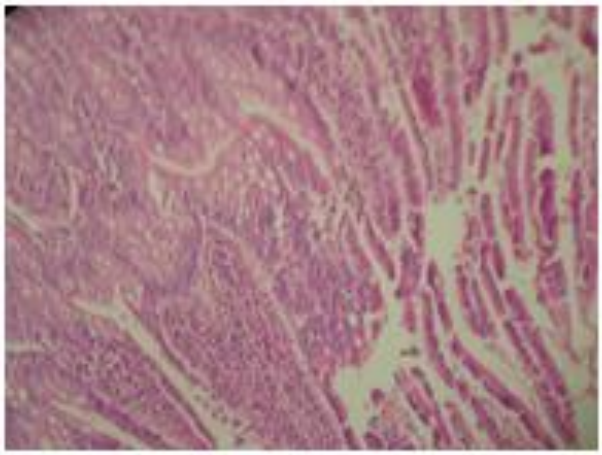

Fig. 35

Fig. 34 Lung – Fresh juice from 10 g leaves - Peribronchial lymphoid hyperplasia H&E x 100

Fig. 35 Intestine – Fresh juice from 10 g leaves - Goblet cell hyperplasia and fragmentation of villi H&E x 100

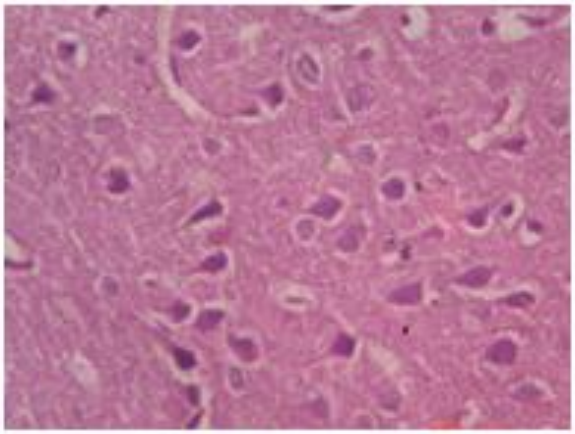

Fig. 36

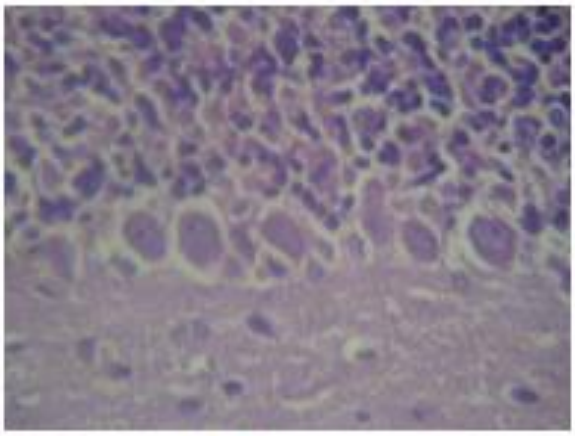

Fig. 37

Fig. 36 Cerebrum – Fresh juice from 10 g leaves - no histological lesions H&E x 400
Fig. 37 Cerebellum – Fresh juice from 10 g leaves - no histological lesions H&E x 400

