IDENTIFICATION OF TOXIC FRACTIONS OF *Mimosa invisa* (ANATHOTTAVADI) AND ITS TOXICITY IN RABBITS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis, entitled **"IDENTIFICATION OF TOXIC FRACTIONS OF *Mimosa invisa* (ANATHOTTAVADI) AND ITS TOXICITY IN RABBITS"** is a bonafied record of research work done by me during the course of research and that the 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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Usha. P.T.A.

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Introduction

1. INTRODUCTION

Phytotoxins have caused extensive losses to the livestock industry in many parts of the world. Even though toxic principles from many plants have been isolated, the scientific validation is still incomplete.

Toxic plants may grow together with forage plants and are therefore readily accessible to grazing animals. Only few toxic plants can be considered sufficiently palatable to be eaten in preference to better class of forage. Animals allowed free choice usually select the more desirable forage and avoid the plants that are toxic. Overgrazing with periodic draughts has impaired or destroyed the growth of palatable and nutritious forage plants and has permitted the spread of less palatable often poisonous species that are well adapted to withstand semiarid conditions existing over most of the areas. The danger of overgrazing is always greatly increased in periods of moisture deficiencies that reduce forage production.

There is great variation in the toxicity of poisonous plants and in amounts necessary to cause injury or death. Some act as acute poison, while others must be consumed over a considerable period of time to produce harmful effects. With majority of poisonous plants, amounts well below toxic limits may be eaten even for a considerable period of time with little or no effects.

Mimosa is a shrubby herbaceous spreading annual plant but it can grow biennial whenever water is available year round. It is commonly known as "Giant sensitive plant", "Creeping sensitive plant" or Nila grass (Sankaran, 2001). In malayalam it is known as "Anathottavadi". Two varieties were found to be present. *Mimosa invisa* (thorny variety) and *Mimosa invisa inermis* (thornless variety). The thornless variety of mimosa is capable of reverting to thorny variety (Vattakkavan *et al.*, 2007).

Mimosa is a native of tropical America and was imported by neighbouring tea gardens from East Asia in 1960s as a nitrogen fixer prior to planting tea. Sankaran (2001) has reported that *Mimosa invisa* is widespread in the central and southern parts of Kerala.

Mimosa invisa toxicity is an emerging problem in Kerala. Poisoning due to this plant is frequently reported in cattle and goats. The phytotoxin present in the plant affects mainly kidneys (Rajan *et al.*, 1986). The main clinical symptom of toxicity was reduced feed and water intake (Alex *et al.*, 1991). Knowledge of chemical nature of the phytotoxin leads to more rational treatment of affected animal. This will also makes it possible to detect the poison in tissues of animals, thus leading to diagnosis in doubtful cases (Radeleff, 1970). The chemical nature of phytotoxin present in *M. invisa* has not been studied. The detailed toxicity study of this plant is also lacking. Symptomatic treatment is being followed in cases of poisoning which fails in most cases. Hence this research was undertaken with the following objectives.

- to screen the stem and leaves of *Mimosa invisa* at various stages of its growth for the active principles present in it.
- to identity the toxic fractions present in the extracts of *Mimosa invisa.*
- to assess the toxicity of fractions in rabbits.
- an attempt has been made to scientifically validate the effect of a combination of *Hygrophila auriculata, Boerhavia diffusa* and *Tribulus terrestris* in mimosa toxicity.

Review of Literature

2. REVIEW OF LITERATURE

2.1. *Mimosa invisa*

Mimosa invisa is a shrubby, herbaceous spready plant that has the capacity to form dense thickets. It is a native of Brazil and is a serious weed in Philippines, South East Asia and Pacific islands.

Plant description

Stems: Conspicuously four angled, branching profusely, 2-4 m long with numerous recurved prickles.

Leaves: Bright green, bipinnate 10-20 cm long consisting of 4-9 pairs of primary segments each with 10-20 pairs of opposite leaflets without any stalk, tapering spear shaped 6-12 mm long 1.5 cm wide. Leaflets fold when touched.

Flowers: Pinkish violet, numerous in globular heads about 1-2 cm in diameter, 1-3 heads arising on separate stalks from the leaf axils. Petal tubes four lobed with 8 pinkish violet stamens that protrude beyond the petals.

Fruits: A softly spiny, 3-4 seeded, flattened pod, born in clusters on the leaf axils.

Seed: Light brown glossy, flattened oval in outline, 2-3 mm long with a 'U' shaped mark on each face.

Root: Robust branched tap root, 1-2 m long, with nitrogen fixing nodules (Schultz, 2000).

Rajan *et al.* (1986) conducted studies on the toxicity of the plant *Mimosa invisa Mart var inermis Adlb.* in calves. Calves were given orally different dose levels of Mimosa (50, 100, 150, 200, 300 g) for 30 days and observed changes. They noted reduction in haemoglobin level on the 11^{th} and 21^{st} day of mimosa

administration. Pathological changes observed were patchy greyish white area on the cortex of kidneys, severe congestion and oedema of abomasal mucosa, congestion of liver and distension of gall bladder. Histopathologically there were varying degrees of degeneration of tubular epithelium, hyaline degeneration of epithelial cells and multiple focal areas of haemorrhage in the medullary region of the kidney.

A clinical case of mimosa poisoning in a heifer was described by Alex *et al.* (1991). The animal was presented in the Veterinary college hospital, Mannuthy with symptom of reduced feed and water intake. Since the animal was not improving with symptomatic treatment, exploratory laparotomy was conducted and found that abomasum was half filled with mimosa leaves. Diffuse oedema of retroperitoneal region, swollen oedematous kidneys with echymosis were the lesions observed. Histopathological examination of kidneys showed severe congestion, focal haemorrhage, and tubular hyalinization. Liver showed sinusoidal congestion and focal areas of degeneration and necrosis.

Tungtrakanpoung and Rhienpanish (1992) reported death of two swamp buffaloes after eating *Mimosa invisa Mart var inermis Adelbert*. The symptoms were salivation, stiffness, lack of mastication, muscular tremors, dyspnoea and recumbency. Post mortem lesions were congestion and petechial haemorrhage of heart, liver, kidneys, rumen and intestine. They suggested that the toxic elements of *M. invisa* are cyanide and nitrates. Water extract was toxic to mice with LD_{50} 0.6 g/kg, while alcoholic extract showed LD_{50} 0.22 g/kg.

The camels grazing on *Mimosa invisa* died within 24-48 hrs after the onset of nervous signs (Li *et al.*, 1996).

Alikutty and Pillai (1992) reported a clinical case of mimosa poisoning in a buffalo. The symptoms observed were reduced appetite and severe oedema on

the ventral aspect of the abdomen and perineum. Blood samples on analysis showed increase in Blood Urea Nitrogen (BUN) and leucopenia.

Jayasree *et al.* (2007) noticed a reduction in body weight, when silage containing more than 50 per cent *Mimosa invisa* was fed to rabbits. Increase in proportion of *Mimosa invisa* showed a proportionate reduction in body weight. The toxicity symptoms were less when dry powdered *Mimosa invisa* was incorporated in the silage.

2.2. PLANTS CAUSING NEPHROTOXICITY AND HEPATOTOXICITY

Barni *et al.* (1990) described the toxicity of *Abrus precatorius* in Nubian goats. Experimental feeding of *Abrus precatorius* at different dose levels showed toxicity symptoms like inappetence, bloody diarrhoea, dyspnoea, dehydration, loss of condition and recumbency. Histopathological examination revealed necrosis of hepatocytes and renal convoluted tubules. These changes were accompanied by increased aspartate amino transferase, gamma glutamyl transferase, urea, creatinine and decrease in total protein.

Craig *et al.* (1991) reported progressive hepatopathy characterized by sequential development of intranuclear inclusions in hepatocytes and biliary hyperplasia after administration tansy ragwort in ponies. The toxicity was due to toxic pyrrolizidine alkaloids. GGT was the most consistent enzyme to rise above control values.

Two out breaks of amaranthus species poisoning in cattle was reported by Ferriera *et al.* (1991). The clinical symptoms observed were depression, anorexia, decreased ruminal activity, diarrhoea, incordination and increased levels of urea and creatinine. Kidneys were oedematous. The histological lesions revealed toxic nephrosis.

Amaranthus species poisoning was characterized by weakness, incordination and death within 48 hrs. Main post mortem finding was tan whitish

kidneys. The main histological findings were acute tubular injury (Selles *et al.*, 1991).

Chakraborty and Mandal (1992) observed Karaja oil induced hepatitis in cattle. The condition was fatal and some animals were severely ill. The main symptoms were diarrhoea, oedema, inappetence and general malaise. The main change observed was extremely high serum levels of urea (six times the normal value) and creatinine (ten times the normal value).

Experimentally induced *Cassia obtusifolia* poisoning has resulted in loss of body weight, muscle atrophy and marked elevation of creatine phosphokinase and aspartate aminotransferase levels (Loung and Mecoy, 1992).

Lugt *et al.* (1992) experimentally induced *Cestrum laevigatum* (Schlenchtd) poisoning in sheep. They observed clinical symptoms like depression, anorexia and ruminal stasis. Increase in activity of aspartate aminotransferase, lactate dehydrogenase and gamma glutamyl transferase were observed.

Malone *et al.* (1992) observed that cows and calves rapidly lost body condition and became dull and anorectic after grazing on pasture containing Bog asphodel during summer. The affected cows had kidney damage as evidenced by elevated plasma urea and creatinine values. Histopathological examination showed diffuse renal tubular necrosis.

Clinicopathological studies were conducted on aran-induced toxicosis in sheep by Nasir and Al Sultan (1992). Sheep were fed with *Trifolium ripens* (aran) in flowering state at different dose levels for 14 days. Both liver and kidney showed congestion with fatty changes in liver.

According to Done and Bain (1993) the algal boom on water was associated with hepatotoxicosis in sheep. Histopathological examination

revealed coagulative necrosis with haemorrhage in liver. There was mild tubular nephrosis in the kidney.

Chopped *Hypericum perforatum* plants were fed to sheep and blood samples were collected at zero, seven and 14 days. After one week of administration the severity of clinical symptoms increased. Blood Urea Nitrogen, bilirubin, aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transferase activities were increased (Kako *et al.,* 1993).

Lemos *et al.* (1993) observed clinical signs after 30days of feeding of *Amaranthus spinosus* in cattle. Clinical signs were depression, anorexia, weight loss, diarrhoea and subcutaneous oedema. Post mortem findings were pale and contracted kidney and perineal oedema.

Sachwiste *(Nolina microcarpa)* blossom toxicity was studied by Rankins *et al.* (1993). They observed that toxicosis was evident within 24 hours and symptoms were inappetence, depression, hypokalemia, hypophosphatemia, increased alkaline phosphatase, creatinine kinase and γ-glutamyl transpeptidase.

Photosensitization reaction in rabbits after administration of *Hypericum perforatum* plant was reported by Al-Khafaji (1994). There were changes in haematological parameters. The biochemical changes observed were increase in alanine aminotransferase, aspartate aminotransferase and decrease in total protein levels. Pathological changes were observed in liver, urinary bladder, kidneys and lungs.

Amaranthus retroflexus induced nephrotoxicity caused death of 48 cattle and 35 animals were clinically affected. Poisoned animals showed serum urea nitrogen between 55 and 284 mg/dl and serum creatinine value was between 6.7 and 29.9 mg/dl. Histopathological examination revealed widespread degeneration and necrosis of proximal and distal convoluted tubules (Casteel *et al.,* 1994).

East *et al.* (1994) observed that consumption of cotton seed meal based mineral supplement and concentrate resulted in gossypol toxicosis in adult dairy goats. Clinical signs noticed were limb swelling and stiffness, ventral abdominal oedema and anorexia. Post mortem examination revealed generalized subcutaneous oedema, acute centrilobular necrosis of liver and myocardial fibrosis.

Bog asphodel poisoning *(Narthecium ossifragum)* poisoning in cattle and sheep was studied by Flaoyen *et al.* (1994). Single dose of the plant showed increase in creatinine and urea values. On histopathological examination kidney damage characterized by tubular degeneration and necrosis was observed.

Flaoyen *et al.* (1995a) demonstrated the toxicity of *Narthecium ossifragum* in seven lambs by feeding 15 gram wet matter per kilogram of the plant for 10 days. They observed increase in serum creatinine from day one to day four followed by a fall to normal by sixth day after feeding started.

Seven calves were fed with a mixture of bog plants containing *Narthecium ossifragum* at a rate of 15g/kg body weight for two consecutive days. The serum levels of creatinine, urea and magnesium increased whereas calcium decreased. Aspartate aminotransferase and gamma glutamyl transferase activitities were increased indicating hepatic dysfunction (Flaoyen *et al.*. 1995b).

Jaramillo-Juarez *et al.* (1995) studied the acute toxicity of tullidora seeds. They noticed decrease in renal blood flow and glomerular filtration rate. Histopathological lesions were cloudy swelling and hydropic degeneration of epithelial lining of proximal convoluted tubules.

Effect of experimental feeding of *Anagalis arvensis* to cattle and buffaloes was done by Sadekar *et al.* (1995). Toxicity symptoms observed were dullness, anorexia, suspended rumination and oedematous swelling observed in the perennial region. Kidney and liver showed pathological lesions.

Sensitivity and specificity of Gamma glutamyl transferase as a screening test for liver disease was described by Curran *et al.* (1996). Crotalaria plant poisoning was used to evaluate various biochemical tests of sub clinical liver disease. They found that gamma glutamyl transferase has sufficient sensitivity (75%) and specificity (90%) to function as a criteria for sub clinical liver disease in horses.

Botha *et al.* (1997) described field outbreak of vermeersiekte, an erosive disease causing production and reproduction losses, caused by *Geigeria burki* Harv subsp. Burki var hirtilla Merxm wherein serum activities of creatinine kinase and gamma glutamyl transferase were slightly elevated along with increase in aspartate aminotransferase, glutamyl dehydrogenase and lactate dehydrogenase.

Deoras *et al.* (1997) reported that single intraperitoneal dose (25 mg/kg body weight) of gossypol to Sprague-Dawley rats produced increase in activity of liver and blood serum gamma glutamyltransferase. Histopathological examination showed degenerative changes and coagulative necrosis of hepatocytes.

Flaoyen *et al.* (1997a) observed increase in serum creatinine and urea levels by day five in goats after administration of a single dose of aqueous extract derived from 30g/kg of *Narthecium ossifragum*. The value reached to normal by day 20. Histopathological examination of kidneys of goats died revealed tubular epithelial cell degeneration and necrosis.

Effect of aqueous extract and insoluble plant residue of *Northecium ossifragum* plant was described by Flaoyen *et al.* (1997b). The aqueous extract caused increase in serum creatinine and magnesium while the activity of glutamate dehydrogenase increased only in animals dosed with insoluble plant residue. Calves fed with flower stem developed increased creatinine, urea, serum glutamate dehydrogenase, alanine aminotransferase, aspartate aminotransferase

and gamma glutamyl transferase activities. Serum creatinine significantly increased in 14 day old calf showing intrinsic nephrotoxicity of the plant.

Flaoyen *et al.* (1997c) reported nephrotoxicity in goats caused by dosing with a water extract from stems of *Narthecium ossifragum* plant. A single dose of 30g of the plant caused increase in creatinine and urea up to fourth day and then decreased to normal value by day 10. Histopathological examination of the kidney showed tubular epithelial cell degeneration and necrosis.

Effect of aqueous extract of *Solanum malacoxylon* was studied in rabbits by Marcolla *et al.* (1997). Microscopic examination of the myocardium showed swollen group of muscle fibres with sarcoplasmic vacuolations.

Somavanshi and Gounalen (1997) observed death of rabbits after feeding a diet containing 30 per cent *P. squarrosum*. Clinical signs noted were anaemia, cachexia and 50 per cent loss of body weight. Histopathology of lungs, liver and kidneys showed marked vesicular changes, oedema and haemorrhages.

Fresh *Iphonia aucheri* (500 g) was crushed and suspended in 500 ml water and given orally using stomach tube to a three year old sheep. Blood analysis showed sharp increase in alanine and aspartate aminotransferase indicating severe liver damage (Wernery *et al.*, 1997).

Medical records of 60 dogs with evidence of cycad ingestion were reviewed by Albertson *et al.* (1998). *Cycad involuta* was the common cause of poisoning to these animals and 95 per cent developed liver and gastrointestinal problems, serum biochemical abnormalities like high serum bilirubin, alkaline phosphatase and alanine aminotransferase.

Raposo *et al.* (1998) studied the experimental intoxication of *Myoporum laetum* in sheep. All the animals were showing photodermatitis and increase in serum levels of alanine aminotransferase, gamma glutamyl transferase and bilirubin. Histopathology was characterized by periportal liver necrosis.

An outbreak of toxicosis due to Oak leaves (*Quercus caltiprinos*) was described by Yeruham *et al.* (1998). Symptoms observed were progressive wasting, dullness, anorexia, polyurea, nephrosis, constipation and recumbency. The clinico-pathological findings revealed increase in blood urea, creatinine, aspartate aminotransferase, gamma glutamyl transferase, creatine kinase, lactate dehydrogenase and alkaline phosphatase. Pathological findings were severe nephrosis.

According to Gadir and Adams (1998) pearl millet caused development of goiter and enterohepatonephropathy in Nubian goats. There were changes in serum and tissue iodine and selenium concentration, alterations in serum aspartate transaminase, gamma glutamyl transferase, total protein, total lipids and haematological values.

Langseth *et al.* (1999) reported that the principle toxic substance in *Narthecium ossifragum*, a wild plant of lilaceae family responsible for nephrotoxic effect on cattle, goats and other ruminants was isolated and identified by X-ray Crystallography as 3 methoxy- 2(5H) furanone.

Sorby and Flaoyen (1999) observed that three cattle that had consumed large quantities of acorn showed inappetence and reduced ruminal activity. Blood samples revealed greatly increased levels of creatinine and urea. All the animals died and post mortem examination showed perirenal oedema and pale kidney with petechial haemorrhages. Histopathological examination indicated nephrosis which was attributed to tannins in the ingested acorn.

Combined effect of *citrullus colocynthis* and *Rhazya Stricta* in Najdi sheep was reported by Adams *et al.* (2000). Oral administration of these plants was proved to be fatal. Symptoms observed were profuse diarrhoea, dehydration, ataxia and recumbency followed by enterohepatonephrotoxicity. There were alterations in alanine aminotransferase, serum lactate dehydrogenase, total protein, albumin, globulin, bilirubin, cholesterol and urea.

Citrullus colocynthus L. Schrad. toxicity in rats caused reduction in body weight, feed efficiency, diarrhoea and enterohepanephropathy. These lesions were correlated with alterations in serum lactate dehydrogenase, aspartate aminotransferase and alterations in urea, total protein and albumin (Adams *et al.*, 2001).

Experimentally induced cholangiohepatopathy with fractionated extracts from *Brachiaria decumbens* was reported by Cruz *et al.* (2001). Gross changes in liver were pale foci multi focally distributed in hepatic parenchyma.

Flaoyen *et al.* (2001) observed tolerance to nephrotoxic component of *Narthecium ossifragum* in sheep. Nephrotoxic syndrome after administration of *Narthecium ossifragum* were increased levels of serum creatinine and urea. A single intraperitoneal challenge after seven to twelve days of intraruminal dosage did not lead to increased serum creatinine and urine concentration. This indicated that oral treatment had resulted in an increased tolerance to nephrotoxic principles in *Narthecium ossifragum.*

Hepatotoxic effect of *Panicum virgatum* was reported by Lee *et al.* (2001). The study revealed presence of steroidal saponins in the sample consumed by animals.

Arnagallis arvensis poisoning in cattle and sheep caused lethality in 91.3 to 100 per cent. Clinical signs observed were weakness, staggers, diarrhoea, coma and death. Gross lesions in various organs were petechiae, fluid in the cavities, mesenteric and perineal oedema. Kidneys were pale with petechiae in renal cortex. Histologically severe nephrosis was observed. There were elevation of levels of urea and creatinine (Rivero *et al.*, 2001).

Toxic effect of a mixture of 0.25 g/kg of each of *Nerium oleander* and *Rhazya stricta* leaves in sheep was studied by Adams *et al.* (2002). The combination of these two plants was proved to be fatal in animals within 24 hours. The main

post mortem lesions were congestion, haemorrhage, emphysema and hepatonephropathy.

Parthenium histerophorus was drenched at a rate of 250 mg/rabbit/day for 21 days. There was decrease in body weight, alopecia. Haematocrit values reduced significantly after two weeks. Serum creatinine levels, alanine aminotransferase and aspartate aminotransferase levels were increased whereas total protein and albumin decreased. It is concluded that the plant is hepatoxic and nephrotoxic (Prakash *et al.*, 2002).

Yeruham *et al.* (2002) reported two cases of urinary retention syndrome caused by ingestion of a shrub *Cistus salvifolius* in beef cattle. Clinical syndrome observed were progressive wasting, anorexia, distension of bladder and nephrosis. Clinicopathological findings revealed increased blood urea, creatinine, aspartate aminotransferase, creatine kinase, alkaline phosphatase, total protein, albumin, potassium, sodium and chloride. Pathological findings were cystitis, pyelonephritis and increase in urinary bladder thickness.

Cats ingested Daylilies are at a risk of gastrointestinal distress and acute renal failure (Hardley *et al.*, 2003).

Toxicological studies of *Prosopis juliflora* were carried out by Misri *et al.* (2003). They administered the plants to 18 goats at a rate 400 g/animal/day and found that there were increase in blood urea nitrogen and aspartate aminotransferase activity. Histopathological studies revealed necrotic lesions in liver, bile duct degeneration and degenerative changes in renal tubule.

Fan *et al.* (2005) studied the toxic effect of *Oxytropis glacialis* in goats. Animals were fed with 10g/kg/day of *Oxytropis glacialis* and histopathological and biochemical parameters were assessed. RBC, haemoglobin, PCV, MCH and MCHC were significantly decreased. The activities of serum alkaline phosphatase, SGOT, lactate dehydrogenase and blood urea nitrogen values were significantly higher than controls.

Meintjes *et al.* (2005) noticed sub acute symptoms in sheep like depression, inappetence, teeth grinding and tachycardia after feeding *Nolletia gariepenia* (DC) Mattf. There were decline in glomerular filtration rate, increased sodium excretion and rise in gamma glutamyl transferase levels.

Experimental intoxication of *Stryphnodendron fissuratum* fruits in cattle at different dose levels produced moderate increase in aspartate aminotransferase, creatinine kinase and gamma glutamyl transferase (Rodrogues *et al.,* 2005).

Spontaneous poisoning by *Trema micranthea* in goats was reported by Traverso and Driemeier (2005). The affected goats showed anorexia, drooling, coma and death. There were gross lesions in kidney. One of the animal showed perirenal oedema and pale kidney with petechial haemorrhages.

Anagallis arvensis toxicity in cattle and buffaloes due to accidental ingestion of the weed was reported by Harish *et al.* (2006). They observed clinical signs of dullness, anorexia, delayed rumination, restlessness, decreased body condition, dehydration and haemorrhagic gastroenteritis. Gross pathological examination revealed haemorrhagic lesions in lung, liver, kidney and heart confirming cytotoxic and nephrotoxic effects of blue pimpernel in livestock.

2.3. PLANTS USED FOR TREATMENT OF TOXICITY

2.3.1. Hygrophila Species

Clinical investigation of the effects of *Hygrophila spinosa* root was done by Mazumdar *et al.* (1999). They reported the presence of greasy mass lupeol and lupenone in petroleum ether extract. They also observed that sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone chlordiazepoxide which was potentiated by crude petroleum ether extract of *Hygrophila spinosa.*

Haematinic effects of *Hygrophila spinosa* on experimental rodents were studied by Gomes *et al.* (2001). Effects of ethanolic extract of aerial parts of *H. spinosa* was examined on male albino rats for certain haemotological changes.

The ethanolic extract at the rate of 100 and 200 mg/kg orally in male albino rats significantly increased the haemoglobin, haematocrit, RBC and WBC. Serum iron and serum total iron binding capacity also significantly increased.

Misra *et al.* (2001) isolated constituents of *Asteracantha longifolia*. They isolated two aliphatic esters and betulin from areal parts of the plant. Previously isolated constituents were flavonoids, terpenoids and sterols.

Protective effects of *Asteracantha longifolia* alcoholic extract in mouse liver injury induced by carbon tetrachloride and paracetamol was studied by Hewawasam *et al.* (2003). Asteracantha reduced alanine aminotransferase level (ALT) by 69.32 per cent, increased liver reduced glutathione level by 64.65 per cent in the pretreated group in both cases of toxicity. Histopathological studies showed marked improvement in the liver texture. Pretreatment showed better results than post treatment.

Shanmugasundaram and Venkataraman (2005) studied the antinociceptive property of both *Hygrophila auriculata* aerial parts and roots by chemical and thermal method in mice. In chemical method acetic acid writhing test and thermal method hot plate and tail flick tests were performed. Both the extracts at doses 100 and 200 mg kg orally inhibited abdominal constrictions induced by acetic acid and increased threshold of mice towards the thermal source in a dose dependent manner. The activity exhibited by the extracts was comparable to that of standard drug aspirin.

Hepatoprotective effects of aqueous extracts of *Hygrophila auriculata* root on carbon tetrachloride induced liver toxicity in rats were investigated by Shanmugasundaram and Venkataraman (2006). The levels of ALT, AST, ALP, LDH and total bilirubin were significantly increased in carbon tetrachloride treated controls whereas the levels of above enzymes were significantly reversed on treatment with *Hygrophila auriculata.* Histopathology also showed regenerative changes of the damage produced by carbon tetrachloride.

2.3.2. *Boerhavia diffusa*

Aqueous extracts of twigs of *Boerhavia diffusa* showed moderate diuretic activity. The diuretic activity was attributed to the presence of potassium content in the plant (Bhide *et al.,* 1958).

Chopra *et al.* (1958) reported that the active principle of *B. diffusa* was compound of alkaloidal nature called "Punarnavine" large quantities of potassium nitrate and other salts contained in the plant might contribute to its diuretic effect. Intravenous injection of alkaloids in cats produced distinct and persistent rise in blood pressure and diuresis.

Abraham (1975) reported that the plant *Boerhavia diffusa* as a whole was effective in jaundice, oedema, blood pressure and acting as diuretic in mild cases.

Nadkarni (1976) suggested that 1-4 drachm of the liquid extract from the plant produced diuresis in case of oedema and ascitis especially due to liver, peritoneal and kidney conditions. The diuresis was mainly due to the action of Punarnavine – the alkaloid, on renal epithelium.

Different extracts and three isolates from extracts of *B. diffusa* subjected to in vivo and in vitro testing for hepatotoxicity. The petroleum ether, chloroform and methanol extracts and the total alkaloids of the roots were reported to lower the increased SGOT, SGPT in rats treated with carbon tetrachloride. One of the isolates identified as retenoid was reported to lower SGOT and SGPT at dose rates of 100µg/kg in carbon tetrachloride induced hepatotoxicity. A steroid isolated from aerial parts significantly lowered SGPT and SGOT at a dose level of 200 µg/ml (Chakraborthi and Handa, 1989a).

The alcoholic extract of *B. diffusa* at a rate of 500 mg/kg possessed significant protective activity against carbon tetrachloride induced hepatic injury in rats revealed by decrease in levels of SGOT, SGPT, serum bilirubin, plasma prothrombin, and BSP clearance time (Chandan *et al.,* 1991).

The effect of 50 per cent ethanolic extract of the root of *Boerhavia diffusa* on country made liquor induced hepatotoxicity was studied in albino rats by Gulati *et al.* (1991). They suggested that *Boerhavia diffusa* (100mg/100g body weight/day) protected the rats from hepatotoxic action of country made liquor as evidenced by serum ALT, triglycerides, cholesterol and total lipid levels in both serum and tissues.

Rawath *et al.* (1997) investigated the effect of seasons, thickness of roots and form of dosage on the hepatoprotective activity of the roots of *B. diffusa* against thioacetamide induced hepatotoxicity in rats. The results showed that an aqueous extract (2 ml/kg) of roots of diameter 1-3 cm collected in the month of May (summer) exhibited maximum protection of the serum enzymes SGOT, SGPT and ALP. The aqueous form of drug administration had more activity than powder form.

Srivastava *et al.* (1998) reviewed the chemistry, pharmacology and botany of *B. diffusa* extracts and their isolates. They described various chemical constituents of *B. diffusa* and reported that some of them possessed hepatoprotective, adaptogenic, antifibrinolytic, diuretic and antiviral properties.

Bharathan (2002) studied the anti-inflammatory, hepatoprotective and immunostimulant properties of *Boerhavia diffusa.* It was found that at a dose rate of 400mg/kg it exhibited anti-inflammatory property. Also reported its antioxidant and immunostimulant activities at this dose level.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of *Boerhavia diffusa* extract at a dose rate of 2.4 g/kg body weight orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused normal maintenance of level of water intake and urine output after surgery. It also prevented accumulation of peritoneal fluid and gangrene.