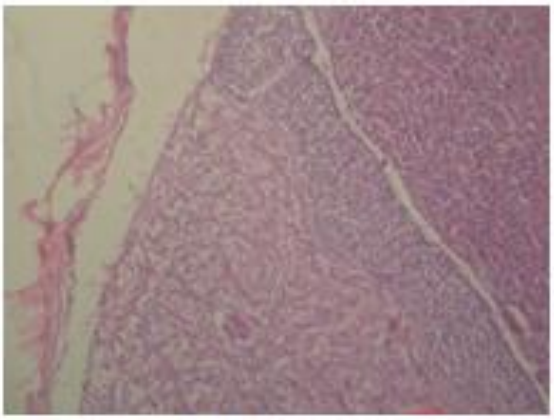

Fig. 38

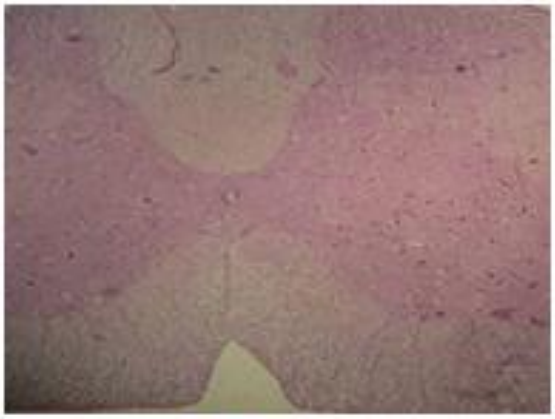

Fig. 39

Fig. 38 Pituitary - Fresh juice from 10 g leaves - no histological lesions H&E x 100
Fig. 39 Spinal cord – Fresh juice from 10 g leaves - no histological lesions H&E x 100

DISCUSSION

5. DISCUSSION

The present study was undertaken to assess the pathological effects of ethanolic extract of *Ficus tsiela* Rox b and its fresh juice in rats. *Ficus tsiela* Rox b is identified to be a toxic plant and had been proved to produce neurotoxicity in calves. Both natural intoxication and experimentally induced toxicities have been reported by Nair *et al.* (1985) and Rajan *et al.* (1986). *Ad libitum* feeding of this plant in calves produced symptoms of toxicity whereas in goats it did not produce any effect (Nair *et al.,* 1999). Further experiments on toxicity could not be carried out because of the stringent rules in subjecting animals to experimentation. Hence it has become imperative to select and identify the suitable laboratory animals for conducting further studies and if the animals pick up the toxicity as observed in the natural hosts, it becomes easy to proceed with amelioration studies and development of a suitable antidote. This study also envisaged to find out whether rat could be a good model for this study. As per O.E.C.D. (Organization for Economic Co-operation and Development) guidelines, rat is the preferred model for oral toxicity studies.

5.1 PHYTOCHEMICAL SCREENING

Phytochemical analysis of the *Ficus tsiela* Rox b fresh juice revealed the presence of detectable levels of tannins, glycosides, diterpenes, triterpenes, alkaloids, flavanoids and phenolic compounds whereas the alcoholic extract revealed the presence of tannins, phenolic compounds, diterpenes and triterpenes.

Many of the above mentioned principles were detected in different Ficus spp. Preliminary screening of the methanolic extract of *Ficus platyphylla* stem bark revealed the presence of flavonoids, tannins and saponins (Amos *et al*., 2001,

Wakeel *et al.,* 2004). Hameed E.S.A. (2009) reported that phenolic compounds were the major principles in six Ficus spp. (*F. sycomorus, F. virens, F. nitida, F. afzelli, F. decora*).

Paterson and Clinch (1994) demonstrated psoralene, proteolytic enzyme ficin, and alkaloids including tylophorine, septicine, autofine and ficuseptine in Ficus spp. They observed that one or more of such chemical groups could be involved in reported animal mortality resulting from the ingestion of *F. tsiela.*

5.2 PHYSIOLOGICAL PARAMETERS

5.2.1 **Body Weight**

The experimental animals did not show any significant variation in body weight as compared with the control animals. All the groups showed a gradual increase in body weight. This indicated that the treatment of animals with fresh juice or extract did not interfere with the growth. They were able to utilize all the nutrients for body energy needs. But in toxicity study of *Mimosa invisa* in rabbits, Jayasree *et al.* (2007) observed rapid reduction in body weight. The extract of *Euphorbia balsamifera*, *E. heterophylla*, *E. hirta*, *E. hyssopifolia* and *E. lateriflora* also caused weight reduction in albino rats (Adedapo *et al.,* 2004).

5.3 BIOCHEMICAL PARAMETERS

5.3.1 **Aspartate aminotransferase (AST)**

The activity of AST is a useful indicator of liver, heart, muscle and kidney function as it is located in the cytoplasm and released into the circulation after cellular damage.

Ficus tsiela ethanolic extract at the doses of 1000 and 1500 mg/kg body weight caused significant increase in the levels of AST on day 14 and 21 of treatment. This is in agreement with Nair *et al.* (1987) as they observed an increase in AST levels in calves in experimental *Ficus tsiela* toxicosis. Singh *et al.* (1988) also observed marked increase in the AST levels in sheep after feeding pure sun-dried water hyacinth which is a hepatotoxin.

In the present study, at higher concentrations of the extract and fresh juice the liver, heart and kidney showed histopathological lesions. Central venous congestion and diffuse multifocal necrosis in the liver, intramuscular haemorrhage in heart and degenerative changes in the kidney were the lesions observed. The increase in serum concentrations of AST levels could be related to these lesions. At the same time it is observed that the flavonoids and terpenes present in the extract were not able to protect these organs probably because of the higher content of the toxic principles.

High levels of AST indicate liver damage, cardiac infarction and muscle injury (Mc. Gregor *et al.*, 2003). In all domestic species, the activity of AST is high in liver and hence hepatic injury causes high AST activity. AST activity is also high in kidney, pancreas and in erythrocytes (Kaneko *et al.*, 1997). The increase in activity of these enzymes in the plasma is often seen following liver damage and it is attributed to the leakage of the enzymes from damaged hepatocytes.

5.3.2 **Alkaline phosphatase (ALP)**

Alkaline phosphatase is found primarily in the intestine, kidney, liver and bone. Kidney and intestine have the greatest activity per gram of the tissue. Renal ALP is not generally found in serum, whereas osseous and hepatic ALP has been

identified in the sera of all species studied. Intestinal ALP may appear in the rat serum due to its slower second phase disappearance (Kaneko *et al.*, 1997).

Rats fed with 1000 and 1500 mg/kg body weight of the extract on day 14 and 21 showed a significant increase in ALP. This could be attributed to the damage to liver, kidney and intestine. Histopathological lesions varying from mild degeneration to necrosis could be observed in these organs. This finding is in accordance with Mandal and Randhawa (1999) who reported significant elevation of ALP and AST in Lantana induced hepatitis in buffalo calves.

5.3.3 **Creatine kinase (CK)**

CK is a sensitive indicator of muscle damage. But only large increases in serum CK activity are of clinical significance. A small increase in serum CK and marked rise in AST serve as an indicator of muscle ischemia incident (Kaneko *et al.*, 1997).

The group of rats which were fed with high doses of extract showed an increase in CK value on 21st day, so also the AST values. This can be related to the cardiac myopathy caused by the toxins. Histopathologically, the cardiac muscles revealed attenuation of fibres, myolysis and intramuscular haemorrhage. Keeler *et al.* (1985) observed an increase in serum AST, CK and lactate dehydrogenase (LDH) in animals poisoned by *Thermopsis montana* which ultimately led to myopathy.

5.3.4 **Creatinine**

Creatinine is formed in the metabolism of muscle creatine phosphate. After the formation it is excreted following filtration by the glomerulus and a high level in the serum indicated kidney damage.

A progressive and statistically significant increase in mean creatinine value was observed in group V and VI on day 7, 14 and 21 whereas group III and IV showed an increase on 21st day when compared to control group. This clearly indicated nephrotoxicity of the toxic principles present. Congestion, haemorrhage, degeneration and necrosis of both tubular epithelial cells and glomeruli were the observed lesions on histopathological examination. Gounalan *et al.* (1999) also reported marked increase in AST, ALP and creatinine values in association with bracken-fern induced nephrotoxicity in rats.

Singh *et al.* (1987) reported that the nephrotoxicity caused by bracken fern showed progressive increase in BUN, uric acid, creatinine and total acid phosphatase activity. Flaoyen *et al.* (1997) found an increase in concentrations of creatinine, urea and magnesium in goats by feeding water soluble nephrotoxic fraction from the stems of *Narthecium ossifragum* plants. Badiei *et al.* (2009) reported increased levels of total bilirubin, conjugated bilirubin, BUN and creatinine concentrations in *Panicum miliaceum* poisoning in sheep.

5.3.5 **Blood Glucose**

No significant change could be observed in the blood glucose of the treatment groups when compared with the control group. Histologically, pancreas in all animals were normal with well preserved acinar and islet cells. This observation is in contradiction to the finding of Nair *et al*. (1987) who observed transient hypoglycemia in calves experimentally induced with *Ficus tsiela* toxicity. This indicated species variation in the response of the organs to toxicity.