Pari and Satheesh (2004) investigated the antidiabetic effect of *Boerhavia diffusa*. They also studied its effect on serum and tissue lipids in experimental diabetes. Oral administration of *B. diffusa* leaf extract at 200mg/kg body weight for 4 weeks resulted in significant reduction in serum and tissue cholesterol, free fatty acids, phospholipids and triglycerides.

2.3.3. *Tribulus terrestris*

Pande *et al.* (2000) investigated hepatoprotective activity of fruits of *Tribulus terrestris* L. The methanolic extract of the fruits was fractionated using column chromatography and the four fractions obtained were evaluated for hepatoprotective activity on albino rats. Effects of fractions were assessed on the basis of biochemical tests and histopathological profile. The acetone fraction was found to possess significant hepatoprotective activity.

Meher *et al.* (2000) conducted studies to evaluate nephroprotective action of *Tribulus terrestris* and *Crataeva niruvala* Bachham in gentamicin induced nephrotoxic models in rats. Serum creatinine and urea levels were determined every week for 6 weeks. *Tribulus terrestris* was fed daily at a rate of 65 mg/kg and 130 mg/kg and *Crataeva niruvala* Bachham at a rate of 70 mg/kg and 140 mg/kg was fed to rats after 8 days of gentamicin administration. The group administered with higher doses revealed maximum nephroprotective effect within a period of four weeks whereas the toxicant group took seven weeks for recovery.

Preliminary study of the diuretic and contractile effects of *Tribulus terrestris* and its comparison with *Zea mays* was done by Al-Ali *et al.* (2003). It was observed that the aqueous extract of the leaves and fruits of *T. terrestris* resulted in marked diuretic effect. There was much increase in urine volume and K^+ excretion. In addition to this *T. terrestris* extract evoked contractive activity in guinea pig ileum. *Zea mays* had potentiated the amount of sodium excretion by *T. terrestris* extract.

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

New Zealand White rabbits procured from the Small Animal Breeding Station, Mannuthy were used for the study. Animals were allowed to acclimatize for one week before the experiment. Feed and water were given *ad libitum*. All the animals were maintained under similar conditions of feeding and management.

3.2. COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACTS

Fresh leaves with stem of *Mimosa invisa* were collected from Veterinary College Campus and cut into small pieces.

3.2.1. Preparation of *Mimosa invisa* Fresh Juice

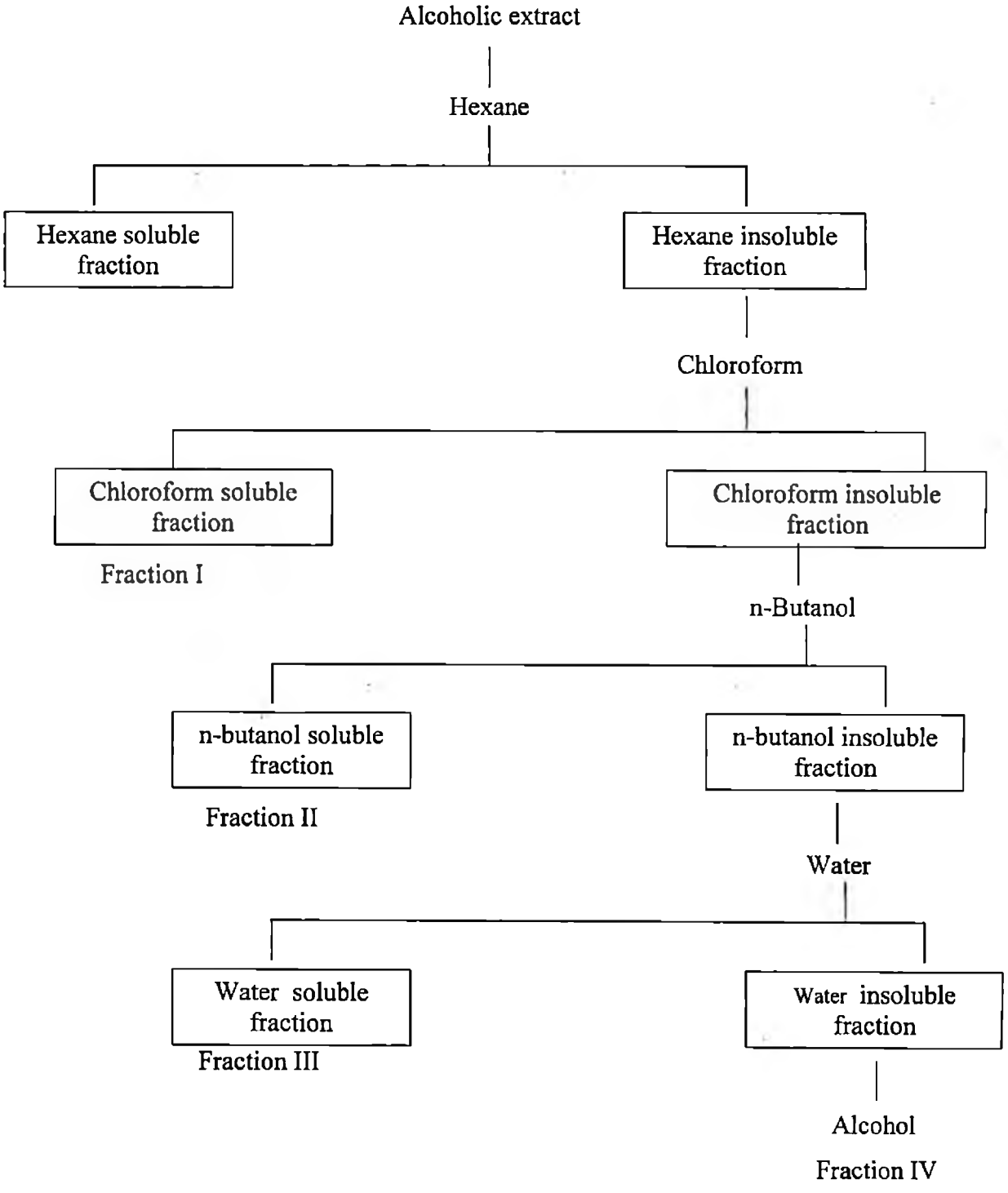
The fresh plant material was macerated and mixed with minimum quantity of water and filtered through a sieve and removed the fibre. From 50 g of plant material 100 ml of juice was prepared.

3.2.2. Preparation of Alcoholic Extract and Decoction

The fresh plant material (15 kg) was soaked in ethanol for 48 hrs with frequent stirring. After 48 hours the plant material was squeezed out and the alcohol was filtered through filter paper. The alcoholic solution was subjected to evaporation under vacuum at a lower temperature (<50°C), so that 15 litres of alcoholic solution was reduced to three litres. Further evaporation was done by keeping the concentrated extract in an open vessel in the refrigerator. The percentage yield was estimated by weighing the plant material before and after extraction. The extract was prepared using both young tender plants and plants at flowering stage.

Fig.1 Mimosa invisa

Preparation of various fractions of alcoholic extract of *Mimosa invisa*



The decoction was prepared by adding 3000 ml of water to the drug (300 gram each of *Boerhavia diffusa,* T*ribulus terrestris* and *Hygrophila auriculata*). Boiled the mixture for two hours and strained the decoction through muslin cloth to remove the coarse materials. Then reduced the decoction to one litre by boiling so that one millilitre of the decoction contain 0.9 gram of the material. The decoction was administered at a rate of 5 g/kg (5.5 ml/kg of the decoction) orally.

3.2.3 Fractionation of the Alcoholic Extract

The alcoholic extract was mixed with silica gel of mesh 60-120 and filled in glass column. The column was first eluted with hexane until there is no colour to the hexane coming out of the column. Hexane fraction was discarded.

Then the column was eluted with chloroform to collect the chloroform soluble portion of the extract. Collected this fraction, evaporated the chloroform and labelled it as Fraction I.

Eluted the column with n-Butanol until all the n-Butanol soluble fraction was being separated. This fraction was collected and evaporated to dryness and preserved it as Fraction II.

Collection of water soluble fraction was done by eluting the column with water until no more colour to the water coming out of the column. This fraction was evaporated to dryness and preserved it as Fraction III.

The portion of extract, that is not soluble in any of the solvents, was collected by eluting the column with alcohol.

This fraction was also dried and stored as Fraction IV.

3.3. PRELIMINARY SCREENING OF *Mimosa invisa* FOR ITS TOXIC EFFECTS IN RABBITS

Before conducting the actual screening study, pilot studies were conducted to find out the approximate dose required to produce toxic effect

3.3.1. Pilot Study Using Fresh Juice of *Mimosa invisa*

Eight adult rabbits were divided into four groups of two animals each. Four dose levels of *Mimosa invisa* (15, 20, 25 and 30 g/kg) as fresh juice was administered to these rabbits. Levels of urea, creatinine, ALT and AST were taken as toxicity criteria.

3.3.2. Pilot Study Using Alcoholic Extract of *Mimosa invisa*

Four groups of rabbits each containing two animals were administered with four dose levels (0.5, 0.75,1.0 and 1.25 g/kg) of *Mimosa invisa* alcoholic extract. Levels of Urea, Creatinine, ALT and AST were taken as toxicity criteria.

3.3.3. Study with Toxic Dose of *Mimosa invisa* Fresh Juice and Alcoholic Extract

Eighteen adult rabbits were divided into three groups of six animals each. Schedule of the experiment is as shown below.

Group I	-	Control diet alone (without any extract)
Group II	-	Toxic dose of *Mimosa invisa* fresh juice
Group III	-	Toxic dose of *Mimosa invisa* alcoholic extract

Blood samples were collected before the experiment (day zero) and at day one, day three, day five, day ten, day fifteen and day twenty for biochemical evaluation. The haematological parameters were also observed on zero day, fifth day, tenth day, fifteenth day and twentieth day for haematological evaluation. Three millilitre blood was collected from median artery using 21 Gauge needle

for separation of serum and one millilitre blood with EDTA was collected for haematological evaluation. For serum collection clotted blood was kept in the refrigerator for half an hour and then centrifuged the blood at 3000 rpm for 10 minutes.

Serum was separated and biochemical parameters like total protein, albumin, urea, creatinine and enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma Glutanyl transferase (GGT), Creatine kinase were estimated. On twentieth day of the experiment all the animals were sacrificed and detailed post mortem was conducted. Liver, kidney and heart were collected for histopathological examination.

3.4. IDENTIFICATION OF TOXIC FRACTION IN RABBITS

Pilot studies were conducted to find out the toxicity of each fraction. Different dose levels of each fraction were administered in rabbits to assess the toxic dose. The fraction/fractions having toxic effect was selected for further study in rabbits. The design of the experiment was as follows.

Group IV	-	Control diet alone
Group V	-	Pooled toxic fraction (Fraction II + III)
Group VI	-	Half the pooled toxic fraction
Group VII	-	Double the pooled toxic fraction
Group VIII	-	Pooled toxic fraction followed by a decoction of a combination of *Hygrophila auriculata*, *Tribulus terrestris* and *Boerhavia diffusa* at a rate of 5g/kg body weight for 20 days.

Blood samples were collected before the experiment (zero day) and on first day, third day, fifth day, tenth day, fifteenth day and twentieth day of the experiment for the estimation of biochemical parameters and serum enzymes. Haematological parameters were examined zero day, fifth day, tenth day, fifteenth day and twentieth day. The animals were sacrificed on twentieth day of the experiment and conducted postmortem. Internal organs were examined for any gross lesions and liver, kidney and heart were taken and preserved in ten per cent formalin for histopathological examination.

3.5 PHYTOCHEMICAL SCREENING OF EXTRACT AND FRACTIONS FOR ACTIVE PRINCIPLES

The phytochemical screening was done as per the methods described by Harborne (1991).

3.5.1 Tests for Detection of Steroids

Salkowski test

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

Lieberman Burchardt test

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

3.5.2. Tests for Detection of Alkaloids

About 5 mg of extract was mixed with 5 ml of Ammonia and then extracted with equal volume of chloroform. Five millilitre dilute hydrochloric acid

was added to this. The acid layer obtained was used for chemical tests for the alkaloids.

Mayer's test

To one ml of extract, few drops of Mayers reagent (1.358 g of Mercuric chloride dissolved in 60 ml of water and poured into a solution of five gram of potassium iodide in 10 ml of water and then make up the volume to 100 ml with distilled water). Development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

Few drops of Wagners reagent (two gram of iodine and six gram of potassium iodide dissolved in 100 ml of water) were added to 1 ml of acid extract. Development of reddish brown precipitate indicates the presence of alkaloids.

Hager's test

Few drops of Hagers reagent (one gram of picric acid dissolved in 100 ml of water) were added to 1 ml of the acid extract. Development of yellow precipitate indicates the presence of alkaloids.

Dragendorff's test

Few drops of Dragendorffs reagent (stock solution (1) 0.6 grams of bismuth subnitrate was dissolved in 2 ml concentrated hydrochloric acid and 10 ml water was added. Stock solution (2) Six grams of potassium iodide was dissolved in 10 ml of water. Then both stock solutions were mixed together and then mixed with seven millilitre of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to make up the volume to 400 ml) was mixed with one millilitre of acid extract. Development of brown precipitate indicates the presence of alkaloids.

3.5.3. Test for Detection of Phenolic Compounds

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

3.5.4. Test for Detection of Tannins

Ferric chloride test

Two milligram of the extract was mixed with 3 ml of one per cent ferric chloride solution. Development of blue, green, or brownish color indicates the presence of tannins.

Gelatin test

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

3.5.5. Tests for Detection of Flavonoids

Ferric chloride test

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

Lead acetate test

Few drops of neutral ten per cent lead acetate was mixed with 2 ml of alcoholic solution of the extract. Development of yellow colour indicates the presence of flavonoids.

3.5.6. Tests for Detection of Glycosides

Sodium hydroxide test

Dissolved a small amount of extract (about 5 mg) in one millilitre of water and added five to six drops of sodium hydroxide (10 per cent). Development of a yellow colour indicates the presence of glycosides.

Benedicts test

To about 1ml of the extract (0.5 gram extract in 1 ml of water) 5 ml of Benedicts reagent was added. The mixture was boiled for 2 minutes. Development of brown to red colour indicates presence of glycosides.

3.5.7. Test for Detection of Diterpenes

Five milligram of the extract was dissolved in 3 ml of copper acetate solution (five per cent). Development of green colour indicates the presence of diterpenes.

3.5.8 Test for Detection of Triterpenes

Salkowski test

About five milligram of the extract was dissolved in 3 ml chloroform and then it was mixed with 3 ml of concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicates the presence of triterpenes.

Leiberman Burchardt test

To three millilitre of chloroform solution of extract added few drops of acetic acid and 1 ml of concentrated sulphuric acid. Development of a deep ring at the junction of two layers indicates the presence of triterpenes.

3.5.9. Tests for Detection of Saponins

Foam test

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

Haemolysis test

To two millilitre normal saline in test tubes added 2 ml distilled water to one and 2 ml one per cent extract to the other. Then five drops of blood was added to each test tube and centrifuged. The resulting haemolysis revealed the presence of saponin.

3.5.10. Test for Detection of Mimosine

Fresh Mimosa leaves were collected and dried under shade until no weight loss. Weighed accurately 1 g of leaflets and put it in a standard 100 ml flask and filled the flask with 100 ml 0.1 N HCl and macerated using a homogenizer. 10 ml of this macerate was placed in a boiling bath tube and added 15 ml of 0.1 N HCl with charcoal and boiled for 15 min. Filtered through No.2 whatman filter paper. Took 2 ml of this filtrate and added 5 ml Na_2EDTA solution (1g Na_2EDTA in 4 litres of water) and 1 ml of 60% ferric chloride. Allowed the mixture to remain in dark for 15 minutes. Mimosine standard and a positive sample of Mimosine (*Lucaenia leucocephala*) were taken for reference. Development of bluish violet colour was taken as positive test (James and Kaye, 1981).

3.5.11. Test for Detection of Cyanide

Finely chopped fresh plant materials (5 g) were added to100 ml boiling tube. Four to 12 drops of chloroform was added. To the stoppered lid, a picrate paper was anchored in such a way that this picrate paper was hanging away from

the bottom of the liquid. The test tube was kept on a water bath at 30 to 37° C for about three hours. The test is positive if yellow picrate paper turn to red (Bark, 1963).

3.5.12. Test for Nitrate

The plant material was tested with diphenylamine reagent (Diphenylamine stock solution was prepared by dissolving 0.5 g of diphenylamine in 20 ml of water and concentrated sulphuric acid was added to bring the volume to 100 ml. Equal part of this stock solution was mixed with 80 per cent sulphuric acid). The test was conducted by adding few drops of the reagent to the crushed plant material. A bright blue colour indicate presence of nitrate (Householder *et al.*, 1966).

3.6. SERUM BIOCHEMISTRY

3.6.1. Total Serum Protein (Albumin-Globulin Ratio)

Total serum protein was estimated by Biuret method (Henry *et al.*, 1957) was carried out in GENESYS-IO- UV-Thermospectronic. Albumin was estimated by Doumas method (Doumas *et al.*, 1971) using Ecoline kit (M/s E. Merck India Limited, Mumbai).

3.6.2 Creatinine, Urea

Serum creatinine and urea estimation was carried out in semiautomatic blood analyser ('Microlab 200') using Ecoline® kits manufactured by E. Merck (India) Ltd.

3.6.3. Serum Enzymes

Serum enzymes like ALT, AST, r-glutamyl transferase, creatine kinase and alkaline phosphatase were estimated using Ecoline kits manufactured by E. Merck (India) Limited in semi automatic blood analyser ('Microlab 200') (Alan,1988).

3.7. HAEMATOLOGICAL PARAMETERS

Haematological parameters were studied using blood with EDTA as an anticoagulant.

3.7.1. Packed Cell Volume (PCV)

Packed cell volume was estimated by filling the wintrobe haematocrit tube with a uniform column of blood devoid of air bubbles. The tubes were centrifuged at 6000 rpm for 15 minutes (Benjamin, 1985).

3.7.2. Haemoglobin Concentration

The haemoglobin concentration was measured by acid haematin method (Benjamin, 1985).

3.7.3. Total Leucocyte Count

The leucocytes were counted by standard dilution technique using Thomas Fluid as diluent. Counting was done using the haemocytometer placed under low power of the microscope (Benjamin, 1985).

3.7.4. Total Erythrocyte Count

The erythrocytes were counted by diluting the blood with haem's fluid. It was done by counting keeping the haemocytometer under high power of the microscope (Benjamin, 1985).

3.7.5. Differential Leucocyte Count

Blood smears were prepared from freshly drawn blood by slide method. Stained the smears with Wright's stain and counted the leucocytes under the oil immersion objective of the microscope (Benjamin, 1985).

3.8. POST MORTEM EXAMINATION

On twentieth day of the experiment all the animals were sacrificed and detailed postmortem examination was conducted. Liver, kidney and heart were observed for any gross lesions.

3.9. HISTOPATHOLOGICAL EXAMINATION

The histopathological examination of liver, kidney and heart were done to assess the extend of damage caused by *Mimosa invisa*. The tissues were collected and fixed in 10 per cent formalin and processed through routine paraffin embedding process, stained with haematoxylin and eosin and studied the histopathology (Sheehan and Hrapchak, 1980).

3.10. STATISTICAL ANALYSIS

The data were analysed statistically by independent t-test and paired t-test (Snedcor and Cochran, 1985)

Results

4. RESULTS

4.1. IDENTIFICATION, COLLECTION OF PLANT MATERIAL AND EXTRACTION

The plant was taxonomically identified (Fig.1) and collected from Veterinary College Campus for the preparation of the extract. The fresh juice was prepared by macerating plant material and mixing with water, cold alcoholic extract was prepared and yield was approximately 5 per cent. The alcoholic extract was further subjected to fractionation and the percentage of various fractions was chloroform fraction 9 per cent, butanol fraction 40 per cent, aqueous fraction 50 per cent and insoluble residue less than one per cent.

4.2 PRELIMINARY SCREENING OF *Mimosa invisa* FOR ITS TOXIC EFFECTS IN RABBITS

4.2.1. Pilot Study Using Fresh Juice of *Mimosa invisa*

Out of the four different doses tried (15, 20, 25 and 30 g/kg), 25 g/kg per body weight was found to be toxic, but not lethal to rabbits. Serum biochemical parameters like urea, creatinine, ALT, AST were significantly increased at this dose rate, which indicate it as toxic dose.

4.2.2. Pilot Study Using Alcoholic Extract of *Mimosa invisa*

Trial experiments using dose levels of 0.5, 0.75, 1.0, 1.25 g/kg of alcoholic extract showed that 1.0 g/kg body weight was toxic to rabbits. The toxicity was indicated by elevated values of urea, creatinine, ALT and AST. But this dose did not produce any lethality.

4.3. EFFECT OF TOXIC DOSE OF *Mimosa invisa* FRESH JUICE

Toxic effect of fresh juice of *Mimosa invisa* was studied by administering the juice obtained from 25 g/kg of fresh Mimosa plant. The toxicity was assessed by studying the following parameters.

4.3.1. Clinical Observations

Clinical symptoms observed were off-feed, animal looked dull, lethargic, mostly in sternal recumbent posture.

4.3.2. Biochemical Parameters

4.3.2.1. Serum Alanine Aminotransferase (ALT) Levels

Serum ALT levels on 0, 1, 3, 5, 10, 15 and 20^{th} day were 40.67 ± 4.45, 212.5 ± 33.26, 165.67 ± 15.63, 143.5 ± 15.63, 116.33 ± 9.93, 55.17 ± 5.66 and 50.33 ± 1.74 u/l respectively. The results are presented in Table 1 and Fig. 2. A significant increase ($P<0.01$) was observed from day one onwards and it was showing a diminishing trend from 15^{th} day onwards. Significant increase ($P<0.01$) was observed when compared with control group also (Table 1a, Fig.9).

4.3.2.2. Serum Aspartate Aminotransferase (AST) Levels

Serum AST levels showed significant increase ($P<0.05$) on third day and fifth day and the values on other days were not significantly different when compared to the values before administration of *Mimosa invisa* and also with control. The serum AST levels at 0, 1, 3, 5, 10, 15 and 20^{th} day were 43.5 ± 4.18, 91.67 ± 18.74, 141.15 ± 14.11, 111.83 ± 8.28, 56.33 ± 10.93, 50.5 ± 7.28 and 46.67 ± 4.65 u/l respectively (Table 2 and Fig.3). The control values are shown in Table 2a and Fig.10.

4.3.2.3. Serum Gamma Glutamyl Transferase (GGT) Levels

A significant rise ($P<0.01$) in serum GGT levels were observed on day 1, 3, 5 and 10 after administration of *Mimosa invisa* fresh juice when compared to day zero values and control values. The GGT levels observed were 4.67 ± 0.33, 8.5 ± 0.43, 10.67 ± 0.49, 8 ± 0.58, 7 ± 0.37, 5.17 ± 0.33 and 4.5 ± 0.22 u/l on 0, 1, 3, 5, 10, 15 and 20th day respectively. The results are presented in Table 3 and Fig.4. The control values are shown in Table 3a and Fig.11.

4.3.2.4. Serum Creatine Kinase Levels

The creatine kinase levels observed were 148.0 ± 16.69, 173.33 ± 20.43, 183.5 ± 23.92, 143.33 ± 22.95, 152.17 ± 19.43, 147.83 ± 20.12 and 151.33 ± 17.75 u/l on 0, 1, 3, 5, 10, 15 and 20th day respectively (Table 4 and Fig. 5). There were no significant differences in creatine kinase levels when compared to zero day value and control values even though slight increase was noticed on 1st and 3rd day. The control values are depicted in Table 4a and Fig.12.

4.3.2.5. Serum Alkaline Phosphatase (ALP) Levels

Serum ALP level on day zero was 74 ± 5.87 u/l and on 1, 3, 5, 10, 15 and 20th day were 126 ± 12.25, 136.83 ± 12.88, 119.33 ± 7.97, 84.5 ± 3.35, 65.5 ± 3.97 and 57 ± 2.97 u/l respectively. There was significant increase ($P<0.01$) in ALP levels on day 1, 3 and 5 after Mimosa administration when compared with zero day value. A significant increase ($P<0.01$) was also observed on day 1, 3, 5 and 10 when compared to control. The results are presented in Table 5, Fig. 6 and in Table 5a, Fig.13.

4.3.2.6. Serum Creatinine Levels

The serum creatinine levels significantly increased ($P<0.01$) when compared to zero day values and control values. The levels noted were 2.33 ± 0.21, 8.33 ± 0.49, 10.83 ± 0.70, 9.17 ± 0.75, 6 ± 0.21, 3.33 ± 0.21 and 2.5 ± 0.22 mg/dl on days 0, 1, 3, 5, 10, 15 and 20 respectively. The values tend to decrease

from 10th day onwards. The results are presented Table 6, Fig.7 and in Table 6a, Fig.14.

4.3.2.7. Serum Urea Levels

Serum urea levels on 0, 1, 3, 5, 10, 15 and 20th day were 40.83 ± 0.87, 106.5 ± 6.06, 113.33 ± 4.98, 96.5 ± 4.41, 90.67 ± 3.92, 76.83 ± 4.88 and 49.0 ± 1.0 mg/dl respectively (Table 7, Fig.8). These observations showed that there was significant increase ($P<0.01$) in serum urea levels after mimosa administration. However there was a decline in the levels of urea from 5th day onwards. The increase in level was significant when compared to the control (Table 7a and Fig.15).

4.3.2.8. Serum Total Protein Levels

Total protein levels did not show significant difference when compared to control and zero day values. The observed values for albumin were 5.81 ± 0.15, 6.07 ± 0.08, 6.02 ± 0.09, 5.96 ± 0.10, 5.97 ± 0.11, 5.85 ± 0.18 and 5.93 ± 0.06 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. The values are presented in Tables 8 and 8a.

4.3.2.9. Serum Albumin Levels

There were no significant differences in albumin levels when compared to zero day and control values. The values of total protein observed were 3.58 ± 0.10, 3.62 ± 0.12, 3.48 ± 0.14, 3.75 ± 0.04, 3.33 ± 0.06, 3.12±0.08 and 3.12 ± 0.03 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Tables 9 and 9a).

4.3.2.10. Serum Globulin Levels

The serum globulin levels observed were 1.74 ± 0.14, 1.65 ± 0.15, 1.68 ± 0.15, 1.45 ± 0.01, 1.31 ± 0.01, 1.25 ± 0.10 and 1.20 ± 0.13 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. There were no significant differences in values

observed when compared with zero day values and control values (Tables 10 and 10a).

4.3.2.11. Albumin-Globulin Ratio

Albumin-Globulin ratio on day 0, 1, 3, 5,10, 15 and 20 were 2.18 ± 0.13, 2.28 ± 0.20, 2.15 ± 0.20, 2.28 ± 0.08, 2.57 ± 0.06, 2.67 ± 0.20 and 2.67 ± 0.20 respectively. There were no significant variations observed (Tables 11and 11a).

4.3.3. Haemogram

4.3.3.1 Volume of Packed Red Blood Cells (VPRC)

Volume of Packed Red Blood Cells on 0, 1^{st}, 3^{rd}, 5^{th}, 10^{th}, 15^{th} and 20^{th} day were 44.17 ± 1.49, 39.17 ± 1.42, 40.33 ± 1.33, 41.00 ± 1.12 and 39.5 ± 0.89 % respectively (Table12). All the values after administration of *Mimosa invisa* showed significant decrease ($P<0.01$) with maximum decrease on fifth day. There was significant decrease on comparison with control values on day 5, 10 and 15 (Table 12a).

4.3.3.2. Haemoglobin (Hb) Concentration

Haemoglobin concentration before administration of *Mimosa invisa* was 13.17 ± 0.48 g/dl whereas the values after administration on 5^{th}, 10^{th}, 15^{th} and 20^{th} day were 12.5 ± 0.45, 12.0 ± 0.53, 12.67 ± 0.33 and 12.08 ± 0.35 g/dl respectively. The values were not statistically significant. On comparison with control values, it showed a significant decrease ($P<0.05$) on 10^{th} day. The results are presented in Tables 13 and 13a.

4.3.3.3. Red Blood Cell (RBC) Counts

There was significant decrease ($P<0.05$) in RBC count on 10^{th}, 15^{th} and 20^{th} day after *Mimosa invisa* fresh juice administration. But there were no significant difference when compared to control group. The mean RBC count

(millions/mm^3) on day 0, 1, 3, 5, 10, 15 and day 20 were 9.71 ± 0.29, 9.22 ± 0.21, 8.92 ± 0.21, 8.45 ± 0.22 and 8.25 ± 0.23 respectively (Tables 14 and 14a).

4.3.3.4. Erythrocyte Indices

The calculated values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) fall within normal limits when the values before and after administration of *Mimosa invisa* were compared. On comparison with control values also there were no significant difference.

The values noted for MCV were 45.61 ± 1.71, 42.53 ± 1.65, 45.23 ± 1.53, 48.57 ± 1.13 and 47.88 ± 4.6 μm^3 on day 0, 5, 10, 15 and 20.

The MCH values were 13.65 ± 0.65, 13.58 ± 0.57, 13.45 ± 0.42, 15.0 ± 0.4 and 14.68 ± 0.41 µg on day 0, 5, 10, 15 and 20 respectively.

The values of MCHC observed on day 0, 5, 10, 15 and 20 were 30.33 ± 1.63, 32.45 ± 1.84, 29.98 ± 1.33, 30.95 ± 0.71 and 30.62 ± 0.89 g% respectively. The results are presented in Tables 15, 15a, 16, 16a, 17 and 17a.

4.3.3.5. Total Leucocyte Count (TLC)

Total leucocyte count (numbers/mm^3) observed were 5133.33 ± 474.28, 7825 ± 603.98, 6133.33 ± 475.51, 6050 ± 759.39 and 5650 ± 437.22 on day 0, 5, 10, 15 and day 20 (Table18). A significant increase ($P<0.05$) in TLC was noted on 5^{th} and 10^{th} day after mimosa administration. The control values are shown in Table 18a.

4.3.3.6. Differential Leucocyte Count (DLC)

No significant differences in DLC were noted after administration of fresh juice of *Mimosa invisa* in rabbits.

The lymphocyte count observed were 56.5 ± 0.92, 56.33 ± 0.88, 56.67 ± 1.20, 56.83 ± 0.70 and 56.33 ± 0.56 % on day 0, 5, 10, 15 and day 20 respectively.

The observed values for neutrophil count were 42.5 ± 0.80, 43.17 ± 1.01, 42.67 ± 1.02, 42.5 ± 0.76 and 43 ± 0.45 % on 0^{th}, 5^{th}, 10^{th}, 15^{th} and 20^{th} day respectively.

The values noted for eosinophil count were 1.0 ± 0.26, 0.5 ± 0.22, 0.67 ± 0.21, 0.67 ± 0.33 and 0.67 ± 0.21 % on day 0, 5, 10, 15 and day 20 respectively. The results are presented in Tables 19, 19a, 20, 20a, 21 and 21a.

4.3.4. Post Mortem Examination

On 20^{th} day of the experiment, the animals were sacrificed and detailed post mortem examination was conducted. No gross lesions could be noticed. But the animals died due to administration of higher dose (30 g/kg) showed diffuse greyish white areas of necrosis in liver and kidney and lung haemorrhages. Tracheal smears were taken and examined for pasturella and was found to be negative. There were no gross lesions in heart. All other organs remained apparently normal.

4.3.5 Histopathological Examination

The histological section of liver, kidney and heart are shown in figure16, 17 and 18 respectively. There were no histopathological lesions in organs collected from animals sacrificed on 20^{th} day of the experiment. But the animals died after administration of higher dose (30g/kg body weight) of *Mimosa invisa* showed the following lesions: Microscopical examination of the sections of kidney revealed diffuse congestion and dilatation of tubules, tubular degeneration, necrosis, hyalinization of the cortical tubules and occasional necrosis of glomeruli. (Fig.19). Majority of the medullary cords remained intact. Liver showed extensive fatty change and necrosis (Fig.20). Diffuse degeneration

Fig. 16 - Kidney - Control H & E x 100

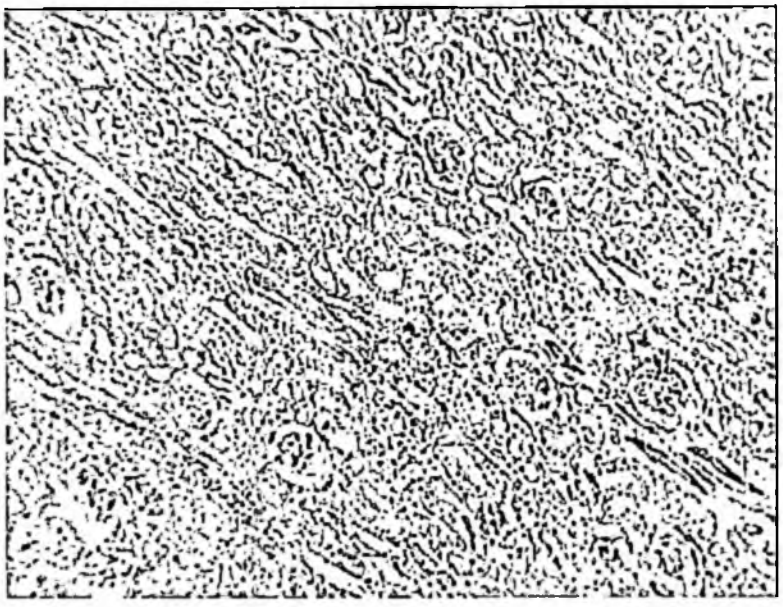


Fig. 17 - Liver - Control H & E x 400

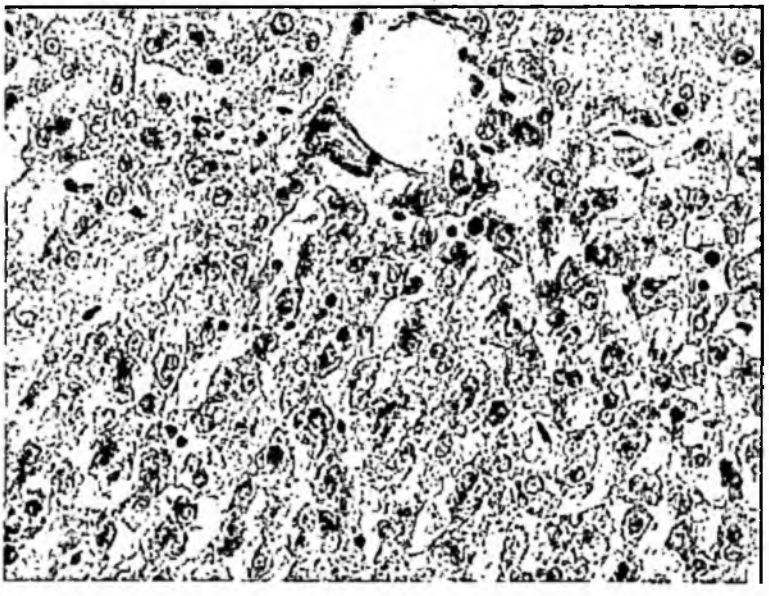
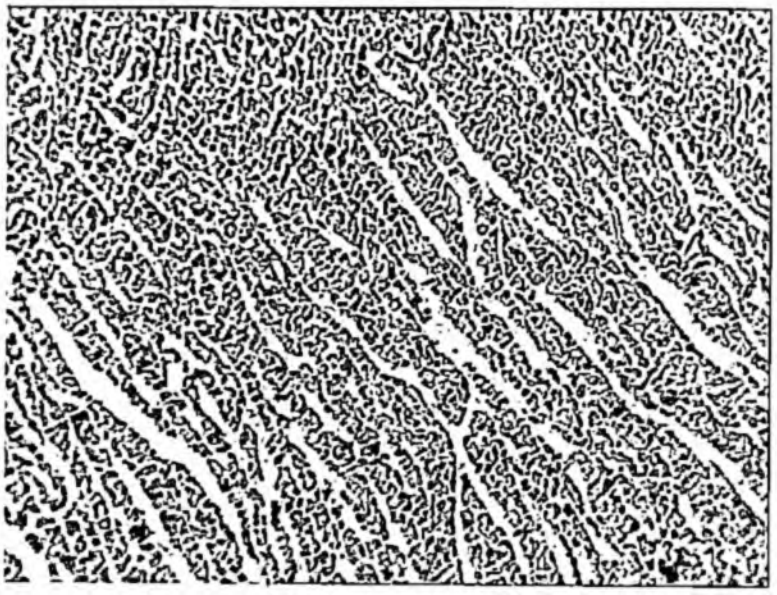


Fig. 18 - Heart - Control H & E x 100



of muscle fibres, widening of the intermuscular space and intermuscular haemorrhages were the changes observed in heart (Fig.21).

4.4. EFFECT OF TOXIC DOSE OF ALCOHOLIC EXTRACT OF *Mimosa invisa*

The toxic dose (1 g/kg) derived from the pilot study was used to study toxicity in rabbits.

4.4.1. Clinical Observations

The main clinical symptoms were inappetence, dullness, lethargy and sternal recumbent posture.

4.4.2. Biochemical Parameters

The biochemical parameters assessed were ALT, AST, GGT, creatine kinase CK, ALP, urea, creatinine, total serum protein, albumin, globulin and albumin-globulin ratio.

4.4.2.1. Serum ALT Levels

Serum ALT levels were 38.33 ± 2.63, 157.5 ± 19.7, 172.33 ± 13.32, 185.33 ± 10.22, 130.33 ± 7.92, 66.17 ± 7.87 and 43.5 ± 7.26 u/l on day 0, 1, 3, 5, 10, 15 and 20 respectively. A significant increase ($P<0.01$) in serum ALT levels observed after administration of alcoholic extract of *Mimosa invisa* (Table 1, Fig.2). On comparison with control values also it showed a significant increase (Table 1a, Fig.9). But the values decreased from 10^{th} day onwards.

4.4.2.2. Serum AST Levels

There were significant increase in serum AST levels on comparison with zero day values and control values. The values observed were 32.67 ± 2.99, 98.5 ± 14.67, 101.00 ± 4.65, 123.67 ± 7.97, 81.67 ± 6.10, 48.17 ± 5.7 and 35.83 ± 4.13 u/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively. Here also the values

appeared decreasing from 10th day. The results are shown in Tables 2, 2a and Fig. 3, 10.

4.4.2.3. Serum GGT Levels

Serum GGT levels after administration of alcoholic extract of *Mimosa invisa* were 5.83 ± 0.48, 8.83 ± 0.70, 9.67 ± 0.88, 10.83 ± 0.70, 8.17 ± 0.7, 6 ± 0.37 and 5.83 ± 0.31 u/l on day 0, 1, 3, 5, 10, 15 and 20 respectively. From 15th day onwards the values are showing a tendency to regain normal values. The results are tabulated in Table 3.3a and Fig. 4.11.

4.4.2.4. Creatine Kinase Levels

The creatine kinase levels observed were 181.17 ± 15.28, 201.33 ± 6.59, 219.67 ± 3.65, 213.83 ± 8.64, 189.83 ± 7.68, 165.33 ± 11.10 and 155.33 ± 11.61 u/l on day 0, 1, 3, 5, 10, 15, 20 respectively. A significant increase ($P<0.05$) was observed on 3rd day after Mimosa, thereafter values showed a decreasing tendency. Similar results are observed on comparison with the control. The results are shown in Table 4, 4a and Fig. 5, 12.

4.4.2.5. Serum ALP Levels

The serum ALP level before the administration of extract was 59 ± 7.90 u/l whereas the values on day 1, 3, 5, 10, 15 and 20 were 72 ± 6.4, 76.33 ± 7.82, 66.5 ± 6.35, 56.33 ± 5.21, 54.83 ± 3.13 and 57.57 ± 4.94 u/l respectively. The ALP level on 3rd day showed a significant ($P<0.05$) increase, but the values on other days showed moderate increase even though the increase was not statistically significant. There were no significant differences when the values were compared with control values. The results are presented in Tables 5, 5a and Fig. 6, 13.

4.4.2.6. Serum Creatinine Levels

The observed values of serum creatinine were 2.17 ± 0.17, 9.17 ± 0.48, 9.83 ± 0.6, 7.33 ± 0.88, 5.83 ± 0.47, 3.67 ± 0.33, 2.83 ± 0.31 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. There was significant increase ($P<0.01$) in creatinine values on 1st, 3rd and 5th days. Thereafter the creatinine values showed a tendency to decrease. A similar increase followed by decrease was observed when the values were compared with control values. The results are shown in Table 6, 6a and Fig. 7, 14.

4.4.2.7. Serum Urea Levels

There were significant increase ($P<0.05$) in serum urea level from 1st day to 5th day, thereafter it showed a tendency to decrease and attained normal value by 20th day. On comparison with control, significant increase ($P<0.01$) was noticed up to 15th day and afterwards the values became normal. The observed values were 45.67 ± 5.27, 82.17 ± 3.85, 105.17 ± 2.97, 86.67 ± 4.16, 73 ± 2.47, 67.5 ± 2.96 and 51.17 ± 2.7 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Tables 7, 7a and Fig.8, 15).

4.4.2.8. Serum Total Protein

There were no significant differences in total protein values when compared to zero day value and control values. Total protein values noted were 5.08 ± 0.17, 5.12 ± 0.11, 5 ± 0.07, 5.12 ± 0.14, 5 ± 0.08, 4.95 ± 0.09 and 5.05 ± 0.11 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Table 8, 8a).

4.4.2.9. Serum Albumin Levels

The values noted were 3.85 ± 0.04, 3.8 ± 0.08, 3.6 ± 0.13, 3.77 ± 0.08, 3.73 ± 0.09, 3.7 ± 0.11 and 3.65 ± 0.09 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Table 9). There were no significant differences in values when compared with control values and zero day values (Table 9a).

4.4.2.10. Serum Globulin Levels

Serum globulin level also did not show any significant difference when compared with control values and zero day values.

The values of serum globulin observed were 1.23 ± 0.19, 1.32 ± 0.17, 1.5 ± 0.19, 1.27 ± 0.14, 1.28 ± 0.13, 1.25 ± 0.17 and 1.4 ± 0.14 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 10, 10a).

4.4.2.11. Albumin-Globulin Ratio

The albumin globulin ratio obtained in the present study were 3.5 ± 0.49, 3.11 ± 0.36, 2.67 ± 0.41, 3.17 ± 0.41, 3.17 ±0.41, 3.33 ± 0.55 and 2.75 ± 0.27 on day 0, 1, 3, 5, 10, 15 and day 20 respectively. There were no significant differences in values observed when compared with zero day values and control. The results are tabulated in Table 11and 11a.

4.4.3. Haemogram

4.4.3.1. Volume of Packed Red Cells (VPRC)

The volume of Packed Red Cells on day 0, 5, 10, 15 and day 20 were 46 ± 1.39, 41.33 ± 1.17, 39.67 ± 0.80, 38.67 ± 0.76 and 37.33 ± 0.80 % respectively. There was significant decrease ($P<0.05$) in VPRC levels when compared with zero day values and control values. The results are presented in Table 12 and 12a.

4.4.3.2. Haemoglobin Concentration

Haemoglobin concentration did not show significant variations. The values obtained were 13.65 ± 0.58, 13.5 ± 0.79, 13.87 ± 0.68, 14.85 ± 0.69 and 14.8 ± 0.19 g/dl on day 0, 5, 10, 15 and day 20 respectively (Tables 13 and 13a).

4.4.3.3. Red Blood Cell (RBC) Count

There were no significant variations in red blood cell count observed when compared with zero day values and control values. The values noted were 7.35 ± 1.06, 7.64 ± 1.02, 7.87 ± 0.99, 7.45 ± 0.77 and 7.19 ± 0.74 millions/mm^3 on day 0, 5, 10, 15 and day 20 respectively (Tables 14 and 14a).

4.4.3.4. Erythrocyte Indices

The erythrocyte indices did not show much variation when compared to zero day value and control values.

The observed values of MCV were 51.52 ± 1.70, 49.1 ± 1.96, 49.77 ± 2.42, 51 ± 1.55 and 51.08 ± 2.02 (μm^3) on day 0, 5, 10, 15 and day 20 respectively.

The values obtained for MCH were 12.25 ± 0.44, 11.33 ± 0.36, 11.5 ± 0.37, 11.25 ± 0.38 and 10.83 ± 0.31 µg for 0, 5, 10, 15 and day 20 respectively.

The MCHC values obtained were 26.68 ± 0.66, 27.53 ± 1.20, 29 ± 0.21, 29.12 ± 0.84 and 29.05 ± 0.62 g% on day 0, 5, 10, 15 and day 20 (Tables 15, 15a, 16, 16a, 17 and 17a)

4.4.3.5. Total Leucocyte Count (TLC)

Total leucocyte count (number/mm^3) showed a mild increase on comparison with control values and zero day values even though the increase was not statistically significant. The values obtained were 5941.66 ± 575.53, 6675 ± 452.72, 7108.33 ± 420.20, 7358.33 ± 357.64 and 7650 ± 358 on day 0, 5, 10, 15 and day 20 (Tables 18 and 18a).

4.4.3.6 Differential Leucocyte Count (DLC)

The values of Lymphocyte and neutrophil count show significant difference ($P<0.05$) when compared to zero day value. Lymphocytosis with neutropenia was observed. Eosinophil count did not show any variation.

The values of Lymphocyte count noted were 54.67 ± 1.4, 68.83 ± 0.87, 69 ± 0.63, 66.00 ± 0.93 and 65 ± 1.29 on 0^{th}, 5^{th}, 10^{th}, 15^{th} and 20^{th} day respectively.

The neutrophil count was 44.67 ± 1.52, 31.67 ± 1.45, 30.67 ± 0.61, 33.33 ± 1.11 and 34.65 ± 1.56 on day 0, 5, 10, 15 and day 20 respectively.

The eosinophil count was 0.83 ± 0.30, 0.5 ± 0.22, 0.33 ± 0.21, 0.67 ± 0.21 and 0.5 ± 0.30 on day 0, 5, 10, 15 and day 20 respectively. The results are shown in Tables 19, 10a, 20, 20a, 21 and 21a.

4.4.4. Post Mortem Examination

On 20^{th} day of administration of alcoholic extract of *Mimosa invisa*, all the animals in the group were sacrificed and conducted detailed post mortem examination. No gross lesions were observed. But the animals died due to higher dose (1.25 g/kg) showed diffuse necrotic patches in kidney. Liver showed congestion and greyish white patches of necrosis. There were no gross lesions on heart and other organs.

4.4.5. Histopathological Examination

The animals sacrificed on 20^{th} day of the experiment did not show any lesion. But the animal died after administration of higher dose (1.25g/kg body weight) during trial experiments showed pathological changes in liver, kidney and heart. The changes observed in kidney were tubular epithelial necrosis and extensive hyalinisation. Coagulation necrosis of the tubular and glomerular components was seen in focal areas. Extensive glomerular damage with mesangial cell necrosis and presence of granular eosinophilic deposits in the

bowman's space were the other lesions observed (Fig.22). Central venous congestion, paracentral and centrilobular necrosis, extensive fatty change and bile duct proliferation were the changes observed in the liver (Fig.23). Heart showed focal degeneration of muscle fibres, widening of intermuscular space, venous congestion and diffuse haemorrhage (Fig.24).

4.5. EFFECT OF TOXIC DOSE OF POOLED TOXIC FRACTION OF *Mimosa invisa* ALCOHOLIC EXTRACT

From the trial experiments it was found that the toxic dose of the pooled fraction was 0.4 g/kg. This dose was administered in six rabbits to assess the toxicity. The following parameters were studied to assess the toxicity.

4.5.1 Clinical Observations

All the animals were showing inappetence, dullness, lethargy and loss of body condition from the second day of administration of pooled toxic fraction of mimosa.

4.5.2. Biochemical Parameters

4.5.2.1. Serum Alanine Aminotransferase (ALT) Levels

The results are presented in Table 1, 1b and Fig 2, 1b. The serum ALT levels obtained were 55.5 ± 5.69, 85.5 ± 8.05, 126.5 ± 10.55, 136 ± 13.6, 150.17 ± 12.81, 162.67 ± 8.09 and 170.5 ± 5.54 u/l on day zero, 1, 3, 5, 10, 15 and day 20 respectively. A very significant ($P<0.01$) increase in serum ALT was observed after administration of *Mimosa invisa* pooled toxic fraction. On comparison with control also these values showed significant increase (Table 1b and Fig.25).

4.5.2.2. Serum Aspartate Aminotransferase (AST) Levels

A significant ($P<0.01$) increase in the serum AST levels was observed after administration of pooled toxic fraction of the alcoholic extract of *Mimosa*

Fig. 22 - Kidney - Alcoholic extract - hyalinisation, glomerular and tubular necrosis H & E x 100

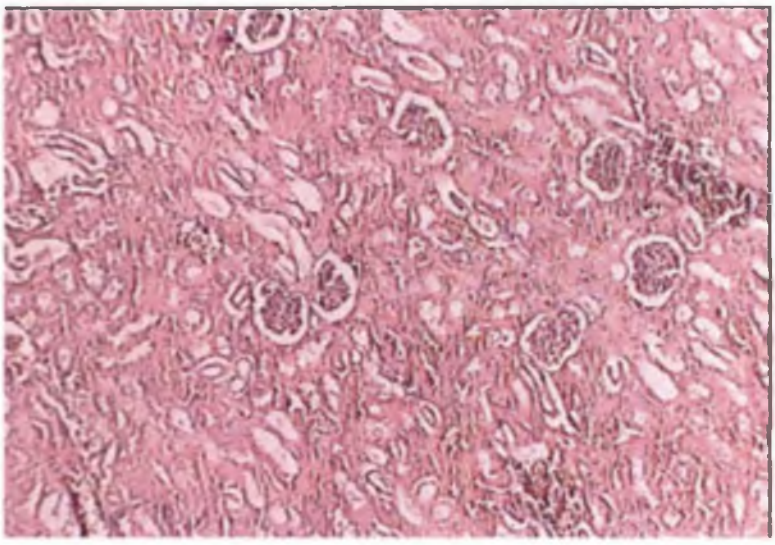


Fig. 23 - Liver - Alcoholic extract - fatty change and hepatocyte necrosis H & E x 400

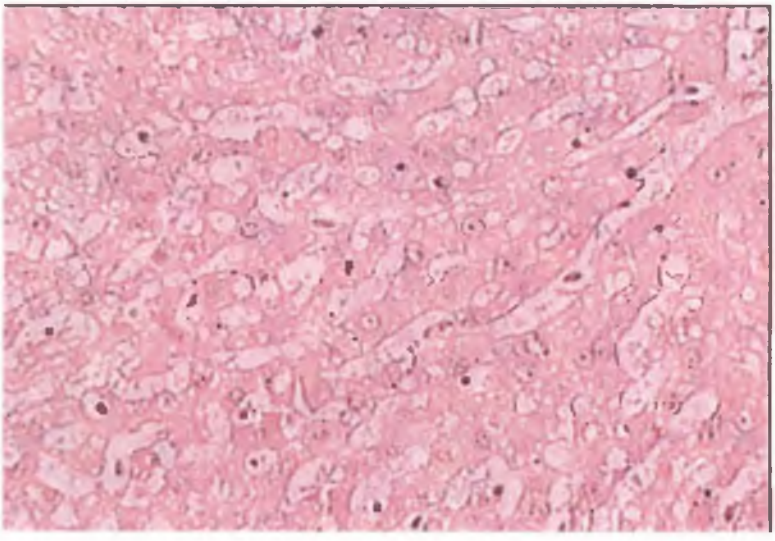
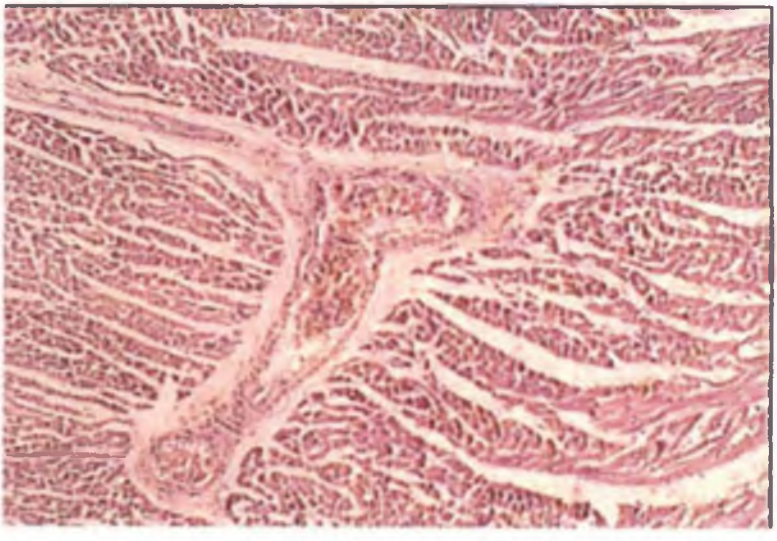


Fig. 24 - Heart -Congestion and focal degeneration of muscle fibres H & E x 100



invisa. The values observed were 51 ± 3.49, 110 ± 8.14, 154.17 ± 15.08, 167.67 ± 17.45, 188.5 ± 11.65, 174.5±5.37 and 186.33 ± 5.78 u/l on day zero, 1, 3, 5, 10, 15 and day 20 respectively (Table 2 and Fig 3). On comparison with control also significant increase could be detected (Table 2b and Fig. 26).

4.5.2.3. Serum Gamma Glutamyl Transferase (GGT) Levels

The values obtained for serum GGT were 5.5 ± 0.56, 6.67 ± 0.42, 8.17 ± 0.60, 9.83 ± 0.33, 10.67 ± 0.33, 11.5 ± 0.22 and 11 ± 0.2 u/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively. A significant increase ($P<0.01$) was noted from 3rd onwards. Significant increase in GGT could be observed on comparison with control values also. The results are shown in Table 3, 3b and Fig. 4, 27.

4.5.2.4. Serum Creatine Kinase Levels

The serum creatine kinase levels noted were 133.83 ± 10.11, 170.67 ± 15.60, 199.33 ± 13.76, 215.33 ± 15.81, 170.33 ± 6.98, 161.5 ± 3.28 and 140.5 ± 3.43 u/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 4 Fig.5). Significant increase was observed from day one to day ten, when compared to zero day value, while comparison with control, the significant difference was up to 15th day (Table 4b and Fig.28).

4.5.2.5. Serum Alkaline Phosphatase Levels (ALP)

There was significant difference ($P<0.05$) in serum ALP levels from 3rd to 15th day after *Mimosa invisa*, pooled toxic fraction, whereas there were no changes when compared to control. The values were 42.83 ± 10.64, 46.5 ± 11.05, 62.17 ± 12.25, 79.67 ± 14.58, 63.67 ± 10.63, 64.5 ± 9.59 and 53.67 ± 8.19 u/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Tables 5, 5b and Fig. 6, 29).

4.5.2.6. Serum Creatinine Values

The creatinine values showed a very significant increase ($P<0.01$) when compared to zero day values and control values. The values noted were 2.17 ±

0.31, 4.67 ± 0.33, 7.17 ± 0.40, 8.33 ± 0.49, 9.83 ± 0.31, 10.83 ± 0.4 and 12.17 ± 0.31 mg/dl on day 0, 1, 3, 5, 15 and day 20 respectively (Table 6, 6b and Fig.7, 30).

4.5.2.7. Serum Urea Levels

The serum urea levels showed a significant increase ($P<0.01$) from 3rd day onwards. The values observed were 59.17 ± 3.7, 64.33 ± 3.90, 142.83 ± 26.20, 157.33 ± 23.92, 175.67 ± 12.07, 195.83 ± 8.99 and 217 ± 10.96 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 7 and Fig 8). The control values are shown in Table 7b and Fig.31.

4.5.2.8. Serum Total Protein Levels

The serum total protein values did not show significant variation when compared to control values and zero day values. The values obtained were 5.6 ± 0.15, 5.37 ± 0.15, 5.95 ± 0.11, 5.67 ± 0.29, 5.45 ± 0.21, 5.37 ± 0.22 and 5.4 ± 0.16 g/dl respectively on day 0, 1, 3, 5, 10, 15 and day 20 (Table 8 and 8b).

4.5.2.9 Serum Albumin Levels

The results are presented in Tables 9 and 9b.

The values of serum albumin were 3.58 ± 0.14, 3.47 ± 0.14, 3.48 ± 0.17, 3.5 ± 0.01, 3.47 ± 0.01, 3.73 ± 0.11 and 3.67 ± 0.12 g/dl on day 0, 1, 3, 5, 10, 15 and day 20. No significant variations were observed on comparison with zero day values and control values.

4.5.2.10 Serum Globulin Levels

The serum globulin levels were calculated from serum total protein and serum albumin values. The calculated values were1.97 ± 0.18, 1.93 ± 0.22, 1.57 ± 0.17, 1.75 ± 0.23, 1.87 ± 0.19, 1.64 ± 0.22 and 1.73 ± 0.20 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 10). These values did not show any

significant variation on comparison with zero day values and control values (Table 10b).

4.5.2.11. Albumin-Globulin Ratio

The calculated values for albumin-globulin in the present study were 1.92 ± 1.9, 1.90 ± 0.5, 2.37 ± 0.19, 2.18 ± 0.29, 1.95 ± 0.21, 1.70 ± 0.15 and 1.92 ± 0.15 respectively on day 0, 1, 3, 5, 10, 15 and day 20. No significant variations could be observed in albumin-globulin ratio. The results are presented in Table 11 and 11b.