5.4 HAEMATOLOGICAL PARAMETERS

The parameters like Hb, PCV, TLC and DLC showed no significant variations in treatment groups compared to control. This indicated that both the

extract and fresh juice from leaves of *Ficus tsiela* have no adverse effect on the haemopoietic system at the given doses. But Gounalan *et al.* (1999) observed an increase in erythrocyte count, PCV and Hb along with a distinct leucopenia, lymphopenia and relative neutrophilia in bracken fern induced toxicity in rats. The crude extract of *Euphorbia balsamifera, E. heterophylla, E. hirta, E. hyssopifolia* and *E. laterifolia* produced anaemia when administered orally in rats (Adedapo *et al.*, 2004).

5.5 OXIDATIVE EFFECTS

Oxidative effect of the plant juice and extract was determined by measuring the alterations in the values of lipid peroxides and reduced glutathione in liver homogenates. In this study, it was observed that both the extract and fresh juice of *Ficus tsiela* could cause oxidative damage as there was a statistically significant increase in lipid peroxides and a concurrent decrease in reduced glutathione. The flavonoids, diterpenes and phenolic compounds though have antioxidant activity, could not resist the damage probably because of the higher concentrations of the toxic principles.

5.5.1 **Lipid Peroxides**

Oxidation of lipid molecules of biological membrane causes membrane damage resulting in the development of the several pathological disorders (Chaturvedi and Segale, 2007). Malondialdehyde, a secondary product of lipid peroxidation is known to cause cross-linkage of membrane compounds containing amino groups that makes the membrane fragile. Lipid peroxidation causes severe damage to cell membrane and increased fragility thereby causing leakage of intracellular enzymes and other compounds resulting in loss of function and cell death. Estimation of thiobarbituric acid reactive substances is diagnostic indices of lipid peroxidation and tissue injury due to oxidative stress (Blaha *et al.*, 2004).

A significant increase in the level of lipid peroxides was observed in the liver of group III and VI when compared to control group. This indicated that the extract and fresh juice of *Ficus tsiela* is able to produce oxidative damage to the liver. Histopathological lesions observcd in the present study confirmed liver damage. One of the comparable observations to this finding is that of Sood *et al.* (2003). They observed increase in the preformed lipid peroxides in the urinary bladder of guinea-pigs in *Onychium contiguum* fern toxicity.

5.5.2 **Reduced Glutathione**

Glutathione is an important antioxidant which functions as free radical scavenger. Decreased glutathione levels have been considered to be an indicator of oxidative stress.

In the present study, group VI (highest dose-1500 mg/kg body weight) showed statistically significant decrease in mean values ($P<0.01$) of reduced glutathione when compared to control group. This indicated oxidative damage of the liver. This is in accordance with the finding of Sarathchandra and Balakrishnamurthy (1997). They observed that *Cleistanthus collinus* leaf extract at a dose rate of 8 g/kg body weight produced acute oral toxicosis and the glutathione was depleted significantly in liver, kidney, heart, brain and skeletal muscles.

5.6 GROSS AND HISTOPATHOLOGICAL OBSERVATION OF THE TISSUES

All the rats irrespective of the dose of the extract given showed no symptoms and survived throughout the study period and remained healthy. At the end of the experiments, all the animals were euthanised and detailed post mortem examination was conducted. Rats administered with the highest dose of extract and fresh juice showed gross lesions in the liver and kidney. Moderate hepatomegaly with

congestion and pin point spots of necrosis were the lesions observed in the liver. The kidneys were appeared severely congested and heart was dilated and flabby.

Liver, kidney, stomach, intestine, cerebrum, cerebellum, pituitary, spinal cord, spleen, adrenal, heart, trachea, lungs, thyroid, parathyroid, pancreas and urinary bladder of all groups of animals were subjected to detailed histopathological examination.

Both the extract and the fresh juice were found to be nephrotoxic which was manifested by varying degrees of tubular degeneration and necrosis. An increase in the serum creatinine level also reflected the kidney damage. Glomerular damage along with congestion and haemorrhage of both cortical and medullary tubules were present. Liver showed diffuse and multifocal necrosis and central venous congestion with prominent Kupffer cell reaction. There was also an increase in the serum AST. These findings indicated the hepatotoxicity of the compounds present in the extract. Oxidative damage of the hepatocytes was evident, as there was increase in lipid peroxides and reduced glutathione. Available literature does not show any information about the overall toxicity of *Ficus tsiela* except the reports by Nair *et al.* (1985) and later the observations by Rajan *et al.* (1986) in calves. The lesions in the present study were in accordance with the lesions observed in the calves. However no microscopically evident adverse effects were observed in the kidney and liver when the extract and fresh juice was fed at a low dose level.