4.5.3. Haemogram

4.5.3.1. Volume of Packed Red Cells (VPRC)

The volume of packed red cells on 0, 5, 10, 15 and day 20 were 51.17 ± 1.14, 46.5 ± 1.23, 43.83 ± 1.54, 43 ± 1.5 and 41.83 ± 2.07 % respectively (Table 12). Significant reduction ($P<0.01$) in VPRC was noticed, after administration of pooled toxic fraction of *Mimosa invisa*. On comparison with control values also similar reduction was observed (Table 12b).

4.5.3.2. Haemoglobin Concentration

The haemoglobin concentration showed a significant decrease ($P<0.01$) after administration of pooled toxic fraction of *Mimosa invisa*. On comparison with control values, the significant decrease was noticed on 15^{th} and 20^{th} day only. The values of haemoglobin were 15.08 ± 0.32, 14.25 ± 0.28, 13.42 ± 0.15, 12.58 ± 0.20 and 11.82 ± 0.15 g/dl on day 0, 5, 10, 15 and day 20 respectively (Table 13 and 13b).

4.5.3.3. Red Blood Cells (RBC) Count

The RBC count showed a significant decrease ($P<0.01$) on 10^{th} day whereas on 15^{th} day the decrease in RBC count at 5 per cent level ($P<0.05$) was observed. The values observed were 10.99 ± 0.39, 10.83 ± 0.28, 9.5 ± 0.19, 9.63

zero day and 67.83 ± 1.49, 68.83 ± 1.14, 68.0 ± 1.03 and 65.5 ± 1.31 % on 5th, 10th, 15th and 20th day respectively.

The neutrophil count was 42.33 ± 1.15, 31.5 ± 1.31, 30.5 ± 1.17, 31.33 ± 0.95 and 33.83 ± 1.19 % on day 0, 5, 10, 15 and 20 respectively.

The eosinophil count was 1.00 ± 0.26, 0.67 ± 0.33, 0.67 ± 0.21, 0.67 ± 0.21 and 0.67 ± 0.26 % on day 0, 5, 10, 15 and day 20 respectively.

4.5.4. Post Mortem Examination

All the animals in this group were sacrificed on the 20th day of experiment and detailed post mortem examination was conducted. Kidney showed focal necrosis and petechial haemorrhages. Liver was highly congested. Heart did not show any gross lesions.

4.5.5 Histopathological Examination

The pathological changes observed in the kidney were congestion, diffuse glomerular and tubular necrosis, dilatation of tubules and hyalinisation of cortical tubules. Some glomeruli appeared shrunken and there was mesangial cell degeneration (Fig.32). Liver showed centrilobular necrosis, fatty changes and widening of the sinusoids (Fig.33). Thinning of muscle fibres, widening of intermuscular space, congestion, focal myolysis were observed in the heart (Fig.34).

4.6 EFFECT OF HALF THE TOXIC DOSE (0.2 g/kg) POOLED TOXIC FRACTION OF *Mimosa invisa* IN RABBITS

Half the toxic dose (0.2 g/kg) of pooled toxic fraction of *Mimosa invisa* was administered in rabbits to study the toxic effects. The following parameters were studied.

± 0.34 and 10.57 ± 0.52 millions/mm^3 on day 0,1,3,5,10,15 and day 20 respectively (Tables 14 and 14b).

4.5.3.4. Erythrocyte Indices

There were no significant variations in erythrocyte indices on comparison with zero day values and control values. The results are shown in Table 15, 15b, 16, 16b, 17 and 17b.The calculated values of MCV were 46.87 ± 2.13, 42.98 ± 1.28, 46.4 ± 1.59, 43.4 ± 1.93 and 38.33 ± 2.38 μm^3. The values showed slight decrease even though the decrease was not significant statistically.

The MCH values were calculated and was found to be 14.03 ± 0.62, 13.05 ± 0.31, 14.15 ± 0.30, 12.78 ± 0.41 and 11.37 ± 0.78 µg on day 0,5,10 and day 20 respectively.

The MCHC values (g%) calculated were 29.38 ± 0.47, 30.35 ± 0.59, 30.75 ± 0.91, 29.58 ± 0.85 and 29.22 ± 0.58 on day 0, 5, 10, 15 and day 20 respectively.

4.5.3.5. Total Leucocyte Count (TLC)

The TLC showed a significant increase ($P<0.05$) on 10^{th}, 15^{th} and 20^{th} day of administration of pooled toxic fraction of *Mimosa invisa*. The values observed were 7481.67± 613.78, 7891.67 ± 486.38, 8416.67 ± 766.12, 8408.33 ± 467.87 and 8708.33 ± 482.77 numbers/cumm. The results are presented in Table 18 and 18b.

4.7.3.6 Differential Leucocyte Count

5

There were significant increase ($P<0.05$) in lymphocyte count and significant decrease ($P<0.05$) in neutrophil count noticed after administration of pooled toxic fraction of *Mimosa invisa* The results are presented in Tables 19, 19b, 20, 20b, 21 and 21b. The lymphocyte count observed were 56.67 ± 1.23 on

zero day and 67.83 ± 1.49, 68.83 ± 1.14, 68.0 ± 1.03 and 65.5 ± 1.31 % on 5th, 10th, 15th and 20th day respectively.

The neutrophil count was 42.33 ± 1.15, 31.5 ± 1.31, 30.5 ± 1.17, 31.33 ± 0.95 and 33.83 ± 1.19 % on day 0, 5, 10, 15 and 20 respectively.

The eosinophil count was 1.00 ± 0.26, 0.67 ± 0.33, 0.67 ± 0.21, 0.67 ± 0.21 and 0.67 ± 0.26 % on day 0, 5, 10, 15 and day 20 respectively.

4.5.4. Post Mortem Examination

All the animals in this group were sacrificed on the 20th day of experiment and detailed post mortem examination was conducted. Kidney showed focal necrosis and petechial haemorrhages. Liver was highly congested. Heart did not show any gross lesions.

4.5.5 Histopathological Examination

The pathological changes observed in the kidney were congestion, diffuse glomerular and tubular necrosis, dilatation of tubules and hyalinisation of cortical tubules. Some glomeruli appeared shrunken and there was mesangial cell degeneration (Fig.32). Liver showed centrilobular necrosis, fatty changes and widening of the sinusoids (Fig.33). Thinning of muscle fibres, widening of intermuscular space, congestion, focal myolysis were observed in the heart (Fig.34).

4.6 EFFECT OF HALF THE TOXIC DOSE (0.2 g/kg) POOLED TOXIC FRACTION OF *Mimosa invisa* IN RABBITS

Half the toxic dose (0.2 g/kg) of pooled toxic fraction of *Mimosa invisa* was administered in rabbits to study the toxic effects. The following parameters were studied.

Fig. 32 - Kidney - Pooled toxic fraction- tubular dilatation, diffuse tubular degeneration, shrunken glomeruli H & E x 100

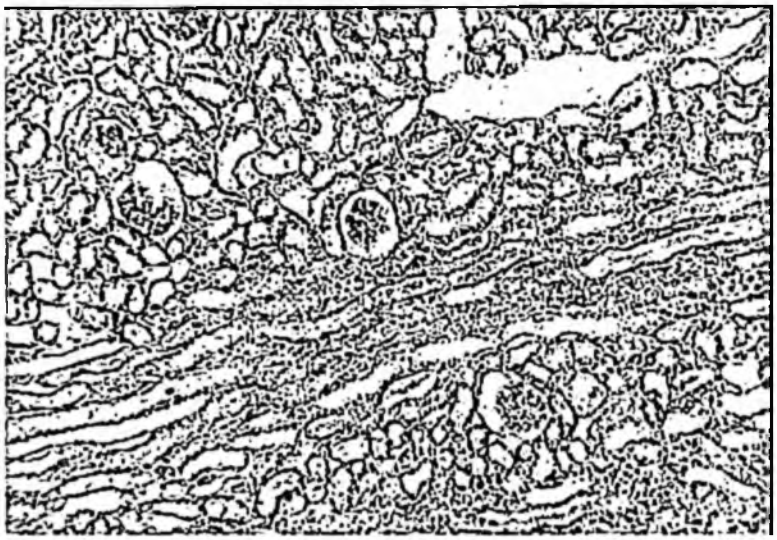


Fig. 33 - Liver - Pooled toxic fraction - fatty change and necrosis H & E x 400

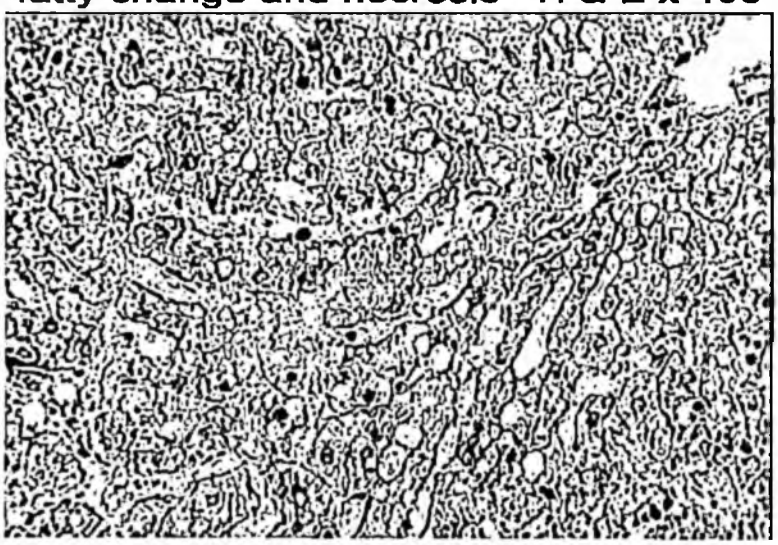
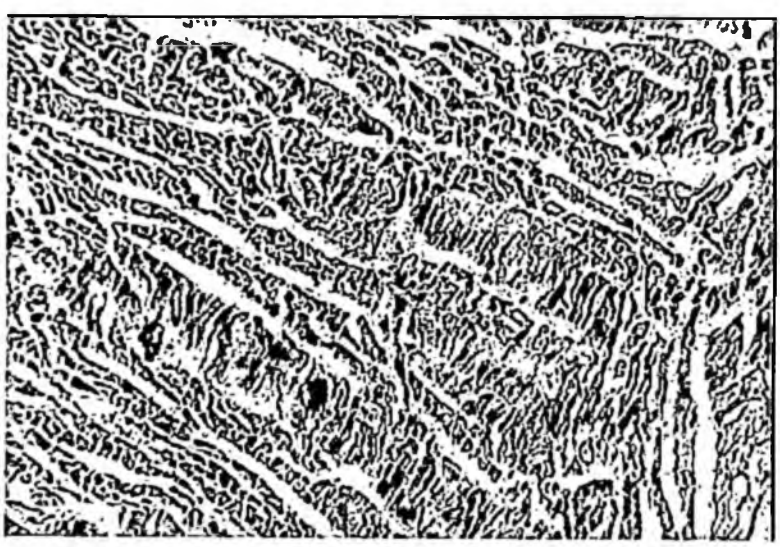


Fig. 34 - Heart - Pooled toxic fraction - focal myolysis and diffuse hyalinisation H & E x 100



4.6.1 Clinical Observations

There were no clinical symptoms observed. The animal was taking feed and water normally.

4.6.2 Biochemical Parameters

4.6.2.1 Serum ALT Levels

The serum ALT levels observed were 41 ± 3.82, 42.33 ± 1.97, 41.5 ± 2.77, 45 ± 1.98, 40.17 ± 0.60, 44.67 ± 2.09 and 41.67 ± 3.17 u/l on day 0, 5, 10, 15 and day 20 respectively. No significant variations could be detected on comparison with control values and zero day values (Tables 1,1b and Fig. 2, 25).

4.6.2.2. Serum AST Levels

The serum AST levels on day 0, 5, 10, 15 and day 20 were 35.5 ± 2.81, 38 ± 1.67, 38.17 ± 2.07, 46.5 ± 2.65, 41.83 ± 1.77, 43.83 ± 1.32, 38.17 ± 2.27 u/l respectively. There was no significant variation in values observed after administration of half the toxic dose of pooled toxic fraction of *Mimosa invisa*. The results are presented in Tables 2, 2b and Fig. 3, 26.

4.6.2.3. Serum GGT Levels

The serum GGT values also fall within normal ranges. The values were 4.33 ± 0.42, 4 ± 0.26, 4 ± 0.26, 3.67 ± 0.21, 4 ± 0.26 and 4.5 ± 0.43 and 4.5 ± 0.34 u/l on day 0, 1, 3, 5, 7, 15 and day 20. The results are shown in Tables 3, 3b and Fig. 4, 27.

4.6.2.4. Serum Creatine Kinase Levels

The serum creatine kinase levels observed were 164.83 ±10.11, 166.83 ± 10.49, 166 ± 14.6, 156.5 ± 8.8, 166.83 ± 8.05, 158.67 ± 9.90 and 168.5 ± 12.83 u/l, on day 0, 1, 3, 5, 10, 15 and day 20 respectively. The values did not show any

significant difference when compared with control values and zero day values (Table 4, 4b and Fig.5, 28)

4.6.2.5. Serum ALP Levels

The results are shown in Table 5, 5b and Fig.6, 29. The serum ALP levels before administration and after on day 1, 3, 5, 10, 15 and 20 were 78 ± 5.56, 78.67 ± 4.84, 76.67 ± 2.43, 80 ± 3.97, 77.17 ± 3.30, 83.67 ± 6.06 and 75.5 ± 6.0 u/l respectively. The values after administration of *Mimosa invisa*, pooled toxic fraction (0.2 g/kg) did not significantly differ from zero day value and control values.

4.6.2.6 Serum Creatinine Levels

The results are presented in Table 6, 6b and Fig. 7, 30. The serum creatinine values observed were 2.00 ± 0.37, 2.33 ± 0.21, 1.67 ± 0.33, 1.67 ± 0.33, 1.5 ± 0.22, 2.33 ± 0.21 and 1.83 ± 0.31 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. The values after administration of *Mimosa invisa* pooled toxic fraction (0.2 g/kg) did not significantly differ from zero day values and control values.

4.6.2.7. Serum Urea Levels

The serum urea levels also remained within normal limit. The values obtained were 51.33 ± 5.17, 54.83 ± 1.96, 53 ± 2.9, 54.67 ± 3.1, 52.33 ± 4.26, 48.33 ± 4.26 and 46.83 ± 4.4 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. The results are shown in Table 7, 7b and Fig.8, 31.

4.6.2.8. Serum Total Proteins

The observed values for total protein were 5.75 ± 0.15, 5.73 ± 0.12, 5.48 ± 0.14, 5.48 ± 0.16, 5.23 ± 0.15, 5.65 ± 0.09 and 5.58 ± 0.14 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively. All the values were within normal ranges. The values of control and experimental group are presented in Table 8 and 8b.

4.6.2.9. Serum Albumin Levels

The serum albumin levels noticed were 3.7 ± 0.11, 3.75 ± 0.09, 3.52 ± 0.12, 3.45 ± 0.07, 3.7 ± 0.05, 3.57 ± 0.08 and 3.5 ± 0.03 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 9). No significant variations could be observed. The values of control are presented in Table 9b.

4.6.2.10. Serum Globulin Levels

The serum globulin was calculated from total protein and albumin values. All the values were found to be within normal ranges. The calculated values were 1.65 ± 0.01, 1.63 ± 0.18, 1.6 ± 0.16, 1.63 ± 0.13, 1.68 ± 0.11, 1.58 ± 0.00, 1.52 ± 0.19 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 10). There were no significant differences noticed. The control values are shown in Table 10b.

4.6.2.11 Albumin-Globulin Ratio

The results are shown in Table 11 and 11b. The albumin-globulin ratio did not show any differences between the values before administration of the drug and after administration of 0.2 g/kg of pooled toxic fraction of *Mimosa invisa*. The calculated values of albumin-globulin ratio were 2.05 ± 0.19, 1.98 ± 0.18, 1.88 ± 0.16, 2.03 ± 0.21, 1.58 ± 0.18, 2.07 ± 0.01 and 2.30 ± 0.15 on day 0, 1, 3, 5, 10 and day 20 respectively. No significant changes could be observed on comparison with control values.

4.6.3. Haemogram

4.6.3.1. Volume of Packed Red Cells (VPRC)

The volume of packed red cells on day 0, 5, 10, 15 and day 20 were 49.33 ± 0.98, 49.33 ± 1.36, 49.5 ± 1.28, 50.33 ± 1.31 and 49 ± 1.03 % respectively. The values observed after administration of 0.2 g/kg of pooled toxic fraction of *Mimosa invisa* did not show significant variations when compared to zero day value and control values. The results are presented in Table 12 and 12b.

4.6.3.2. Haemoglobin Concentration

The haemoglobin levels showed similar values before and after administration of *Mimosa invisa*. The values obtained were 14.42 ± 0.27, 14.5 ± 0.34, 14.42 ± 0.27, 14.41 ± 0.27 and 14.92 ± 0.4 g/dl on day 0, 5, 10, 15 and day 20 respectively. The results are shown in Tables 13 and 13b.

4.6.3.3. Red Blood Cell (RBC) Count

The results are presented in Table 14 and 14b. The RBC count observed were 8.24 ± 0.34, 8 ± 0.33, 7.96 ± 0.42, 8.16 ± 0.38 and 8.29 ± 0.36 millions/mm^3 respectively on day 0, 5, 10, 15 and day 20. There were no changes in RBC count noticed.

4.6.3.4. Erythrocyte Indices

The results are presented in Tables 15, 15b, 16, 16b, 17 and 17b. Significant changes could not be observed in erythrocyte indices calculated. The MCV values were calculated from PCV and RBC and the values were 59.92 ± 0.89, 62.02 ± 1.52, 62.68 ± 1.94, 62 ± 1.40 and 59.4 ± 1.51 (μm^3) on day 0, 5, 10, 15 and day 20 respectively.

The values obtained for MCH were 17.56 ± 0.61, 18.23 ± 0.50, 18.45 ± 0.98, 18.02 ± 0.69 and 17.72 ± 0.61 (µg) on day 0, 5, 10, 15 and day 20 respectively.

The calculated values of MCHC were 29.38 ± 0.50, 29.4 ± 0.47, 29.36 ± 0.65, 28.87 ± 0.66 and 29.77 ± 0.23 (g%) on day 0, 5, 10, 15 and 20 respectively.

4.6.3.5 Total Leucocyte Count (TLC)

No significant changes in TLC were observed, when the values after administration of pooled toxic fraction of *Mimosa invisa* was compared with zero day value and control values. The observed values were 6400± 462.78, 6441.67 ±

401.13, 6325 ± 417.68, 6325 ± 457.12 and 6366.67 ± 394.69 numbers/cumm on day 0, 5, 10, 15 and day 20

4.6.3.6 Differential Leucocyte Count (DLC)

The results are presented in Tables 19, 19b, 20, 20b, 21 and 21b. No significant differences in DLC could be observed after administration of half of the pooled toxic dose of *Mimosa invisa*. The lymphocyte counts noted were 57.83 ± 1.01, 54.5 ± 1.91, 57.00 ± 1.91, 54.83 ± 1.25 and 57.33 ± 0.92 % on day 0, 5, 10, 15 and day 20 respectively.

The observed values for neutrophils count were 41.33 ± 0.88, 44.83 ± 1.97, 42.67 ± 1.82, 44.67 ± 1.09 and 41.83 ± 0.87 % on day 0, 5, 10, 15 and day 20 respectively.

The eosinophil count were 0.83 ± 0.31, 0.67 ± 0.21, 0.33 ± 0.21, 0.50 ± 0.22 and 0.83 ± 0.17 % on day 0, 5, 10, 15 and day 20 respectively.

4.6.4. Post Mortem Examination

On 20th day of the experiment all the animals were sacrificed and detailed post mortem examination was conducted. No gross lesions could be observed in any of the organs.

4.6.5 Histopathological Examination

No changes could be observed on histopathological examination of kidney, liver and heart.

4.7. EFFECT OF TREATMENT ON TOXICITY PRODUCED BY TOXIC DOSE (0.4 g/kg) OF POOLED TOXIC FRACTION OF *Mimosa invisa*

A decoction is prepared from *Boerhavia diffusa*, *Tribulus terrestris* and *Hygrophila auriculata*. This decoction was administered at a rate of 5mg/kg

orally along with toxic dose of Mimosa. The following parameters were studied to assess the efficacy of the treatment.

4.7.1. Clinical Observations

The animals stopped taking feed and water from second day onwards. All the animals were dull and lethargic. But from fifth day onwards they started taking feed and water and became active.

4.7.2. Biochemical Parameters

4.7.2.1. The Serum ALT Levels

The results are presented in Table 22 and Fig.35. The ALT levels noted were 37 ± 2.72, 110 ± 3.6, 74.83 ± 3.64, 65.67 ± 4.51, 49.83 ± 0.60, 42 ± 0.82 and 39 ± 2.02 U/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively. The value on day one showed significant increase ($P<0.01$) whereas the values on third and fifth showed a significant increase at 5% level ($P<0.05$). From 10^{th} day onwards the values became normal.

4.7.2.2. Serum AST Levels

A significant increase ($P<0.05$) in serum AST levels was observed on day 1, 3 and 5, thereafter the values tend to normalize. The values measured were 45.17 ± 1.20, 81.67 ± 2.68, 82 ± 3.48, 66 ± 2.10, 57.83 ± 2.37, 42.17 ± 1.33 and 42.83 ± 1.91 U/l on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 23 and Fig.36).

4.7.2.3. Serum GGT Levels

The values obtained for serum GGT were 4.83 ± 0.40, 5 ± 0.52, 5.0 ± 0.45, 6.5 ± 0.34, 6.17 ± 0.48, 6.83 ± 0.31 and 4.5 ± 0.34 on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 24 and Fig.37). A significant increase ($P<0.05$) was noticed on day 5, 10 and day 15. On 20^{th} day the values returned to normal.

4.7.2.4. Serum Creatine Kinase Levels

The creatine kinase values were 199.67 ± 6.04, 211 ± 3.99, 201.5 ± 4.2, 204 ± 4.54, 197.5 ± 4.9, 200.5 ± 4.17 and 200.83 ± 5.67 U/l on day 0, 1, 3, 5, 10, 15 and day 20 (Table 25 and Fig.38). There were no significant variations in serum creatine kinase values observed.

4.7.2.5. Serum Alkaline Phosphatase (ALP) Levels

The ALP showed a significant increase ($P<0.05$) on first day after administration of toxin and treatment. Thereafter the values remained within normal range. The values were 50.67 ± 5.18, 84 ± 4.49, 53.83 ± 2.30, 52.5 ± 2.05, 53.5 ±2.05, 47.5 ± 2.26 and 47.17 ± 1.66 U/l on day 0, 1, 3, 5, 10, 15 and day 20 (Table 26 and Fig.39).

4.7.2.6. Serum Creatinine Levels

A significant increase ($P<0.05$) in serum creatinine values was observed from day 1 to day 15. On 20th day the values returned to normal. The values were 2.67 ± 0.21, 4.5 ± 0.22, 5 ± 0.26, 5.5 ± 0.22, 4.83 ± 0.17, 4.67 ± 0.21 and 2.5 ± 0.22 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Table 27 and Fig. 40).

4.7.2.7. Serum Urea Levels

A significant increase ($P<0.05$) in serum urea levels noticed on day 1 to day 15. On 20th the urea level returned to normal. The values observed were 42.83 ± 1.54, 71.83 ± 4.71, 69.17 ± 2.12, 67.5 ± 2.36, 63 ± 1.61, 55.35 ± 3.48 and 47 ± 5.01 mg/dl on day 0, 1, 3, 5, 10, 15 and day 20 respectively (Table 28 and Fig.41).

4.7.2.8. Serum Total Protein

The total protein did not show any variation between normal control values and zero day values. The values obtained were 5.45 ± 0.15, 5.42 ± 0.14,

5.18 ± 0.00, 5.22 ± 0.00, 5.2 ± 0.00 and 5.56 ± 0.14 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Table 29).

4.7.2.9. Serum Albumin

All the values of albumin levels were within normal ranges. The values noted were 3.55 ± 0.10, 3.55 ± 0.10, 3.53 ± 0.09, 3.51 ± 0.09, 3.5 ± 0.13, 3.83 ± 0.11 and 3.67 ± 0.12 on day 0, 1, 3, 5, 10, 15 and day 20 (Table 30).

4.7.2.10. Serum Globulin

There were no significant differences in serum globulin levels noticed. The calculated values were 1.93 ± 0.34, 1.95 ± 0.30, 1.65 ± 0.14, 1.71 ± 0.23, 1.7 ± 0.24, 1.29 ± 0.28 and 1.89 ± 0.12 g/dl on day 0, 1, 3, 5, 10, 15 and day 20 (Table 31).

4.7.2.11. Albumin-Globulin Ratio

The albumin-globulin ratio calculated were 2.02 ± 0.23, 1.93 ± 0.20, 1.65 ± 0.01, 1.58 ± 0.12, 1.7 ± 0.12, 1.28 ± 0.11 and 1.9 ± 0.09 on day 0, 1, 3, 5, 10, 15 and day 20 (Table 32). No significant differences in values noticed.

4.7.3. Haemogram

4.7.3.1. Volume of Packed Red Cells (VPRC)

The results are shown in Table 33. The volume of Packed red cell observed were 49 ± 1.23, 49.17 ± 1.45, 47.5 ± 1.38, 48.33 ± 1.50 and 49 ± 1.21% on day 0, 5, 10, 15 and day 20 respectively. No significant variations could be observed.

4.7.3.2. Haemoglobin Concentration

There were no significant variations in values after administration of decoction. The values obtained were 14.33 ± 0.72, 14.25 ± 0.57, 14.33 ± 0.67, 14 ± 0.45 and 14.08 ± 0.60 g/dl on day 0, 5, 10, 15 and day 20 (Table 34).

4.7.3.3. Red Blood Cell (RBC) Count

The RBC count did not show significant variation when compared with zero day value and control values. The observed values were 8.36 ± 0.34, 8.49 ± 0.45, 8.53 ± 0.35, 8.38 ± 0.32 and 8.39 ± 0.28 millions/mm^3 on day 0, 5, 10, 15 and day 20 (Table 35).

4.7.3.4. Erythrocyte Indices

The results are presented in Table 36, 37 and 38. There was no significant variation in erythrocyte indices when the values after administration of decoction were compared with zero day value and control values.

The MCV values were calculated from PCV and RBC and the values obtained were 58.8 ± 1.09, 58.37 ± 1.88, 58.87 ± 0.96, 57.77 ± 0.86 and 58.58 ± 1.42μm^3 on day 0, 5, 10, 15 and day 20. No significant variation could be observed when compared with zero day but all the values differed significantly when compared to control values.

The MCH values also did not show significant variation when compared with zero day values but significantly higher values when compared with control values. The values calculated were 16.87 ± 0.44, 16.87 ± 0.42, 16.77 ± 0.39, 16.73 ± 0.25 and 16.78 ± 0.16 µg on day 0, 5, 10, 15 and day 20 respectively.

The MCHC values were calculated from haemoglobin and PCV and the values did not differ from control values and zero day values. The values were 29.17 ± 0.80, 28.97 ± 0.80, 30.13 ± 0.71, 28.98 ± 0.43 and 28.73 ± 0.82 g % on day 0, 5, 10, 15 and day 20 respectively.

4.7.3.5. Total Leucocyte Count (TLC)

The TLC did show significant variation when it is compared with zero day values and control values. The values of TLC were 6316.67± 453.26, 7233.33 ± 378.3, 6666.67 ± 372.53, 6683.33 ± 486.26 and 6475 ± 483 on day 0,5,10.15 and day 20 (Table 39).

4.7.3.6. Differential Leucocyte Count

The results are presented in Table 40, 41 and 42. There were no significant variations in differential leucocyte count. All values were within normal range. The observed values of lymphocyte count were 53.83 ± 1.25, 57 ± 0.82, 47.83 ± 0.60, 57.5 ± 1.57 and 58.5 ± 1.20 % on day 0, 5, 10, 15 and day 20 respectively.

The neutrophil count noted were 45.67 ± 1.30, 42.17 ± 1.01, 41.67 ± 0.61, 41.83 ± 1.62 and 40.83 ± 1.01 % on day 0, 5, 10, 15 and day 20 respectively.

The eosinophil counts observed were 0.5 ± 0.22, 0.83 ± 0.31, 0.50 ± 0.22, 0.50 ± 0.22 and 0.50 ± 0.22 % on day 0, 5, 10, 15 and 20 respectively.

4.7.4. Post Mortem Examination

Animals were sacrificed on 20th day of the experiment and conducted detailed post mortem examination. No gross lesions could be observed in any of the internal organs. Liver, kidney and heart were taken and preserved in 10 per cent formalin for histopathological examination.