Nair *et al.* (1985) and Rajan *et al.* (1986) observed predominant lesions of toxicity in brain and reported that the plant is highly neurotoxic. They observed cerebral congestion, perivascular and perineuronal oedema, chromatolysis, demyelination and degenerative changes of the grey matter. Contrary to this, rats treated with high doses of fresh juice and extract did not develop any neuronal lesions which could be attributed to the species variation in response to toxicity with

particular reference to target organs. From this investigation, it is observed that rats do not qualify as a good laboratory animal model for the study of the neurotoxicity of *Ficus tsiela*. Therefore, there is a need for further studies in other laboratory animals to choose the best model for further research and development of proper remedies. In this context, it is worth mentioning the observations made by Chanda and Bhaid (1987). They reported that plants that are poisonous to some animals can be harmless to others. In general, some plants are more toxic to ruminants, than are to non-ruminants. Even among ruminants, there are striking differences in tolerance as goats were able to survive on a diet of *F. tsiela* at least for a limited period.

Consistent changes were also observed in the thyroid and adrenal gland of those given high doses of the fresh juice and extract. There was depletion of colloid in the thyroid gland of all rats as compared with the control. The colloid appeared scanty in some follicles and in some others there was no colloid. There was also variation in the follicular size. The presence of goitrogens and other substances affecting the thyroid gland needs to be evaluated. Sastry and Singh (2008) reported similar thyroid lesions in *Leucaena leucocephala* toxicity in female goats. Adrenal gland showed dilatation of sinusoids and medullary cyst. These observations indicated the need for further study on the endocrine effects of this toxic plant. The reactive spleen with well formed white pulp with germinal centres and the greater accumulation of the lymphoid cells in the peribronchial areas as compared with the normal control animal indicated a positive immune response. Whether it is due to the toxic principles or the antioxidant components present in the plant need further investigation. Vijayan (1998) reported that chemicals like carbofuran can act as an immuno-stimulant at very low doses in ducks.

Hyperkeratinisation of non-glandular stomach and damage of the villus epithelium of the intestine, goblet cell hyperplasia, pulmonary congestion and collapse, intermuscular haemorrhage and attenuation of fibres of heart were the other

lesions observed. These findings are in accordance with the observations of Nair *et al.* (1985) and Rajan *et al*. (1986) in toxicity of the plant *Ficus tsiela* Rox b in calves.

From the present investigation, it can be concluded that the plant *Ficus tsiela* is both nephrotoxic and hepatotoxic to rats and not neurotoxic. Since rats failed to develop neurotoxicity which is the most prominent and established change in calves, it is further concluded that rats cannot be considered as a suitable laboratory animal model for the complete evaluation of the toxicity of *Ficus tsiela*.

SUMMARY

6. SUMMARY

The present experiment was undertaken to study the pathological effects of *Ficus tsiela* Rox b in rats.

Forty eight adult female Sprague Dawley rats weighing 150-200 g, divided in to six groups comprising eight animals in each group, were used for the study. Group I animals served as control. Group II and III animals were administered with fresh juice obtained from 5 g and 10 g of leaves of *Ficus tsiela* respectively. Group IV, V and VI animals received ethanolic extract of *Ficus tsiela* leaves at the dose of 750, 1000 and 1500 mg/kg body weight/day respectively in two per cent gum acacia. In all the experimental groups, oral administration was continued up to a period of 21 days and observed for symptoms.

Blood was collected on day 0, 7, 14 and 21 for estimation of Hb, PCV, TLC and DLC and serum was used for the estimation of ALP, AST, CPK, creatinine and blood glucose. At the end of the experiment, all the animals were sacrificed and detailed postmortem examination was conducted and lesions were recorded. Weighed quantity of liver was collected in chilled normal saline for estimation of lipid peroxides and reduced glutathione. Cerebrum, cerebellum, spinal cord, pituitary, thyroid, parathyroid, trachea, heart, stomach, intestine, liver, pancreas, spleen, kidney, adrenal and urinary blabber were examined histopathologically.