4.7.4. Histopathological Examination

In kidney the tubular and glomerular cells appeared normal and there was regeneration of tubular cells in focal areas (Fig.42). No significant histological changes could be seen in the liver. The hepatocytes appeared intact around central vein. The sinusoids appeared moderately dilated with kupffer cell activity. In some of the hepatocytes binucleation and mitosis could be observed indicating

Fig. 42 - Kidney - Pooled toxic fraction+decoction
- tubules with intact normal lining cells H & E x 100

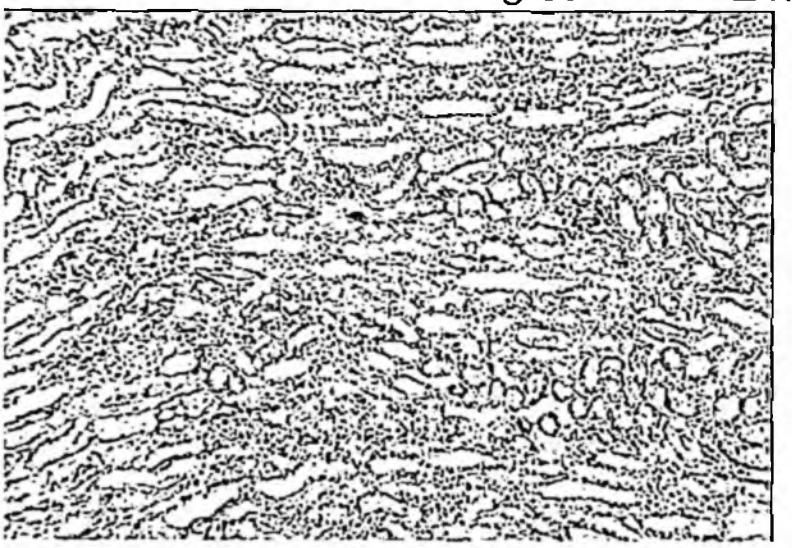


Fig. 43 - Liver - Pooled toxic fraction+decoction
- sinusoidal dilatation, regenerative changes H & E x 400

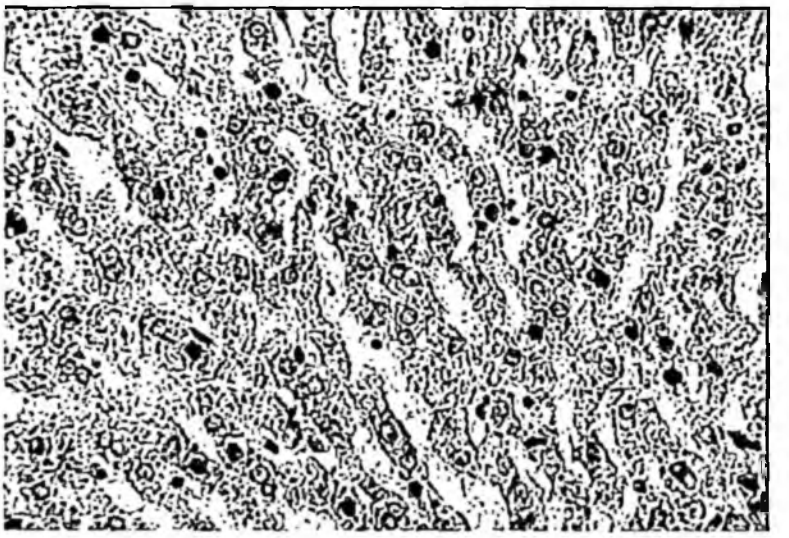
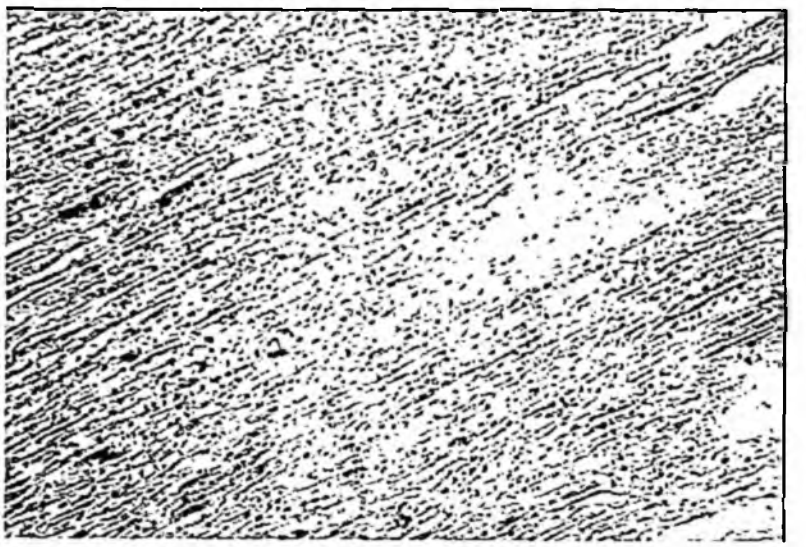


Fig. 44 - Heart - Pooled toxic fraction+decoction
- intact heart muscle fibres H & E x 100



regeneration (Fig.43). No histological lesions could be noticed in heart. The fibres appeared regular (Fig.44).

4.8. SCREENING OF ALCOHOLIC EXTRACT AND VARIOUS FRACTIONS FOR ACTIVE PRINCIPLES

Alcoholic extract and various fractions like chloroform fraction, butanol fraction, aqueous fraction and water insoluble residue were qualitatively tested for various active principles present in it.

4.8.1. Steroids

The alcoholic extract, butanol fraction, and aqueous fraction gave a positive test for steroids as indicated by the development of red colour in Salkowski test and a reddish ring in Leiberman Burchardt test. The chloroform fraction and water insoluble fraction showed a negative reaction to this test.

4.8.2. Alkaloids

The extract and fractions failed to show a creamy white precipitate in Mayer's test, reddish brown precipitate with Wagners reagent, yellow precipitate in Hagers test and reddish brown precipitate in Dragondorff's test. This revealed absence of detectable levels of alkaloids.

4.8.3. Phenolic Compounds

The alcoholic extract, butanol fraction, and aqueous fraction showed a dark blue colour with 10 per cent ferric chloride solution. This indicated the presence phenolic compounds. But chloroform fraction and water insoluble residue gave a negative reaction to this test.

4.8.4. Tannins

When the extract and fractions were mixed with 1 per cent ferric chloride, a blue colour developed which indicated the presence of tannins in the alcoholic

extract, butanol fraction and aqueous fraction. Gelatin test gave a white precipitate. The water insoluble fraction also showed slight positive reaction. But chloroform fraction was negative for this test.

4.8.5. Flavonoids

The green colour developed with neutral ferric chloride and a yellow precipitate with neutral 10 per cent lead acetate which revealed detectable amount of flavonoids in alcoholic extract, butanol fraction, aqueous fraction, chloroform fraction and in water insoluble residue.

4.8.6. Glycosides

When alcoholic extracts, butanol fraction and aqueous fraction were mixed with 10 per cent sodium hydroxide, a yellow colour was developed and with Benedict's reagent it developed a brown to red colour. This revealed presence of detectable level of glycoside in alcoholic extract, butanol fraction and aqueous fraction. Chloroform and water insoluble residue showed a negative reaction to these tests indicating absence of glycosides.

4.8.7. Diterpenes

The diterpene was detected in alcoholic extract, butanol fraction, aqueous fraction since these gave a green colour when mixed with 5 per cent copper acetate solution. But chloroform fraction and water insoluble residue failed to produce the colour with copper acetate indicating absence of diterpenes.

4.8.8. Triterpenes

The Salkowski test gave a yellow colour and Leiberman Burchardt test gave a red ring, which indicated the presence of detectable amount of triterpene in alcoholic extract, butanol fraction and aqueous fraction. Water insoluble residue also revealed slight positive reactions to these tests. Chloroform fraction was negative to this test.

4.8.9. Saponins

The foam test showed a persistent foam which remained for more than 10 minutes indicated the presence of saponin. Haemolytic test was also positive for alcoholic extract, butanol fraction and aqueous fraction.

4.8.10. Cyanides

A light reddish colour appeared on the picrate paper, which indicates traces of cyanide in the plant.

4.8.11. Nitrates

A bluish colour developed by adding the diphenylamine reagent to the crushed plant material, indicating presence of nitrate.

4.8.12. Mimosine

No bluish colour to the mixture after incubating in the dark for 15 minutes which indicated the absence of mimosine.

4.9 EFFECT OF DOUBLE THE TOXIC DOSE (0.8 g/kg) OF POOLED TOXIC FRACTION OF *Mimosa invisa* IN RABBITS

All the animals under this group died within 12-24 hours after administration of the extract. Hence no further observations could be made.

Phytochemical screening of extract and fractions of *Mimosa invisa*

	Test	Alcoholic extract	Chloroform fraction	Butanol fraction	Aqueous fraction	Water insoluble residues
1	Tests for Steroids					
	a. Salkowski test	++	–	++	++	–
	b. Leiberman Burchardt test	–	–	–	–	–
2	Tests for detection of Alkaloids					
	a. Wagners test	–	–	–	–	–
	b. Hager test	–	–	–	–	–
	c. Dragendorff's test	–	–	–	–	–
3	Tests for Phenolic compounds	++	–	++	++	–
4	Tests for Tannins					
	a. Ferric chloride test	++	–	++	++	+
	b. Gelatin test	++	–	++	++	+
5	Tests for Flavonoids					
	a. Ferric chloride test	++	++	++	++	+
	b. Lead acetate test	++	++	++	++	++
6	Tests for Glycosides					
	a. Sodium hydroxide test	++	–	+	++	–
	b. Benedict's test	++	–	+	+++	–
7	Tests for Diterpenes	++	–	++	++	–
8	Tests for Triterpenes					
	a. Salkowski test	++	–	++	++	+
	b. Lieberman Burchardt test	++	–	++	++	+
9	Tests for Saponins					
	a. Foam test	+++	–	++	+++	+
	b. Hemolytic test	++	–	++	++	–

Table 1. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum ALT levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	40.67 ± 4.45	212.5 ± 33.26**	165.67 ± 15.63**	143.5 ± 11.31**	116.33 ± 9.93**	55.17 ± 5.65*	50.33 ± 1.74*
III	38.33 ± 2.63	157.5 ± 19.7**	172.33 ± 13.32**	185.33 ± 10.22**	130.33 ± 7.92**	66.17 ± 7.87*	43.5 ± 7.26*
V	55.5 ± 5.69	85.5 ± 8.05**	126.5 ± 10.55**	136 ± 13.6**	150.17 ± 12.81**	162.67 ± 8.09**	170.5 ± 5.54**
VI	41 ± 3.82	42.33 ± 1.97	41.5 ± 2.77	45 ± 1.98	40.17 ± 0.60	44.67 ± 2.09	41.67 ± 3.17

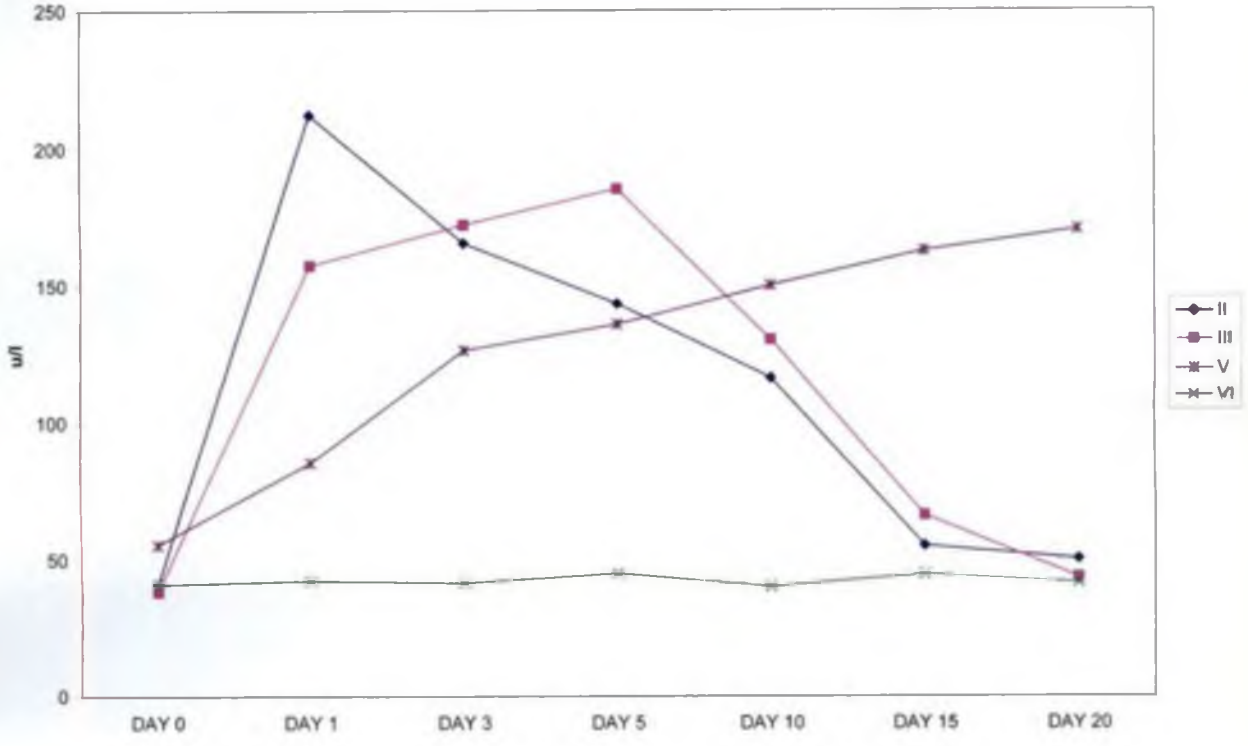
**P<0.01, *P<0.05

Table 2. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum AST levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	43.5 ± 4.18	91.67 ± 18.73	141.5 ± 14.11*	111.83 ± 8.27*	56.33 ± 10.93	50.5 ± 7.28	46.67 ± 4.65
III	32.67 ± 2.99	98.5 ± 14.67*	101.00 ± 4.65*	123.67 ± 7.97*	81.67 ± 6.10*	48.17 ± 5.7	35.83 ± 4.13
V	51 ± 3.49	110 ± 8.14**	154.17 ± 15.08**	167.67 ± 17.45**	188.5 ± 11.65**	174.5 ± 5.37**	186.83 ± 5.78**
VI	35.5 ± 2.81	38 ± 1.67	38.17 ± 2.07	46.5 ± 2.65	41.83 ± 1.77	43.83 ± 1.32	38.17 ± 2.27

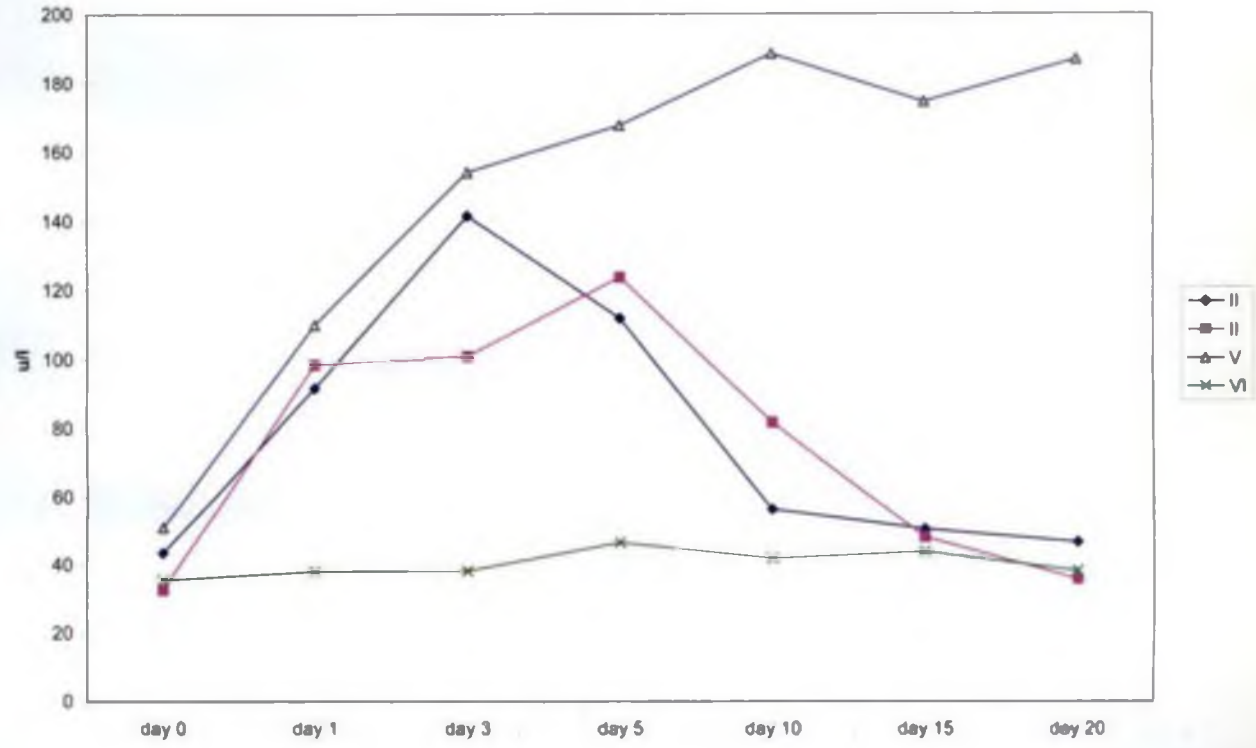
**P<0.01, *P<0.05

Fig. 2 .Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum ALT levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Fig.3.Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum AST levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Table 3. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum GGT levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	4.67 ± 0.33	8.5 ± 0.43**	10.67 ± 0.49**	8.00 ± 0.58**	7.0 ± 0.37**	5.17 ± 0.4	4.5 ± 0.22
III	5.83 ± 0.48	8.83 ± 0.70*	9.67 ± 0.88**	10.83 ± 0.70**	8.17 ± 0.7**	6.00 ± 0.37	5.83 ± 0.31
V	5.5 ± 0.56	6.67 ± 0.42	8.17 ± 0.60**	9.83 ± 0.87**	10.67 ± 0.33**	11.5 ± 0.22**	11.0 ± 0.26**
VI	4.33 ± 0.42	4 ± 0.26	4 ± 0.26	3.67 ± 0.21	4 ± 0.26	4.5 ± 0.43	4.5 ± 0.34

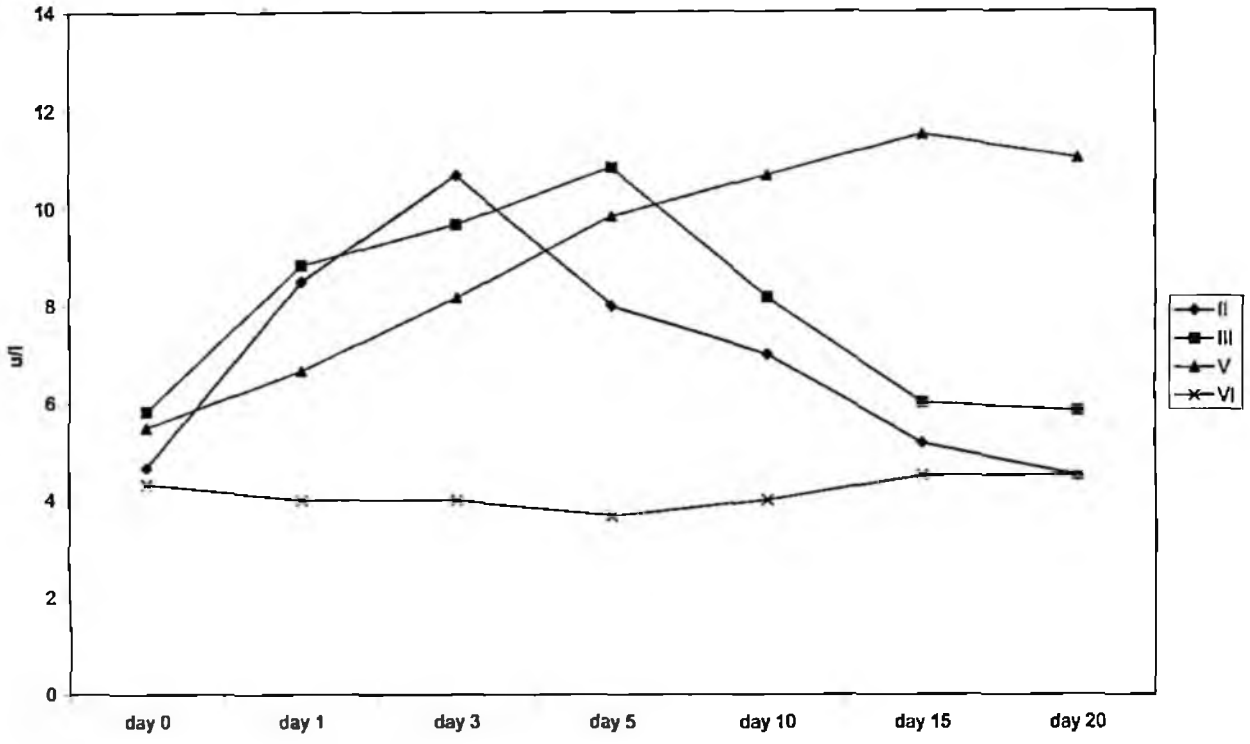
**P<0.01, *P<0.05

Table 4. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum creatine kinase levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	148.0 ± 16.69	173.33 ± 20.43	183.5 ± 23.92*	143.33 ± 22.95	152.17 ± 19.43	147.83 ± 20.13	151.33 ± 17.75
III	181.17 ± 15.28	201.33 ± 6.59	219.67 ± 3.65*	213.83 ± 8.64	189.83 ± 7.68	165.33 ± 11.10	155.33 ± 11.61
V	133.83 ± 10.11	170.67 ± 15.60**	199.33 ± 13.76**	215.33 ± 15.81**	170.33 ± 6.98**	161.5 ± 3.28	140.5 ± 3.43
VI	164.83 ± 10.11	166.83 ± 10.49	166 ± 14.6	156.5 ± 8.80	166.83 ± 8.05	158.67 ± 9.90	168.5 ± 12.83

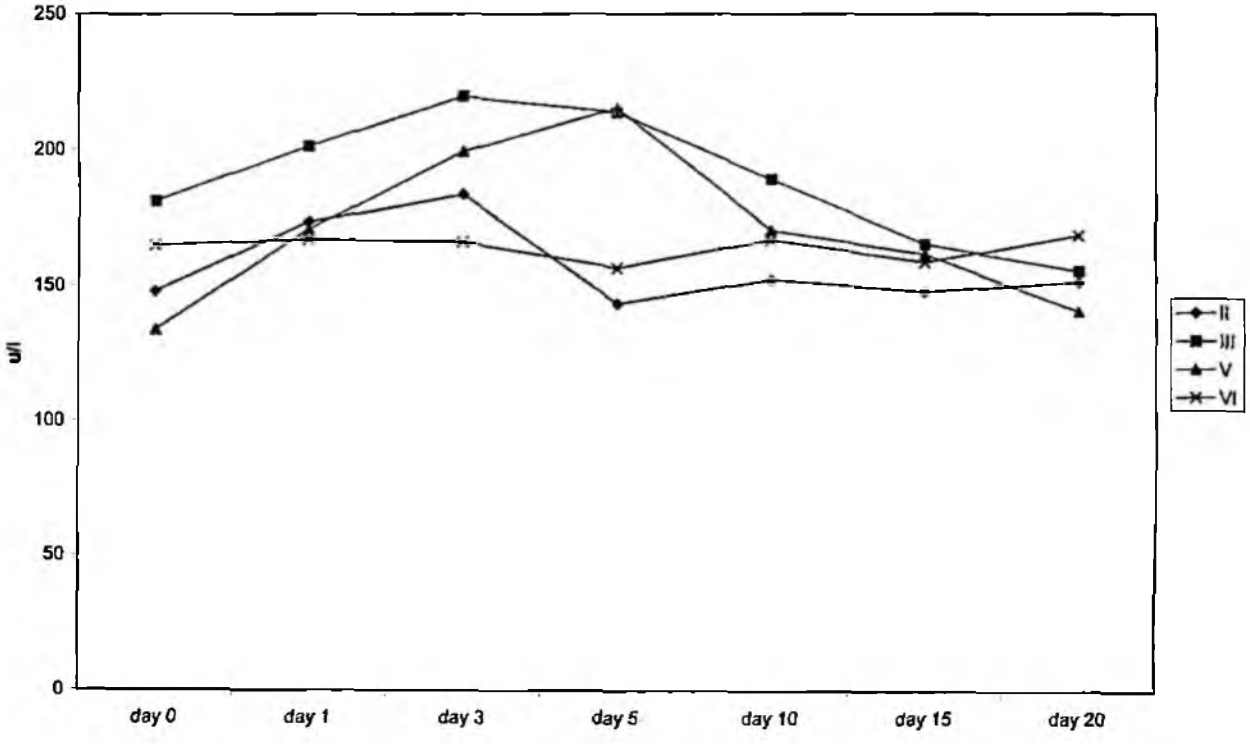
**P<0.01, *P<0.05

Fig.4. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum GGT levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Fig.5. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum creatine kinase levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Table 5. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum alkaline phosphatase level (u/l), Mean±S.E (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	74 ± 5.88	126 ± 12.25**	136.83 ± 12.88**	119.33 ± 7.9**7	84.5 ± 3.35	65.5 ± 3.97	57.00 ± 2.97
III	59 ± 7.90	72 ± 6.40	76.33 ± 7.82*	66.5 ± 6.35	56.33 ± 5.21	54.83 ± 3.13	57.57 ± 4.94
V	42.83 ± 10.64	46.5 ± 11.05	62.17 ± 12.25*	79.67 ± 14.58*	63.67 ± 10.63*	64.5 ± 9.59*	53.67 ± 8.19
VI	78 ± 5.56	78.67 ± 4.84	76.67 ± 2.43	80.0 ± 3.97	77.17 ± 3.30	83.67 ± 6.06	75.5 ± 6.0

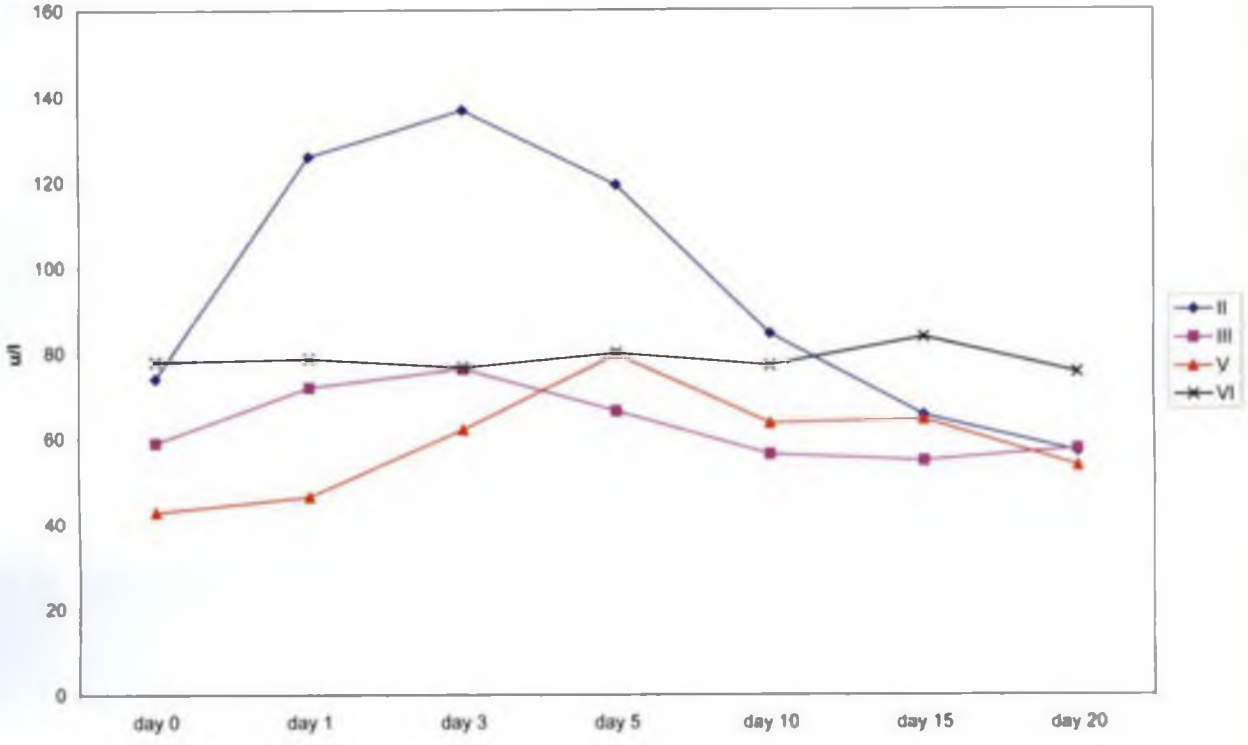
**P<0.01, *P<0.05

Table 6. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum creatinine levels(mg/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	2.33 ± 0.21	8.33 ± 0.49**	10.83 ± 0.70**	9.17 ± 0.75**	6 ± 0.21*	3.33 ± 0.21	2.5 ± 0.22
III	2.17 ± 0.17	9.17 ± 0.48**	9.83 ± 0.6**	7.33 ± 0.88**	5.83 ± 0.47*	3.67 ± 0.33	2.83 ± 0.31
V	2.17 ± 0.31	4.67 ± 0.33*	7.17 ± 0.40**	8.33 ± 0.49**	9.83 ± 0.31**	10.83 ± 0.40**	12.17 ± 0.31**
VI	2.00 ± 0.37	2.33 ± 0.21	1.67 ± 0.33	1.67 ± 0.33	1.5 ± 0.22	2.33 ± 0.21	1.83 ± 0.31

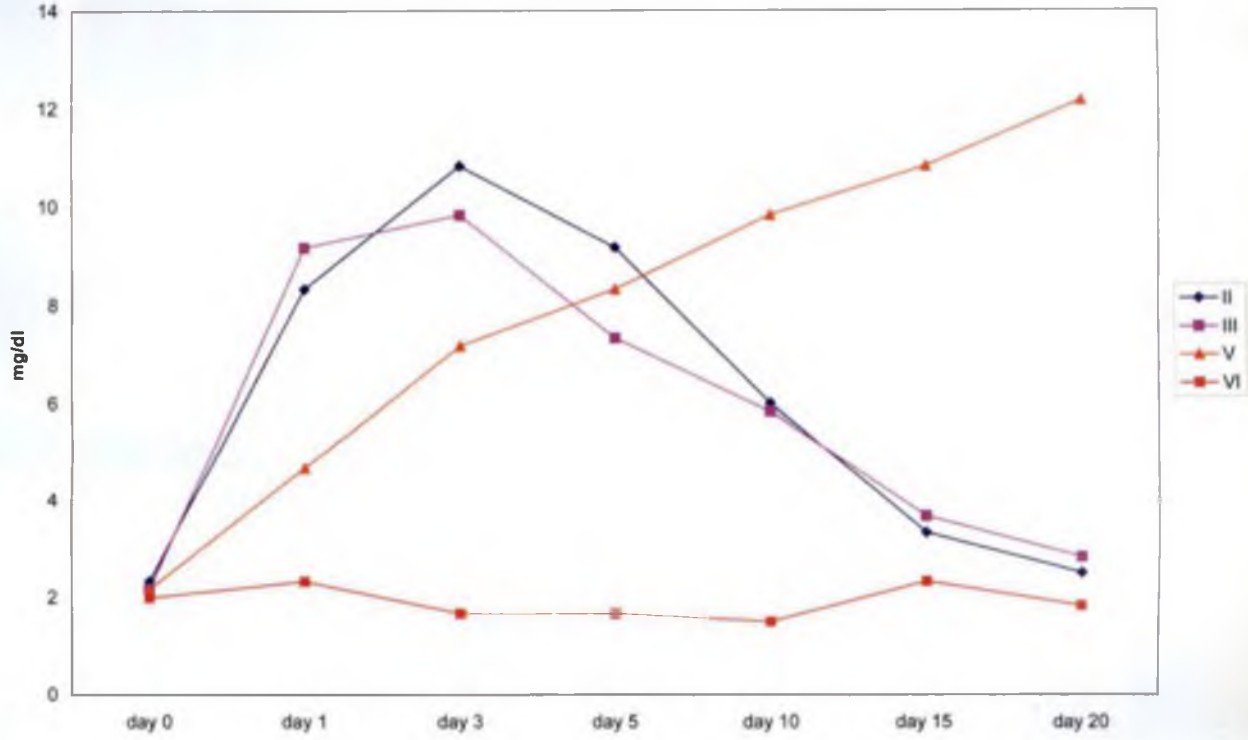
**P<0.01, *P<0.05

Fig.6. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum ALP levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Fig.7. Effect of fresh juice,alcoholic extract and pooled toxic fraction of *M.invisa* serum creatinine levels



II - Fresh Juice of *M. Invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Table 7. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum urea levels (mg/dl), Mean±S.E. (n=6).

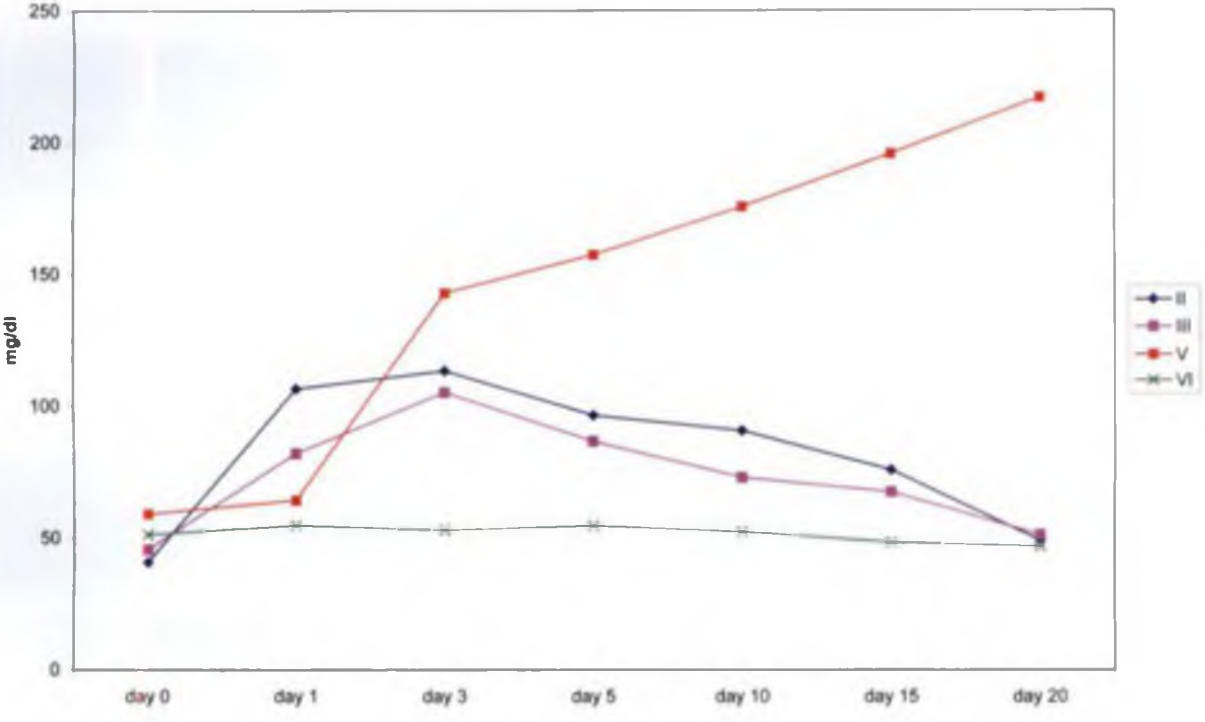
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	40.83 ± 0.87	106.5 ± 6.06**	113.33 ± 4.98**	96.5 ± 4.41**	90.67 ± 3.92**	76.83 ± 4.88**	49 ± 1*
III	45.67 ± 5.27	82.17 ± 3.85*	105.17 ± 2.97**	86.67 ± 4.16**	73 ± 2.47*	67.5 ± 2.96*	51.17 ± 2.7
V	59.17 ± 3.7	64.33 ± 3.90	142.83 ± 26.20**	157.33 ± 23.92**	175.67 ± 12.07**	195.83 ± 8.99**	217.0 ± 10.96**
VI	51.33 ± 5.17	54.83 ± 1.96	53 ± 2.9	54.67 ± 3.1	52.33 ± 4.26	48.33 ± 4.26	46.83 ± 4.44

**P>0.01, *P<0.05

Table 8. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum total protein (g/dl), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	5.81 ± 0.15	6.07 ± 0.08	6.02 ± 0.09	5.96 ± 0.10	5.97 ± 0.11	5.85 ± 0.18	5.93 ± 0.06
III	5.08 ± 0.17	5.12 ± 0.11	5.00 ± 0.07	5.12 ± 0.14	5.00 ± 0.08	4.95 ± 0.09	5.05 ± 0.11
V	5.6 ± 0.15	5.37 ± 0.15	5.95 ± 0.11	5.67 ± 0.29	5.45 ± 0.21	5.37 ± 0.22	5.4 ± 0.16
VI	5.75 ± 0.15	5.73 ± 0.12	5.48 ± 0.14	5.48 ± 0.16	5.23 ± 0.15	5.65 ± 0.09	5.58 ± 0.14

Fig. 8. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *M.invisa* on serum urea levels



II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*
V - Pooled toxic fraction - 0.4g/kg
VI -Pooled toxic fraction - 0.2g/kg

Table 9. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum albumin (g/dl), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	3.58 ± 0.10	3.62 ± 0.12	3.48 ± 0.14	3.75 ± 0.04	3.33 ± 0.06	3.25 ± 0.08	3.12 ± 0.03
III	3.85 ± 0.04	3.8 ± 0.08	3.6 ± 0.13	3.77 ± 0.08	3.73 ± 0.08	3.7 ± 0.11	3.65 ± 0.09
V	3.58 ± 0.14	3.47 ± 0.14	3.48 ± 0.17	3.5 ± 0.09	3.47 ± 0.06	3.73 ± 0.11	3.67 ± 0.12
VI	3.7 ± 0.11	3.75 ± 0.09	3.52 ± 0.12	3.45 ± 0.07	3.70 ± 0.05	3.57 ± 0.08	3.5 ± 0.03

Table 10. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on serum globulin (g/dl), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	1.74 ± 0.14	1.65 ± 0.15	1.68 ± 0.15	1.45 ± 0.01	1.31 ± 0.01	1.25 ± 0.10	1.20 ± 0.13
III	1.23 ± 0.19	1.32 ± 0.17	1.5 ± 0.19	1.27 ± 0.14	1.28 ± 0.13	1.25 ± 0.17	1.40 ± 0.14
V	1.97 ± 0.18	1.93 ± 0.22	1.57 ± 0.17	1.75 ± 0.23	1.87 ± 0.19	1.64 ± 0.22	1.73 ± 0.20
VI	1.65 ± 0.01	1.63 ± 0.18	1.60 ± 0.16	1.63 ± 0.13	1.68 ± 0.11	1.58 ± 0.00	1.52 ± 0.19

Table 11. Effect of fresh juice, alcoholic extract and pooled toxic fraction of *Mimosa invisa* on albumin-globulin ratio, Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
II	2.18 ± 0.13	2.28 ± 0.20	2.15 ± 0.20	2.28 ± 0.08	2.57 ± 0.06	2.67 ± 0.20	2.67 ± 0.20
III	3.50 ± 0.49	3.11 ± 0.36	2.67 ± 0.41	3.17 ± 0.41	3.17 ± 0.41	3.33 ± 0.55	2.75 ± 0.27
V	1.92 ± 1.90	1.90 ± 0.50	2.37 ± 0.19	2.18 ± 0.29	1.95 ± 0.21	1.70 ± 0.15	1.92 ± 0.15
VI	2.05 ± 0.19	1.98 ± 0.18	1.88 ± 0.16	2.03 ± 0.21	1.58 ± 0.18	2.07 ± 0.06	2.30 ± 0.15

Table 12. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on VPRC (%) Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	44.17 ± 1.49	39.17 ± 1.42**	40.33 ± 1.33*	41.00 ± 1.12*	39.5 ± 0.89**
III	46.00 ± 1.39	41.33 ± 1.17**	39.67 ± 0.80**	38.67 ± 0.76**	37.33 ± 0.80**
V	51.17 ± 1.14	46.50 ± 1.23**	43.83 ± 1.54**	43.00 ± 1.50**	41.83 ± 2.07**
VI	49.33 ± 0.98	49.33 ± 1.36	49.50 ± 1.28	50.33 ± 1.31	49.00 ± 1.03

**P<0.01, *P<0.05

Table 13. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on haemoglobin concentration (g/dl), Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	13.17 ± 0.48	12.5 ± 0.45	12 ± 0.53*	12.67 ± 0.33*	12.08 ± 0.35*
III	13.65 ± 0.58	13.5 ± 0.79	13.87 ± 0.68	13.85 ± 0.69	12.8 ± 0.19*
V	15.08 ± 0.32	14.25 ± 0.28*	13.42 ± 0.15**	12.58 ± 0.20**	11.82 ± 0.15**
VI	14.42 ± 0.27	14.5 ± 0.34	14.42 ± 0.27	14.41 ± 0.27	14.92 ± 0.4

**P<0.01, *P<0.05

Table 14. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on RBC count (millions/mm^3) Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	9.71 ± 0.29	9.22 ± 0.21	8.92 ± 0.21*	8.45 ± 0.22*	8.25 ± 0.23*
III	7.35 ± 1.06	7.64 ± 1.02*	7.87 ± 0.99*	7.45 ± 0.77*	7.19 ± 0.74*
V	10.99 ± 0.39	10.83 ± 0.28	9.5 ± 0.19**	9.63 ± 0.34*	10.57 ± 0.52
VI	8.24 ± 0.34	8.0 ± 0.33	7.96 ± 0.42	8.16 ± 0.38	8.29 ± 0.36

**P<0.01, *P<0.05

Table 15. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on MCV (μm^3) Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	45.61 ± 1.71	42.53 ± 1.65	45.23 ± 1.53	48.57 ± 1.13	47.88 ± 4.6
III	51.52 ± 1.70	49.1 ± 1.96	49.77 ± 2.42	51 ± 1.55	51.08 ± 2.02
V	46.87 ± 2.13	42.98 ± 1.28	46.4 ± 1.59	43.4 ± 1.93	38.33 ± 2.38*
VI	59.92 ± 0.89	62.02 ± 1.52	62.68 ± 1.94	62 ± 1.40	59.4 ± 1.51

Table 16. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on MCH (µg) Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	13.65 ± 0.65	13.58 ± 0.57	13.45 ± 0.42	15 ± 0.4	14.68 ± 0.41
III	12.25 ± 0.44	11.33 ± 0.36	11.5 ± 0.37	11.25 ± 0.38	10.83 ± 0.31
V	14.03 ± 0.62	13.05 ± 0.31	14.15 ± 0.30	12.78 ± 0.41	11.37 ± 0.78
VI	17.56 ± 0.61	18.23 ± 0.50	18.45 ± 0.98	18.02 ± 0.69	17.72 ± 0.61

Table 17. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on MCHC (g%) Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	30.33 ± 1.63	32.45 ± 1.84	29.98 ± 1.33	30.95 ± 0.71	30.62 ± 0.89
III	26.68 ± 0.66	27.53 ± 1.20	29 ± 1.21	29.12 ± 0.84	29.05 ± 0.62
V	29.38 ± 0.47	30.35 ± 0.59	30.75 ± 0.91	29.58 ± 0.85	29.22 ± 0.58
VI	29.38 ± 0.50	29.4 ± 0.47	29.36 ± 0.65	28.87 ± 0.66	29.77 ± 0.23

Table 18. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on total leucocyte count (numbers/cumm), Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	5133.33 ± 474.28	7825 ± 603.98*	6133.33 ± 475.51*	6050 ± 759.39	5650 ± 437.22
III	5941.66 ± 575.53	6675 ± 452.72*	7108.33 ± 420.20*	7358.33 ± 357.64*	7650 ± 358*
V	7481.67 ± 613.78	7891.67 ± 486.38	8416.67 ± 766.12*	8408.33 ± 467.87*	8708.33 ± 482.77*
VI	6400 ± 462.78	6441.67 ± 401.13	6325 ± 417.68	6325 ± 457.12	6366.67 ± 394.69

*P<0.05

Table 19. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on lymphocyte count (%), Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	56.5 ± 0.92	66.33 ± 0.88	66.67 ± 1.20	66.83 ± 0.70	66.33 ± 0.56
III	54.67 ± 1.40	68.83 ± 0.87*	69 ± 0.63*	66.00 ± 0.93*	65 ± 1.29*
V	56.67 ± 1.23	67.83 ± 1.49*	68.83 ± 1.14*	68.0 ± 1.03*	65.5 ± 1.31*
VI	57.83 ± 1.01	54.5 ± 1.91	57.00 ± 1.91	54.83 ± 1.25	57.33 ± 0.92

*P<0.05

Table 20. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on neutrophil Count (%), Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	42.5 ± 0.80	33.17 ± 1.01	32.67 ± 1.02	32.5 ± 0.76	33 ± 0.45
III	44.67 ± 1.52	31.67 ± 1.45*	30.67 ± 0.61*	33.33 ± 1.11*	34.65 ± 1.56*
V	42.33 ± 1.15	31.5 ± 1.31*	30.5 ± 1.17*	31.33 ± 0.95*	33.83 ± 1.19*
VI	41.33 ± 0.88	44.83 ± 1.97	42.67 ± 1.82	44.67 ± 1.09	41.83 ± 0.87

*P<0.05

Table 21. Effect of fresh juice, alcoholic extract and pooled toxic fractions of *Mimosa invisa* on eosinophil Count (%), Mean±S.E. (n=6).

Group	Day 0	Day 5	Day 10	Day 15	Day 20
II	1.0 ± 0.26	0.5 ± 0.22	0.67 ± 0.21	0.67 ± 0.33	0.67 ± 0.21
III	0.83 ± 0.30	0.5 ± 0.22	0.33 ± 0.21	0.67 ± 0.21	0.5 ± 0.30
V	1.00 ± 0.26	0.67 ± 0.33	0.67 ± 0.21	0.67 ± 0.21	0.67 ± 0.26
VI	0.83 ± 0.31	0.67 ± 0.21	0.33 ± 0.21	0.50 ± 0.22	0.83 ± 0.17

Table 1a. Serum ALT levels (u/l) – Comparison of Group II and III with Group I (Control) Mean±S.E (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	38.17 ± 2.54	40 ± 0.97	39.17 ± 1.07	40.33 ± 1.20	36.83 ± 2.48	38.00 ± 1.41	35.33 ± 1.76
II	40.67 ± 4.45	212.5 ± 33.26**	**165.67 ± 15.63**	143.5 ± 11.31**	116.33 ± 9.93**	55.17 ± 5.65*	50.33 ± 1.74*
III	38.33 ± 2.63	157.5 ± 19.7**	172.33 ± 13.32**	185.83 ± 10.22**	130.33 ± 7.92**	66.17 ± 7.87*	43.5 ± 7.26

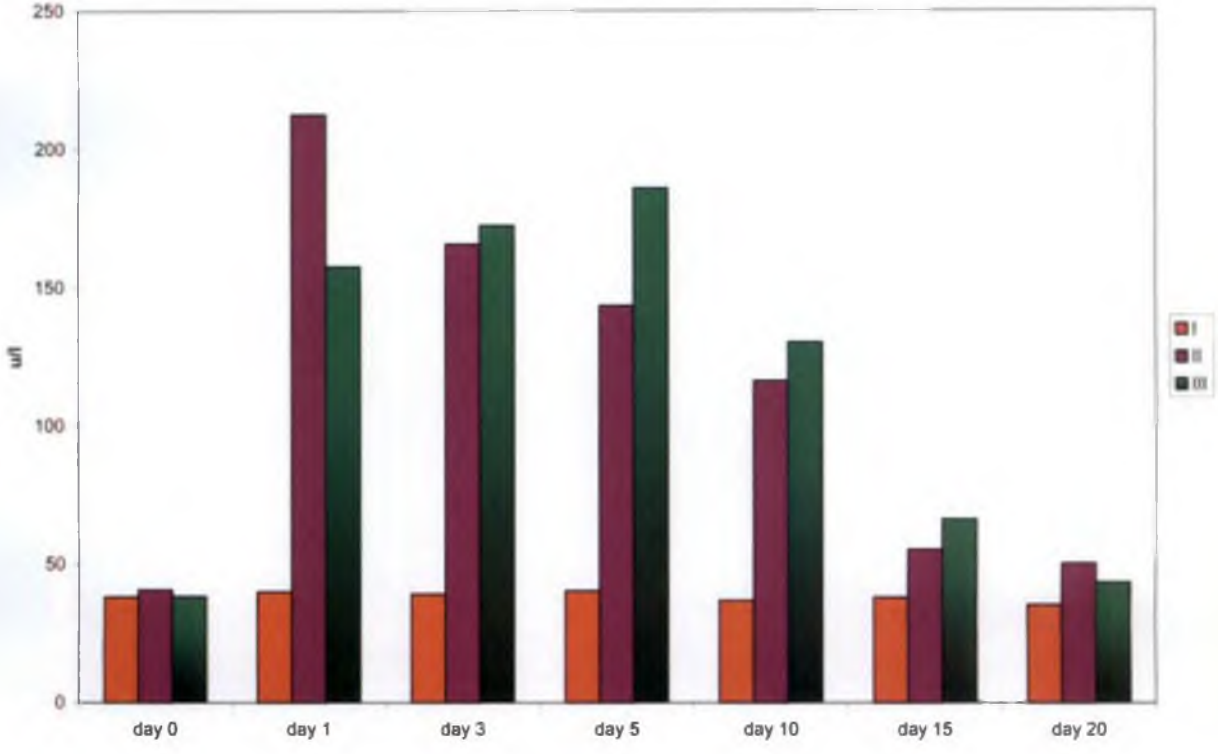
** P<0.01. * P<0.05

Table 2a. Serum AST levels (u/l) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	33.83 ± 2.80	35.5 ± 1.73	31.17 ± 1.14	37 ± 1.26	33.17 ± 2.30	33.83 ± 1.4	33.0 ± 1.39
II	43.5 ± 4.18	91.67 ± 18.73*	141.5 ± 14.11**	111.83 ± 8.27**	56.33 ± 10.93*	50.5 ± 7.28*	46.67 ± 4.65*
III	32.67 ± 2.99	98.5 ± 14.67**	101 ± 4.65**	123.67 ± 7.97**	81.67 ± 6.10*	48.17 ± 5.7	35.83 ± 4.13

**P<0.01, *P<0.05

Fig.9. Effect of fresh juice and alcoholic extract of *M.invisa* on serum ALT levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Table 3a. Serum GGT levels (u/l) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

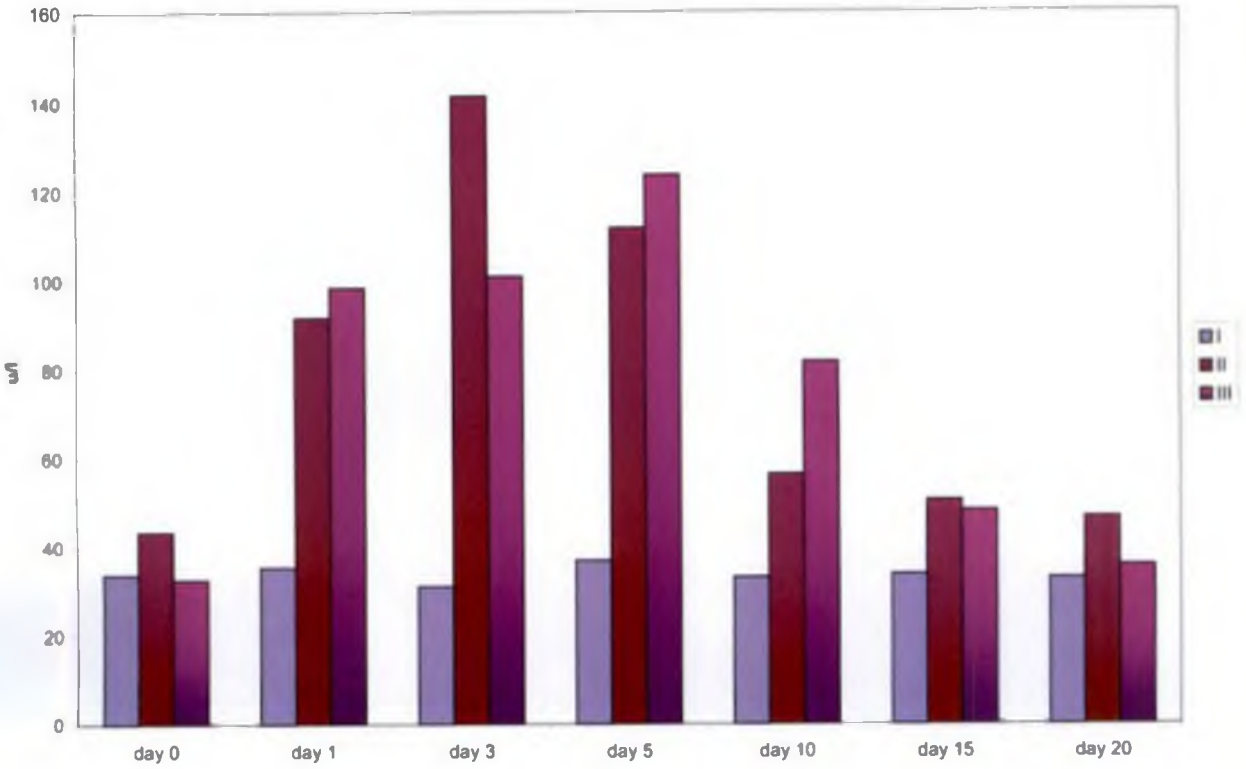
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	5.5 ± 0.43	5.17 ± 0.31	5.5 ± 0.56	4.83 ± 0.30	5.17 ± 0.40	5.17 ± 0.48	5.67 ± 0.33
II	4.67 ± 0.33	8.5 ± 0.43**	10.67 ± 0.49**	8.00 ± 0.58**	7 ± 0.37**	5.17 ± 0.40	4.5 ± 0.22
III	5.83 ± 0.48	8.83 ± 0.70**	9.67 ± 0.88**	10.83 ± 0.70**	8.17 ± 0.70**	6.00 ± 0.37	5.83 ± 0.31

**P<0.01

Table 4a. Serum CK levels (u/l) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

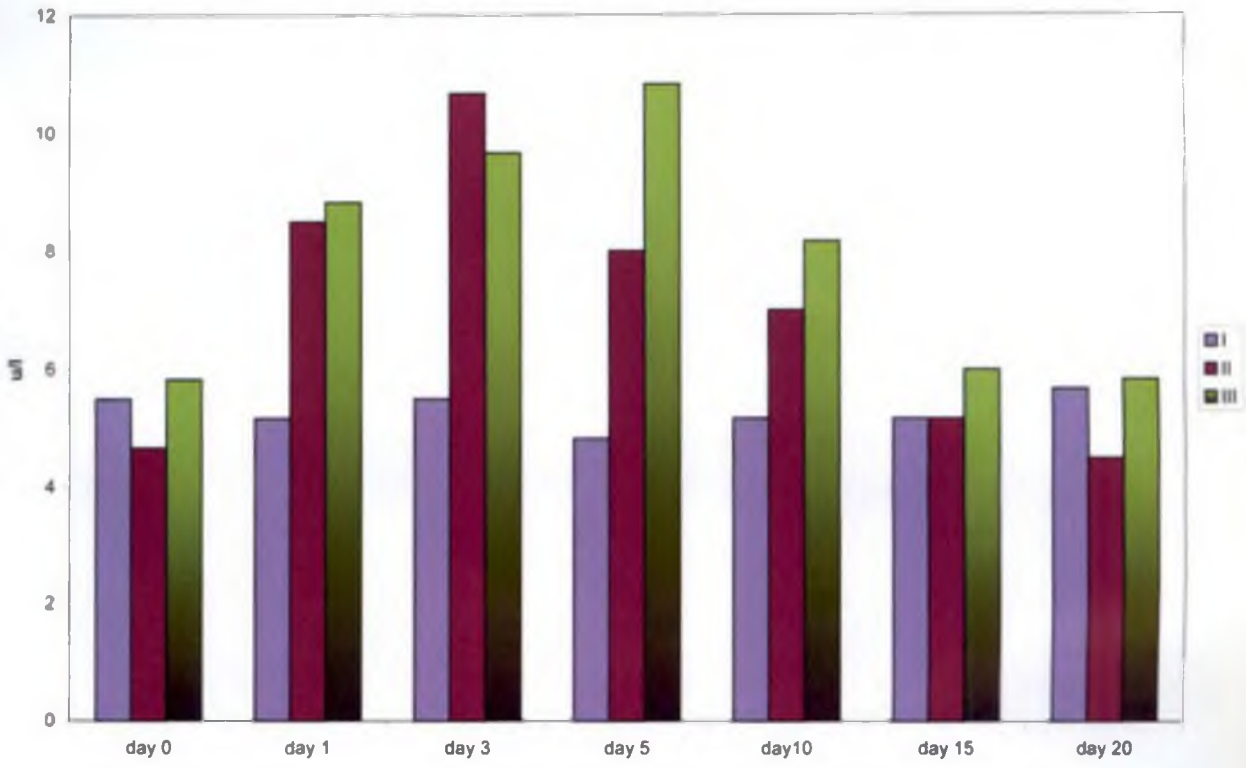
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	183.17 ± 13.48	184.33 ± 11.94	180.667 ± 12.54	180.667 ± 12.05	184.50 ± 13.48	182.5 ± 13.23	187.33 ± 12.65
II	148 ± 16.69	173.33 ± 20.43	183.5 ± 23.92	143.33 ± 22.95	152.17 ± 19.43	147.83 ± 20.13	151.33 ± 17.75
III	181.17± 15.28	201.33 ± 6.59	219.67 ± 3.65	213.83 ± 8.64	189.33 ± 7.68	165.33 ± 11.0	155.33 ± 11.61