Phytochemical analysis of the ethanolic extract revealed the presence of tannins, phenolic compounds, diterpenes and triterpenes. Alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes and triterpenes were present in the fresh juice of *Ficus tsiela.*

Body weight of rats in all the groups showed a gradual increase throughout the experimental period. The study of haematological parameters like Hb, PCV, TLC and DLC revealed that they were not adversely affected. ALP, AST, CK and creatinine values showed significant increase in treatment groups which indicated damage to the liver, kidney and muscle. An increase in the levels of lipid peroxides and a concurrent decrease

in reduced glutathione in liver were observed which indicated oxidative damage to the liver.

Gross lesions were present in liver and kidney of rats which were fed with 1500 mg/kg body weight of extract and fresh juice from 10 g leaves. Liver and kidney were congested. Heart was dilated, flabby and filled with blood. On histological examination, kidney revealed diffuse tubular degeneration and necrosis along with congestion, haemorrhage, degeneration, necrosis and atrophy of glomeruli. Liver showed diffuse and multifocal necrosis, central venous congestion and prominent Kupffer cell reaction. In thyroid, some of the follicles were devoid of colloid. Adrenal gland revealed the presence of medullary cyst. Congestion and diffuse pulmonary collapse along with focal peribronchial lymphoid hyperplasia were present in the lungs. Heart revealed intermuscular haemorrhage and attenuation of muscle fibres.

Intestine showed goblet cell hyperplasia with accumulation of mononuclear cells in the lamina propria. Spleen was reactive with well formed white pulp and germinal centres. In all the animals, cerebrum, cerebellum, spinal cord, pituitary, pancreas and parathyroid were apparently normal.

From this study it was concluded that the extract and fresh juice at higher concentration are both nephrotoxic and hepatotoxic. Higher concentrations of the extract and fresh juice induced toxicological effects on thyroid and adrenal gland too. Since the rats did not develop neurotoxicity which is the predominant and established lesion in calves, it is further concluded that rats does not qualify to be a suitable laboratory animal model for complete toxicological study of *Ficus tsiela* Rox b.

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PATHOLOGICAL OBSERVATIONS OF *Ficus tsiela* (Rox b) TOXICITY IN RATS

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ABSTRACT

The present study entitled 'Pathological Observations of *Ficus tsiela* (Rox b) Toxicity in rats' was undertaken by administering the animals with different concentrations of fresh juice and alcoholic extract for a period of 21 days. The weekly body weights, clinical signs, haematology, biochemical parameters, gross pathology and histopathology of various organs were analysed to study the effect. Phytochemical evaluation of the fresh juice and extract was done and the oxidative damage of the liver was assessed by the estimation of lipid peroxides and reduced glutathione.

Phytochemical evaluation of the fresh juice revealed the presence of detectable levels of tannins, glycosides, diterpenes, triterpenes, alkaloids, flavonoids and phenolic compounds whereas the ethanolic extract revealed the presence of tannins, phenolic compounds, diterpenes and triterpenes.

The animals remained clinically normal throughout the experimental period and the body weight revealed a gradual increase. Hb, PCV, TLC and DLC revealed no variation whereas ALP, creatinine and CK values showed a significant increase in the higher dose groups. There was no variation in the level of blood sugar. There was an increase in the lipid peroxides and reduction in the glutathione in the liver homogenate which indicated oxidative damage.

Gross lesions were not observed in the internal organs except congestion and diffuse enlargement of the kidney and liver in the highest dose group. Focal necrotic spots were present in the liver. Tubular and glomerular degeneration and necrosis of the kidney, sinusoidal congestion and multifocal necrosis of the liver, depletion of colloid and variation in the size of follicles of the thyroid gland, medullary cyst in the adrenal gland, goblet cell hyperplasia of intestine, intermuscular haemorrhage in the heart, reactive spleen with multiple cortical follicles with germinal centres, peribronchial lymphoid hyperplasia in the lungs were the lesions observed. The brain, spinal cord and pituitary did not reveal any signs of intoxication. The study revealed that the fresh juice and ethanolic extract at higher doses are nephro-hepatotoxic but not neurotoxic to rats.