Fig.10. Effect of fresh juice and alcoholic extract of *M.invisa* on serum AST levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Fig.11. Effect of fresh juice and alcoholic extract of *M.invisa* on serum GGT levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Table 5a. Serum alkaline phosphatase levels (u/l) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	66.67 ± 7.47	62.5 ± 6.44	68.67 ± 6.30	65 ± 6.55	64.67 ± 6.15	66.5 ± 6.66	65.67 ± 6.32
II	74 ± 5.88	126 ± 12.25**	136.83 ± 12.88**	119.33 ± 7.97**	84.5 ± 33.5**	65.5 ± 3.97*	57 ± 2.98
III	59.70 ± 7.9	72 ± 6.4	76.33 ± 7.82	66.5± 6.35	56.33 ± 5.21	54.83 ± 3.13	57.67 ± 4.94

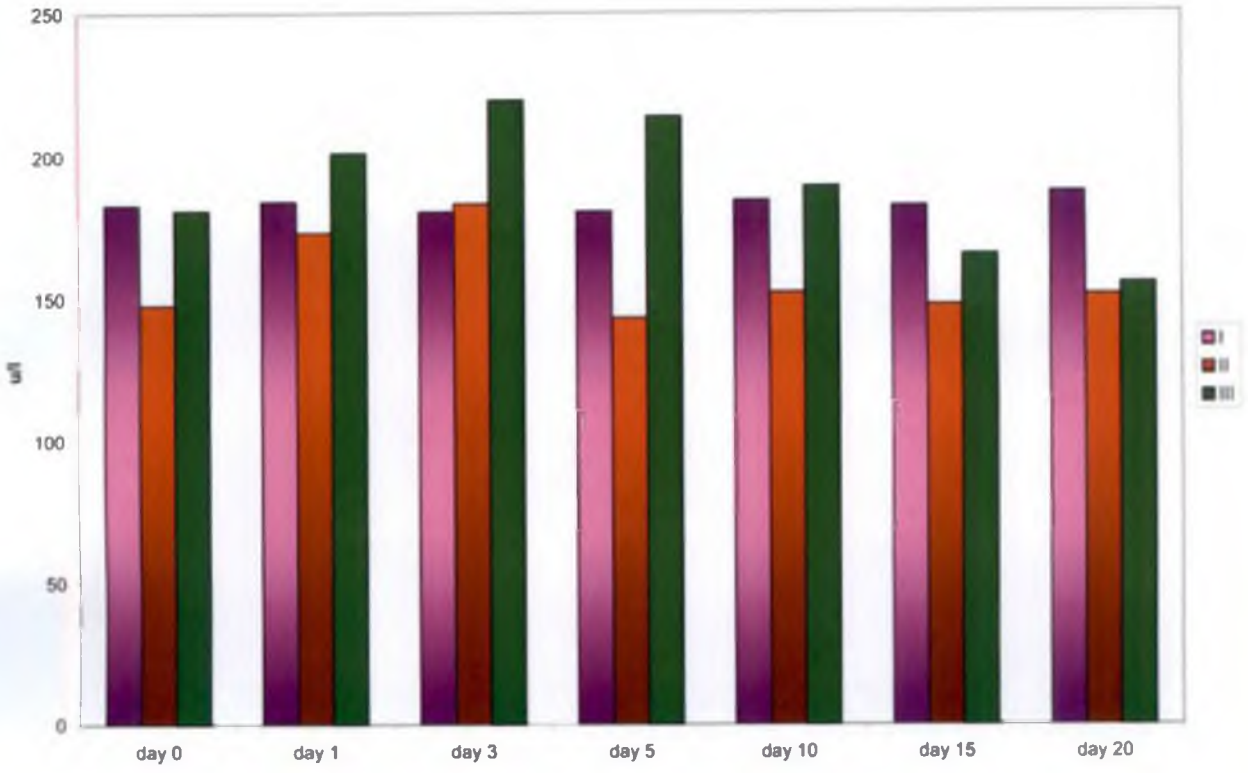
**P<0.01, *P<0.05

Table 6a. Serum creatinine levels (mg/dl) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	2.5 ± 0.22	2.17 ± 0.21	2.33 ± 0.21	2.17 ± 0.17	2.5 ± 0.22	2.17 ± 0.17	2.33 ± 0.21
II	2.33 ± 0.21	8.33 ± 0.4**9	10.83 ± 0.70**	9.67 ± 0.75**	6.00 ± 0.73**	3.33 ± 0.61	2.5 ± 0.22
III	2.17 ± 0.17	9.17 ± 0.48**	9.83 ± 0.6**	7.33 ± 0.88**	5.83 ± 0.47**	3.67 ± 0.33*	2.83 ± 0.31

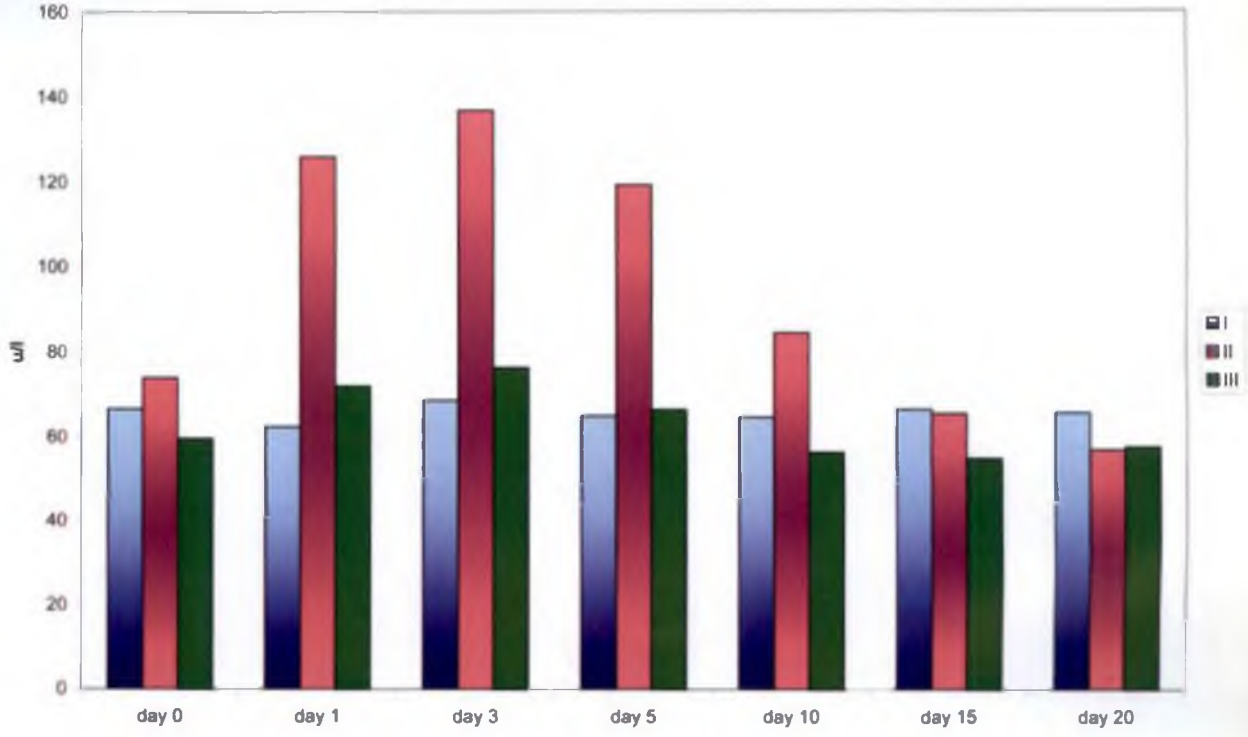
**P<0.01, *P<0.05

Fig.12. Effect of fresh juice and alcoholic extract of *M.invisa* on serum creatine kinase levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Fig. 13. Effect of fresh juice and alcoholic extract of *M.invisa* on serum alkaline phosphatase levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Table 7a. Serum urea levels (mg/dl) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

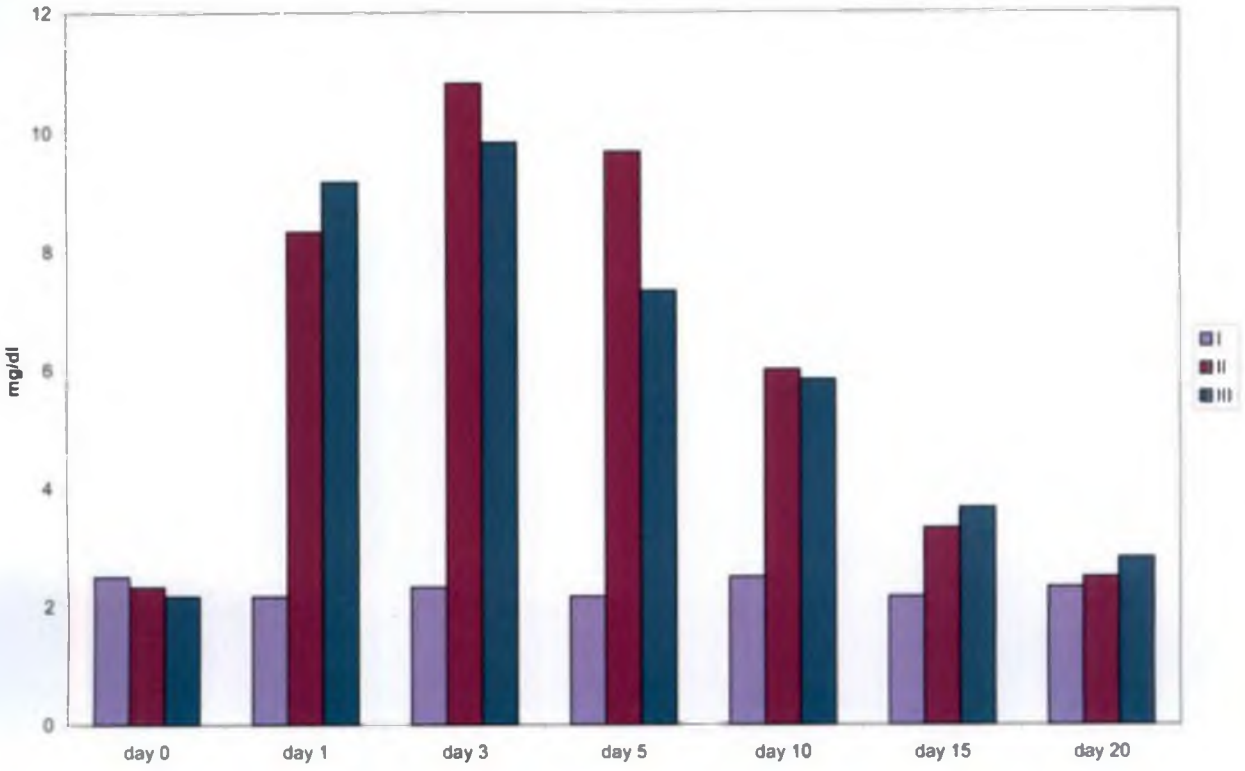
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	35.33 ± 2.14	35.33 ± 1.68	36.5 ± 1.88	35.67 ± 1.67	36.17 ± 2.06	35.0 ± 1.83	35.67 ± 1.86
II	40.83 ± 0.87	106.5 ± 6.06**	113.33 ± 5.0**	96.5 ± 4.42**	90.67 ± 3.92**	76.83 ± 4.88**	49 ± 1.0
III	45.67 ± 5.27	82.17 ± 3.85*	105.17 ± 2.97**	86.67 ± 4.16**	73 ± 2.47*	67.5 ± 2.96*	51.17 ± 2.7

**P<0.01, *P<0.05

Table 8a. Serum total protein levels (g/dl) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

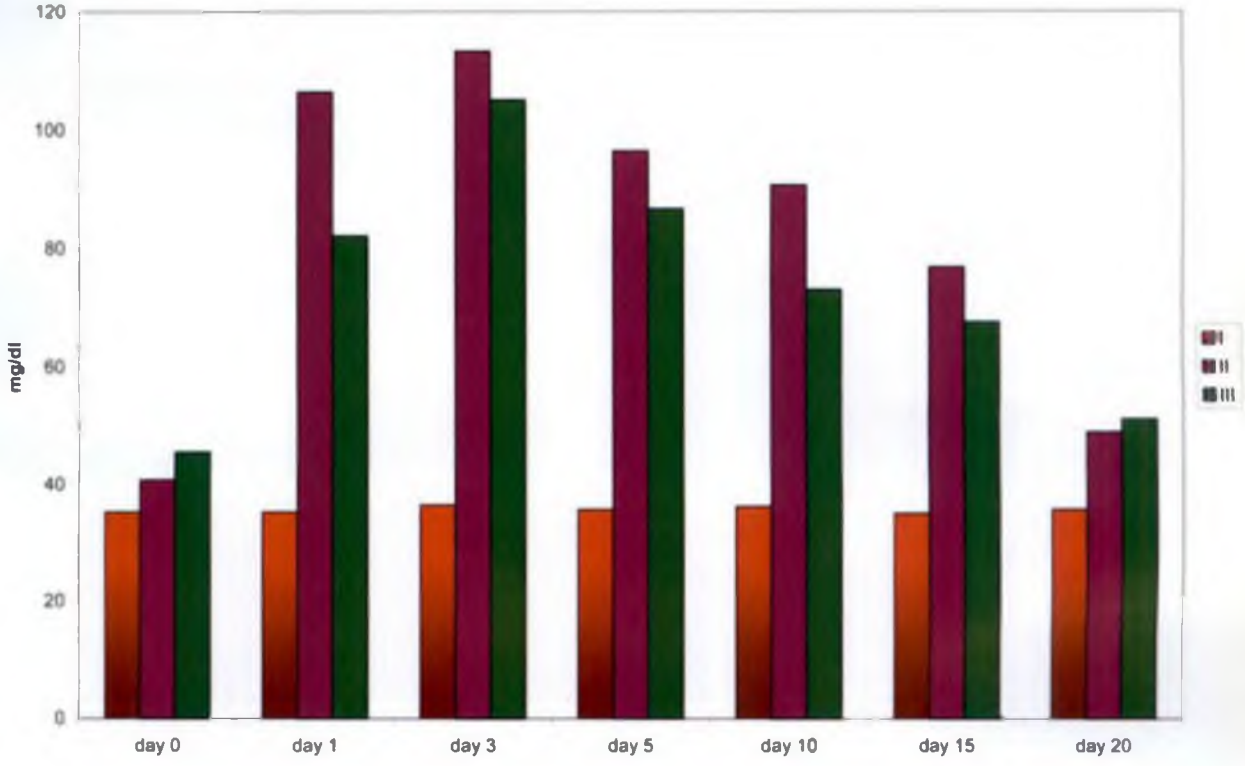
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	5.23 ± 0.15	52 ± 0.10	5.18 ± 0.16	5.25 ± 0.12	5.23 ± 0.13	5.25 ± 0.13	5.27 ± 0.15
II	5.81 ± 0.15	6.07 ± 0.08	6.02 ± 0.10	5.96 ± 0.10	5.97 ± 0.11	5.85 ± 0.18	5.93 ± 0.05
III	5.08 ± 0.17	5.12 ± 0.11	5 ± 0.09	5.12 ± 0.14	5.00 ± 0.08	4.95 ± 0.09	5.05 ± 0.11

Fig. 14. Effect of fresh juice and alcoholic extract of *M.invisa* on serum creatinine levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Fig.15. Effect of fresh juice and alcoholic extract of *M.invisa* on serum urea levels



I - Control
II - Fresh Juice of *M. invisa*
III - Alcoholic extract of *M.invisa*

Table 9a. Serum albumin levels (g/dl) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	3.62 ± 0.09	3.7 ± 0.08	3.62 ± 0.06	3.52 ± 0.04	3.65 ± 0.6	3.62 ± 0.09	3.65 ± 0.09
II	3.58 ± 0.10	3.62 ± 0.12	3.48 ± 0.14	3.75 ± 0.04	3.33 ± 0.06	3.25 ± 0.08	3.12 ± 0.03
III	3.85 ± 0.04	3.8 ± 0.08	3.6 ± 0.13	3.77 ± 0.08	3.73 ± 0.08	3.7 ± 0.11	3.65 ± 0.09

Table 10a. Serum globulin levels (g/dl) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	2.42 ± 0.32	2.53 ± 0.35	2.45 ± 0.27	2.03 ± 0.26	2.5 ± 0.30	2.43 ± 0.35	2.45 ± 0.29
II	1.7 ± 0.14	1.65 ± 0.15	1.68 ± 0.15	1.45 ± 0.10	1.32 ± 0.0065	1.25 ± 0.10	1.2 ± 0.13
III	1.23 ± 0.19	1.32 ± 0.17	1.5 ± 0.19	1.27 ± 0.14	1.28 ± 0.13	1.25 ± 0.17	1.4 ± 0.14

Table 11a. Albumin-Globulin ratio – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
I	1.62 ± 0.18	1.5 ± 0.14	1.5667 ± 0.17	1.9 ± 0.27	1.58 ± 0.18	1.63 ± 0.21	1.62 ± 0.20
II	2.18 ± 0.13	2.28 ± 0.2	2.15 ± 0.20	2.28 ± 0.0083	2.57 ± 0.0092	2.67 ± 0.19	2.67 ± 0.20
III	3.5 ± 0.49	3.11 ± 0.36	2.67 ± 0.41	3.17 ± 0.41	3.17 ± 0.41	3.33 ± 0.55	2.75 ± 0.27

Table 12a. Volume of Packed Red Cells (%) – Comparison of Group II and III with Group I (Control) Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	45.33 ± 1.26	44 ± 1.49	45 ± 1.15	45.5 ± 1.26	45.33 ± 1.23
II	44.17 ± 1.49	39.17 ± 1.42*	40.33 ± 1.33	41 ± 1.13*	39.5 ± 0.89*
III	46 ± 1.39	41.33 ± 1.17	39.67 ± 0.80*	38.67 ± 0.76**	37.33 ± 0.80**

**P<0.01, *P<0.05

Table 13a. Haemoglobin concentration (g/dl) - Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	13.57 ± 0.38	13.28 ± 0.40	13.72 ± 0.30	13.45 ± 0.40	13.93 ± 1.66
II	13.17 ± 0.48	12.50 ± 0.45	12.0 ± 0.53*	12.67 ± 0.33	12.08 ± 0.35
III	13.65 ± 0.58	13.5 ± 0.79	13.87 ± 0.68	14.85 ± 0.69	14.8 ± 0.19

Table 14a. RBC count (millions/mm^3) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	8.93 ± 0.30	8.9 ± 0.24	8.78 ± 0.25	8.73 ± 0.23	8.91 ± 0.28
II	9.71 ± 0.29	9.22 ± 0.21	8.93 ± 0.21	8.45 ± 0.22	8.25 ± 0.23
III	7.36 ± 1.06	7.64 ± 1.02	7.87 ± 0.99	7.45 ± 0.77	7.19 ± 0.74

Table 15a. MCV values (μm^3) – Comparison of Group II and III with Group I (Control). Mean±S.E. (*n=6*)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	51.0 ± 1.79	50 ± 1.03	51.35 ± 1.31	52.22 ± 1.48	50.92 ± 1.46
II	45.62 ± 1.70	42.53 ± 1.65	45.23 ± 1.53	48.57 ± 1.13	47.88 ± 0.46
III	51.52 ± 1.70	49.1 ± 1.96	49.77 ± 2.42	51 ± 1.55	51.08 ± 2.02

Table 16a. MCH values (µg) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	12.08 ± 0.30	11.92 ± 0.24	12 ± 0.22	11.92 ± 0.20	12.08 ± 0.15
II	13.65 ± 0.65	13.58 ± 0.57	13.45 ± 0.42	15 ± 0.40	14.68 ± 0.41
III	12.25 ± 0.44	11.33 ± 0.36	11.5 ± 0.37	11.25 ± 0.38	10.83 ± 0.31

Table 17a. MCHC values (g%) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	26.73 ± 0.85	27.12 ± 0.69	26.76 ± 0.85	26.28 ± 0.82	26.77 ± 0.83
II	30.03 ± 1.63	32.45 ± 1.83	29.98 ± 1.32	30.95 ± 0.71	30.62 ± 0.89
III	26.68 ± 0.66	27.53 ± 1.20	29 ± 1.21	29.12 ± 0.84	29.05 ± 0.62

Table 18a. Total leucocyte count (numbers/cumm) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	5758.33 ± 463.93	5908.33 ± 356.23	5841.67 ± 378.24	5800 ± 414.93	5775 ± 430.84
II	5133.33 ± 474.28	7825.0 ± 603.98*	6133.33 ± 475.51	6050 ± 759.39	5650 ± 437.23
III	5941.66 ± 576.53	6675 ± 452.72	7108.33 ± 420.20	7358.33 ± 357.64*	7650 ± 358*

*P<0.05

Table 19a. Lymphocyte Count (%) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	55.33 ± 1.41	55.67 ± 1.20	55.5 ± 1.48	55.67 ± 1.5	55.33 ± 1.26
II	56.5 ± 0.92	66.33 ± 0.88*	66.67 ± 1.20*	66.83 ± 0.70*	66.33 ± 0.56*
III	54.67 ± 1.40	68.83 ± 0.87*	69 ± 0.63*	66.00 ± 0.93*	65 ± 1.29*

*P<0.05

Table 20a. Neutrophil Count (%) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	44 ± 1.13	43.67 ± 1.15	44.0 ± 1.59	43.83 ± 1.47	44.0 ± 1.03
II	42.5 ± 0.80	33.17 ± 1.01*	32.67 ± 1.02*	32.5 ± 0.76*	33 ± 0.45*
III	44.67 ± 1.52	31.67 ± 1.45*	30.67 ± 0.61*	33.33 ± 1.11*	34.65 ± 1.56*

*P<0.05

Table 21a. Eosinophil Count (%) – Comparison of Group II and III with Group I (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
I	0.67 ± 0.33	0.67 ± 0.33	0.5 ± 0.22	0.5 ± 0.22	0.67 ± 0.33
II	1.0 ± 0.26	0.5 ± 0.22	0.67 ± 0.21	0.67 ± 0.33	0.67 ± 0.21
III	0.83 ± 0.30	0.5 ± 0.22	0.33 ± 0.21	0.67 ± 0.21	0.5 ± 0.30

Table 1b. Serum ALT levels (u/l) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	54.67 ± 4.62	56 ± 4.57	56 ± 3.61	56.67 ± 3.5	55.67 ± 3.34	56.5 ± 3.91	55.83 ± 3.63
V	55.5 ± 5.69	85.5 ± 8.05*	126.5 ± 10.55**	136 ± 13.06**	150.17 ± 12.81**	162.67 ± 8.09**	170.5 ± 5.54**
VI	41 ± 3.82	42.33 ± 1.97	41.5 ± 2.77	45 ± 1.98	40.17 ± 0.60	44.67 ± 2.09	41.67 ± 3.17

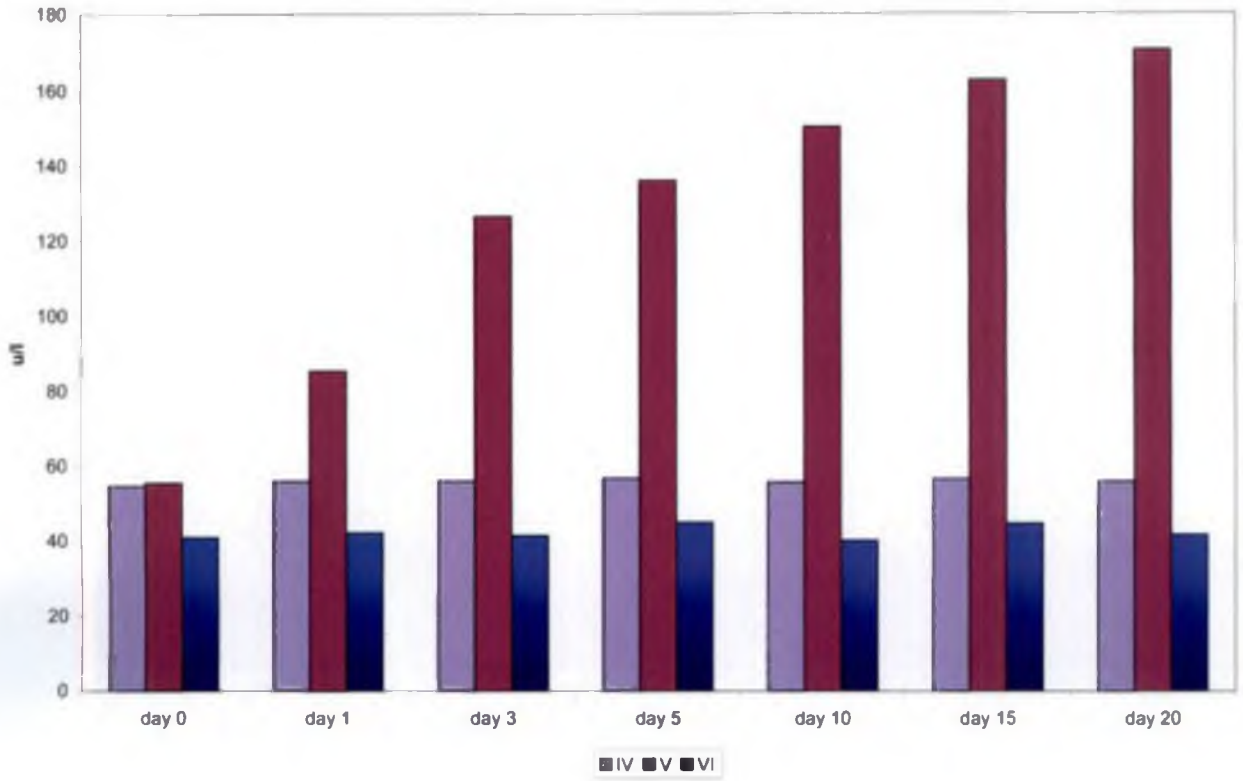
**P<0.01, *P<0.05

Table 2b. Serum AST levels (u/l) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	50.17 ± 3.11	49.5 ± 2.58	49.83 ± 3.0	50.5 ± 3.21	50.83 ± 2.44	51.17 ± 2.65	50.5 ± 2.74
V	51 ± 3.49	110 ± 8.14**	154.17 ± 15.08**	167.67 ± 17.45**	188.5 ± 11.65**	174.5 ± 5.37**	186.83 ± 5.78**
VI	35.5 ± 2.81	38 ± 1.67	38.17 ± 2.07	46.5 ± 2.65	41.83 ± 1.77	43.83 ± 1.32	38.17 ± 2.27

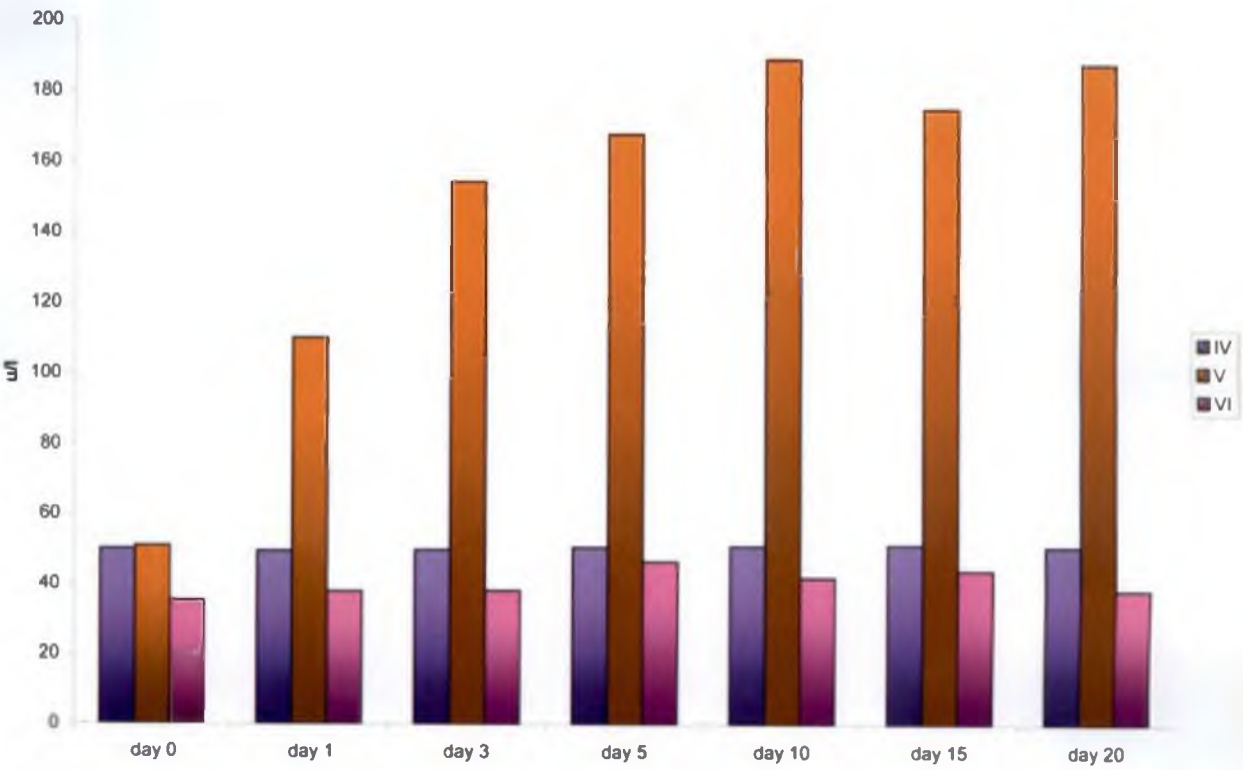
**P<0.01

Fig.25. Effect of pooled toxic fraction of *M.invisa* on serum ALT levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Fig.26. Effect of pooled toxic fraction of *M.invisa* on serum AST levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Table 3b. Serum GGT levels (u/l) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	5.5 ± 0.56	5.67 ± 0.42	5.5 ± 0.43	5.5 ± 0.43	5.17 ± 0.31	5.17 ± 0.48	5.33 ± 0.56
V	5.5 ± 0.56	6.67 ± 0.42	8.17 ± 0.60**	9.83 ± 0.87**	10.67 ± 0.33**	11.5 ± 0.22**	11.0 ± 0.26**
VI	4.33 ± 0.42	4.00 ± 0.26	4.00 ± 0.26	3.67 ± 0.21	4.00 ± 0.26	4.5 ± 0.43	4.5 ± 0.34

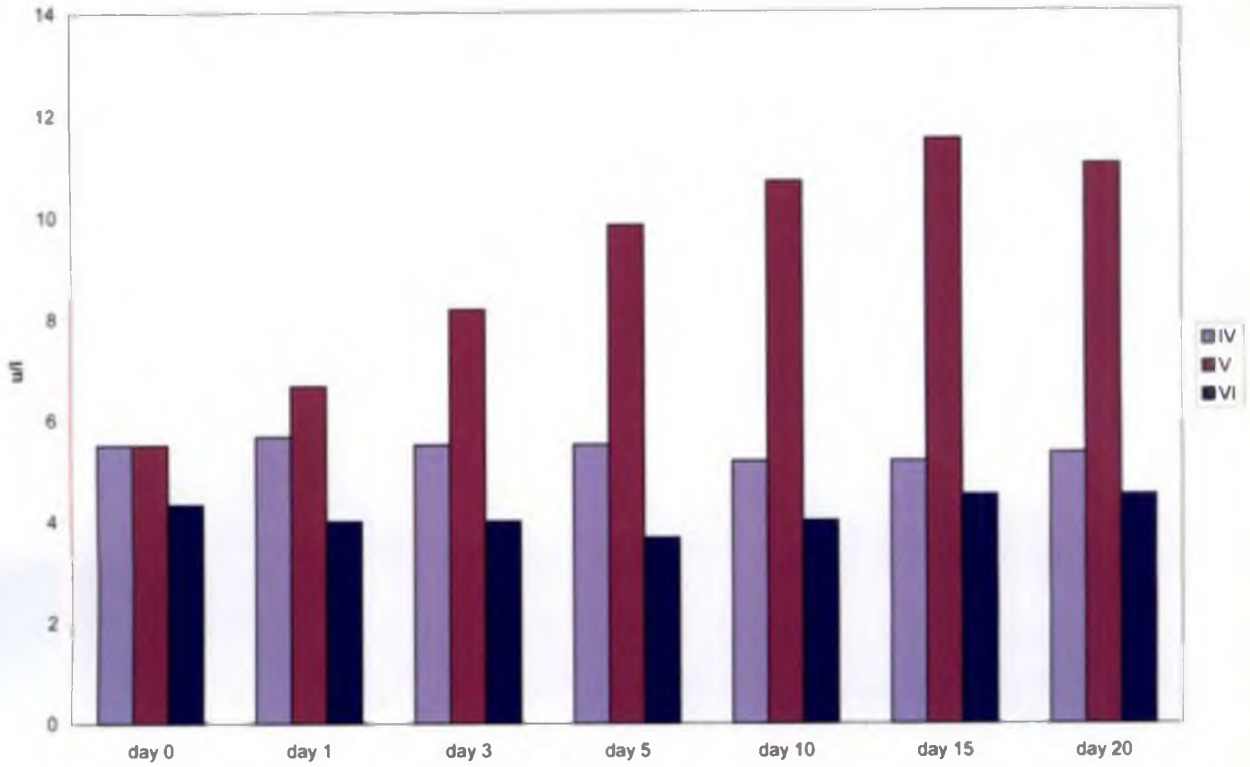
**P<0.01

Table 4b. Serum CK levels (u/l) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	132.67 ± 7.0	134.5 ± 6.39	129.67 ± 6.63	135 ± 6.64	132 ± 7.89	133.5 ± 5.6	131.5 ± 7.0
V	133.83 ± 10.11	170.67 ± 15.6*	199.33 ± 13.76*	215.33 ± 15.81**	170.33 ± 6.98**	161.5 ± 3.28*	140.5 ± 3.43
VI	164.83 ± 14.98	166.83 ± 10.49	166 ± 14.6	156.5 ± 8.80	166.83 ± 8.05	158.67 ± 9.90	168.5 ± 12.83

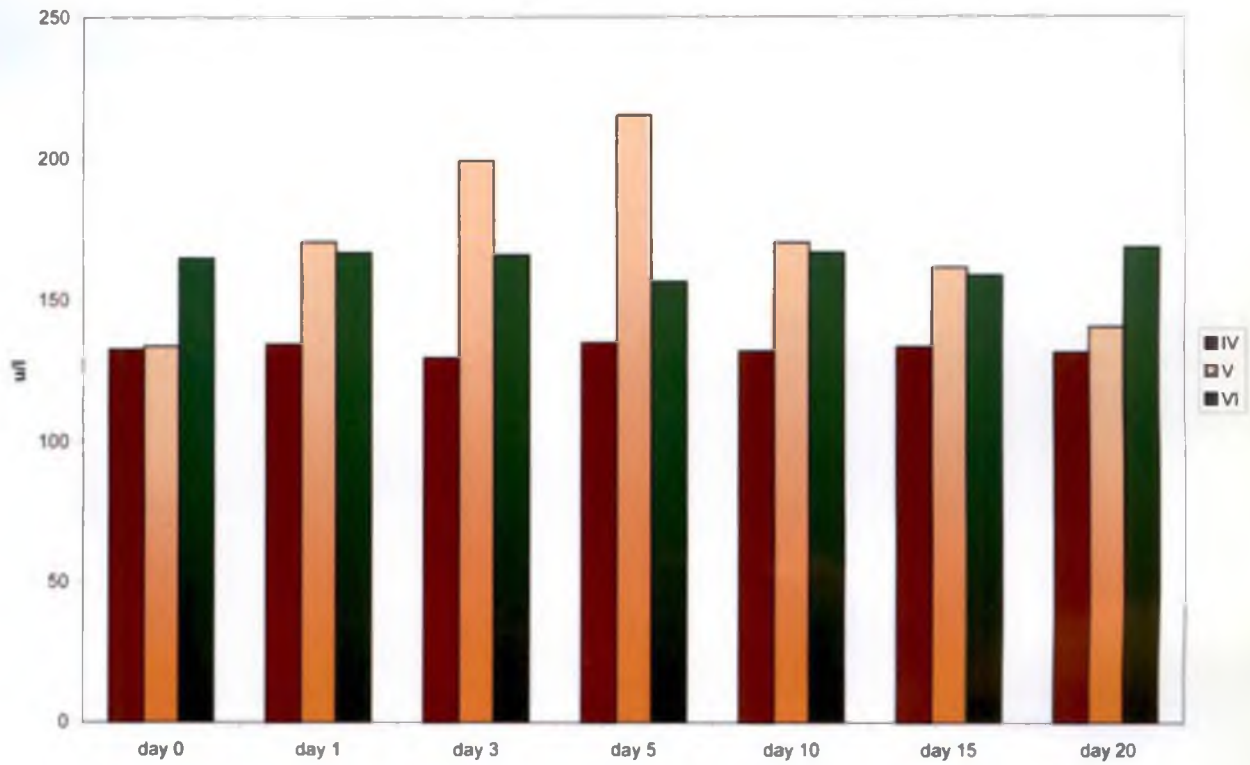
**P<0.01, *P<0.05

Fig.27. Effect of pooled toxic fraction of *M.invisa* on serum GGT levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Fig.28. Effect of pooled toxic fraction of *M.invisa* on serum creatine kinase levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Table 5b. Serum alkaline phosphatase levels (u/l) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

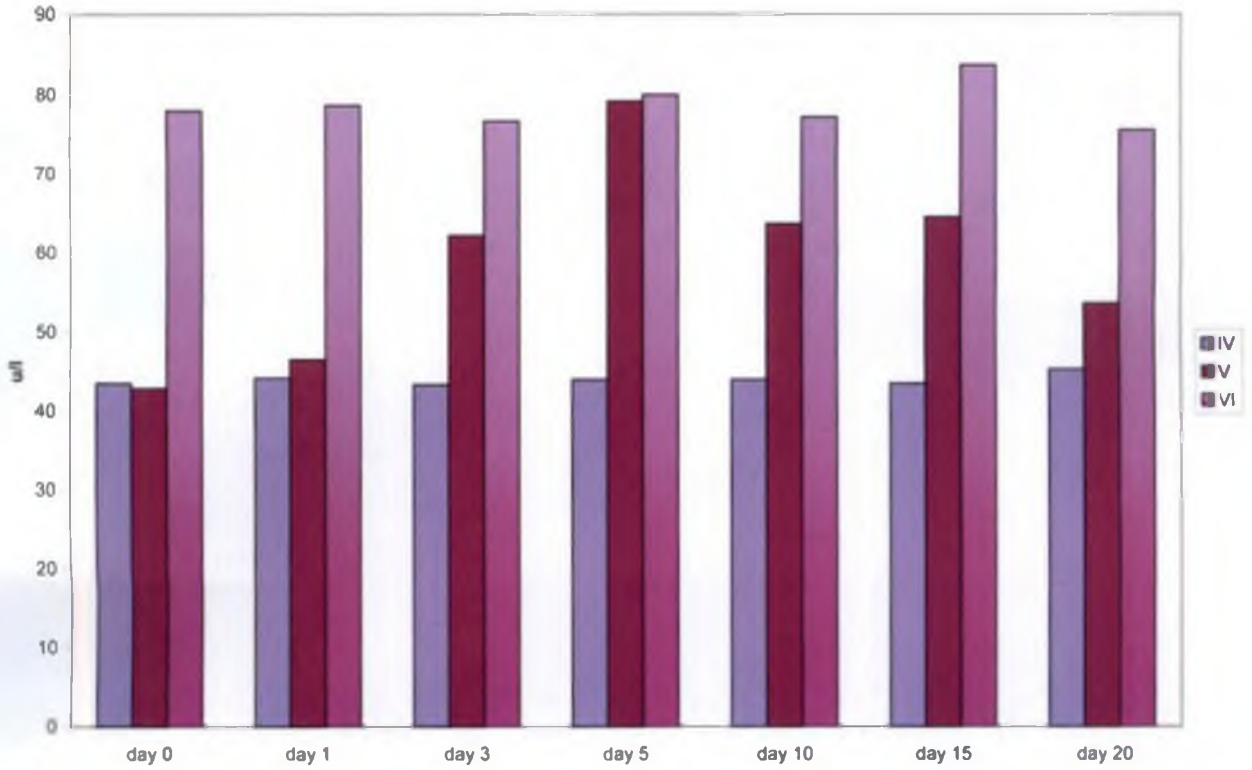
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	43.5 ± 9.19	44.17 ± 8.45	43.33 ± 8.48	44 ± 8.02	44 ± 8.71	43.5 ± 8.53	45.33 ± 8.72
V	42.83 ± 10.64	46.5 ± 11.05	62.17 ± 12.25	79.17 ± 14.58	63.67 ± 10.63	64.5 ± 9.59	53.67 ± 8.19
VI	78 ± 5.56	78.67 ± 4.84	76.67 ± 2.43	80 ± 3.97	77.17 ± 3.30	83.67 ± 6.06	75.5 ± 6.0

Table 6b. Serum creatinine levels (mg/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	2.5 ± 0.22	2.33 ± 0.21	2.67 ± 0.21	2.50 ± 0.22	2.67 ± 0.21	2.67 ± 0.21	2.5 ± 0.22
V	2.17 ± 0.31	4.67 ± 0.33*	7.17 ± 0.40**	8.33 ± 0.49**	9.83 ± 0.31**	10.80 ± 0.40**	12.17 ± 0.31**
VI	2.0 ± 0.37	2.33 ± 0.21	1.67 ± 0.33	1.67 ± 0.33	1.5 ± 0.22	2.33 ± 0.21	1.83 ± 0.31

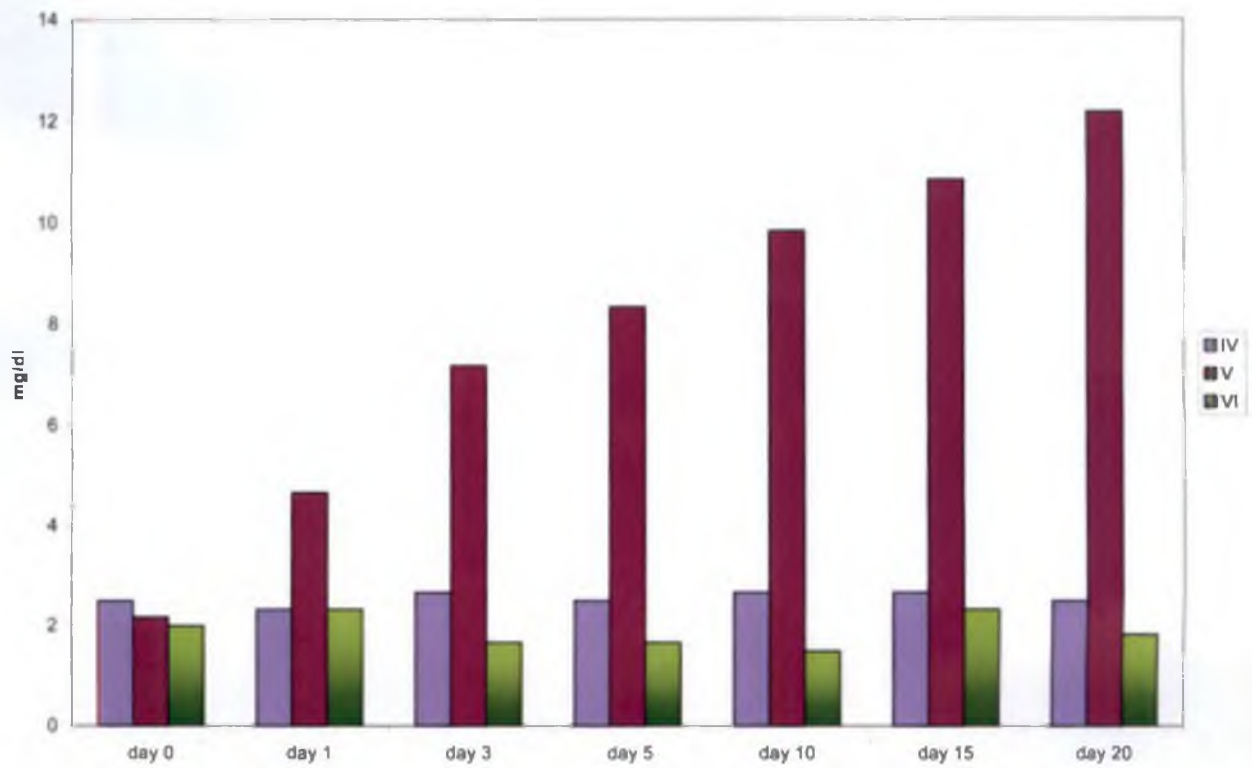
**P<0.01, *P<0.05

Fig.29. Effect of pooled toxic fraction of *M.invisa* on serum ALP levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Fig.30. Effect of pooled toxic fraction of *M.invisa* on serum creatinine levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Table 7b. Serum urea levels (mg/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

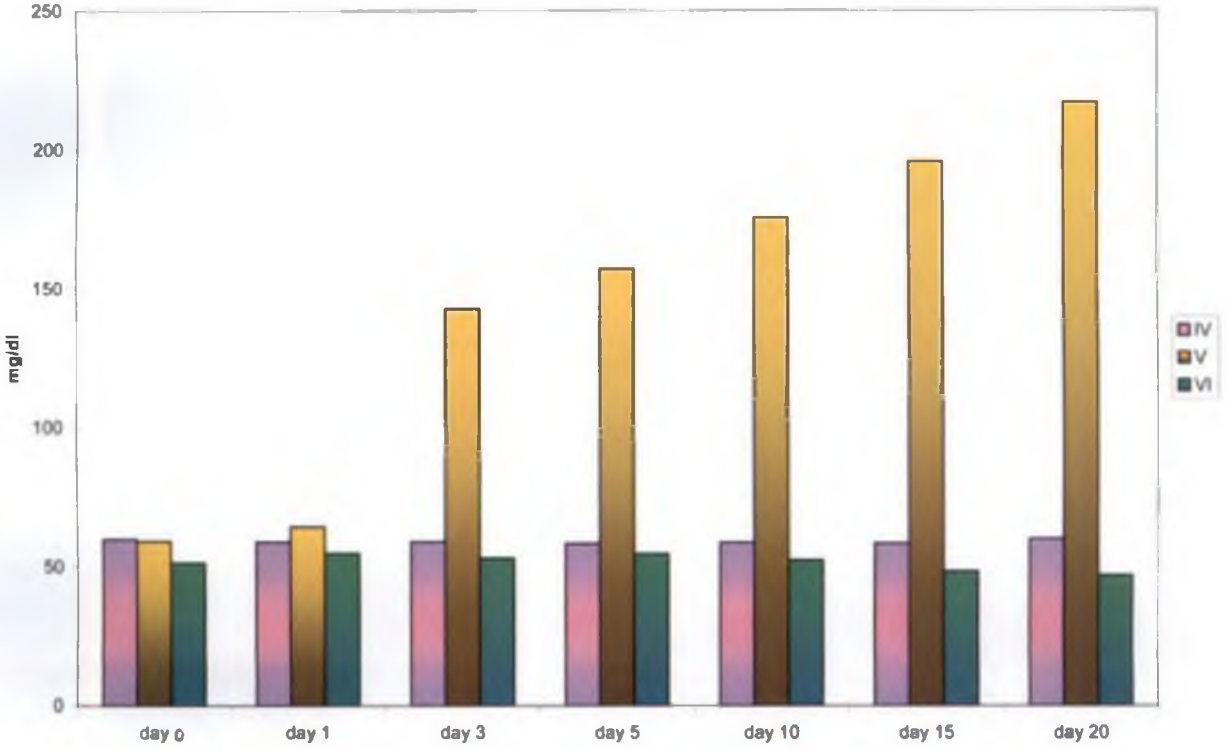
Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	59.83 ± 2.87	58.83 ± 2.41	59 ± 2.41	58.33 ± 2.73	58.67 ± 2.93	58.33 ± 2.90	60.00 ± 2.92
V	59.17 ± 3.7	64.33 ± 3.9	142.83 ± 26.2*	157.33 ± 23.92**	175.67 ± 12.07**	195.83 ± 8.99**	217.0 ± 10.96**
VI	51.33 ± 5.17	54.83 ± 1.96	53 ± 2.9	54.67 ± 3.1	52.33 ± 4.26	48.33 ± 4.26	46.83 ± 4.44

**P<0.01, *P<0.05

Table 8b. Serum total protein levels (g/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	5.58 ± 0.15	5.52 ± 0.14	5.53 ± 0.14	5.5 ± 0.13	5.5 ± 0.15	5.58 ± 0.14	5.62 ± 0.15
V	5.6 ± 0.15	5.37 ± 0.15	5.95 ± 0.11	5.67 ± 0.29	5.45 ± 0.21	5.37 ± 0.22	5.4 ± 0.16
VI	5.75 ± 0.15	5.73 ± 0.12	5.48 ± 0.14	5.48 ± 0.16	5.23 ± 0.15	5.65 ± 0.09	5.58 ± 0.14

Fig.31. Effect of pooled toxic fraction of *M.invisa* on serum urea levels



IV - Control
V - Pooled toxic fraction of *M. invisa* - 0.4g/kg
VI - Pooled toxic fraction of *M.invisa* - 0.2g/kg

Table 9b. Serum albumin levels (g/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	3.3 ± 0.00	3.3 ± 0.00	3.27 ± 0.00	3.28 ± 0.00	3.3 ± 0.00	3.3 ± 0.00	3.32 ± 0.00
V	3.58 ± 0.14	3.47 ± 0.11	3.48 ± 0.17	3.5 ± 0.09	3.47 ± 0.06	3.73 ± 0.11	3.67 ± 0.12
VI	3.7 ± 0.11	3.75 ± 0.10	3.52 ± 0.12	3.45 ± 0.07	3.7 ± 0.05	3.57 ± 0.08	3.5 ± 0.03

Table 10b. Serum Globulin levels (g/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	1.52 ± 0.16	1.57 ± 0.14	1.52 ± 0.14	1.55 ± 0.14	1.6 ± 0.17	1.47 ± 0.00	1.48 ± 0.12
V	1.97 ± 0.18	1.93 ± 0.22	1.57 ± 0.17	1.75 ± 0.23	1.87 ± 0.19	2.3 ± 0.22	2.18 ± 0.20
VI	1.65 ± 0.00	1.63 ± 0.148	1.6 ± 0.16	1.63 ± 0.13	1.68 ± 0.11	1.58 ± 0.00	1.52 ± 0.19

Table 11b. Albumin-Globulin ratio – Comparison of Group V and VI with Group IV (Control). Mean±S.E . (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
IV	2.83 ± 0.18	2.22 ± 0.17	2.27 ± 0.17	2.22 ± 0.16	2.13 ± 0.19	2.28 ± 0.15	2.30 ± 0.16
V	1.92 ± 1.9	1.90 ± 0.5	2.37 ± 0.19	2.18 ± 0.29	1.95 ± 0.21	1.70 ± 0.15	1.92 ± 0.19
VI	2.05 ± 0.19	1.98 ± 0.18	1.88 ± 0.16	2.03 ± 0.21	1.58 ± 0.18	2.07 ± 0.00	2.0 ± 0.15

Table 12b. Volume of Packed Red Cells (%) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	51.17 ± 0.98	50.83 ± 0.48	50.50 ± 0.62	51.0 ± 0.97	51 ± 0.68
V	51.17 ± 1.14	46.5 ± 1.23*	43.83 ± 1.54**	43 ± 1.50**	41.80 ± 2.07*
VI	49.33 ± 0.98	49.33 ± 1.36	49.5 ± 1.28	50.33 ± 1.31	49 ± 1.03

*P<0.05, **P<0.01

Table 13b. Haemoglobin concentration (g/dl) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	15.33 ± 0.25	15.25 ± 0.31	15.08 ± 0.24	15.33 ± 0.25	15.17 ± 0.28
V	15.08 ± 0.32	14.25 ± 0.28	13.42 ± 0.15	12.58 ± 0.20*	11.82 ± 0.15**
VI	14.42 ± 0.27	14.5 ± 0.34	14.42 ± 0.27	14.41 ± 0.27	14.92 ± 0.4

**P<0.01, *P<0.05

Table 14b. RBC count (millions/mm^3) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	11.33 ± 0.20	11.16 ± 0.15	114.26 ± 0.14	11.20 ± 0.21	11.37 ± 0.19
V	10.99 ± 0.39	10.83 ± 0.28	9.5 ± 0.19	9.63 ± 0.34	10.57 ± 0.52
VI	8.24 ± 0.34	8 ± 0.33	7.96 ± 0.42*	8.16 ± 0.38	8.29 ± 0.36

*P<0.05

Table 15b. MCV values.(μm^3) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	45.28 ± 1.33	45.63 ± 1.00	44.83 ± 0.98	45.63 ± 1.14	44.92 ± 1.06
V	46.87 ± 2.13	42.98 ± 1.28	46.4 ± 1.59	43.4 ± 1.93	38.33 ± 2.38
VI	59.92 ± 0.89	62.02 ± 1.52	62.68 ± 1.94	62 ± 1.40	59.4 ± 1.51

Table 16b. MCH values (µg) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	13.57 ± 0.32	13.68 ± 0.34	13.42 ± 0.30	13.72 ± 0.37	13.33 ± 0.29
V	14.03 ± 0.62	13.05 ± 0.31	14.15 ± 0.30	12.78 ± 0.41	11.37 ± 0.78
VI	17.56 ± 0.61	18.23 ± 0.50	18.45 ± 0.98	18.02 ± 0.69	17.72 ± 0.61

Table 17b. MCHC values (g%) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	30.02 ± 0.69	30.0 ± 0.62	29.9 ± 0.69	30.07 ± 0.42	29.77 ± 0.61
V	29.38 ± 0.47	30.35 ± 0.59	30.75 ± 0.91	29.58 ± 0.85	29.22 ± 0.58
VI	29.38 ± 0.050	29.4 ± 0.47	29.36 ± 0.65	28.87 ± 0.66	29.77 ± 0.23

Table 18b. Total leucocyte count (number/cumm) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	8451.67 ± 467.51	8600 ± 447.58	8500 ± 363.55	8541.67 ± 366.83	8525 ± 419.87
V	7481.67 ± 613.78	7891.67 ± 486.38	8416.67 ± 766.12	8408.33 ± 467.87	8708.33 ± 482.77
VI	6400 ± 462.78	6441.67 ± 401.13	6325 ± 417.68	6325 ± 457.12	6366.67 ± 394.69

Table 19b. Lymphocyte Count (%) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	58.17 ± 1.53	57.33 ± 0.71	57.83 ± 0.95	57.33 ± 1.15	57.17 ± 1.33
V	56.67 ± 1.23	67.83 ± 1.49*	68.83 ± 1.14*	68 ± 1.03*	65.5 ± 1.31*
VI	57.83 ± 1.01	54.5 ± 1.91*	57 ± 1.91*	54.83 ± 1.25*	57.33 ± 0.92*

*P<0.05

Table 20b. Neutrophil Count (%) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	41.0 ± 1.24	42.0 ± 0.89	41.67 ± 1.05	42.17 ± 0.98	42.0 ± 1.24
V	42.33 ± 1.15	31.5 ±1.31	30.5 ± 1.17	31.33 ± 0.95	33.83 ± 1.19
VI	41.83 ± 0.88	44.83 ± 1.97	42.67 ± 1.82	44.67 ± 1.09	41.83 ± 0.87

Table 21b. Eosinophil Count (%) – Comparison of Group V and VI with Group IV (Control). Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
IV	0.83 ± 0.31	0.67 ± 0.21	0.50 ± 0.22	0.50 ± 0.22	0.83 ± 0.31
V	1.00 ± 0.26	0.67 ± 0.33	0.67 ± 0.21	0.67 ± 0.21	0.67 ± 0.26
VI	0.83 ± 0.31	0.67 ± 0.21	0.33 ± 0.21	0.50 ± 0.22	0.83 ± 0.17

Table 22. Effect of decoction on serum ALT levels (u/l), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	55.5 ± 5.69	85.5 ± 8.05**	126.5 ± 10.55**	136 ± 13.6**	150.17 ± 12.81**	162.67 ± 8.09**	170.5 ± 5.54**
VIII	37 ± 2.72	110 ± 3.6**	74.83 ± 3.64**	65.67 ± 4.51*	49.83 ± 0.6	42 ± 0.82	39 ± 2.02

**P<0.01, *P<0.05

Table 23. Effect of decoction on serum AST levels (u/l), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	51 ± 3.49	110 ± 8.14**	154.17 ± 15.01**	167.67 ± 17.45**	188.5 ± 11.65**	174.5 ± 5.37**	186.83 ± 5.78**
VIII	45.17 ± 1.20	81.67 ± 2.68*	82 ± 3.48*	66 ± 2.10	57.83 ± 2.37	42.17 ± 1.33	42.83 ± 1.91

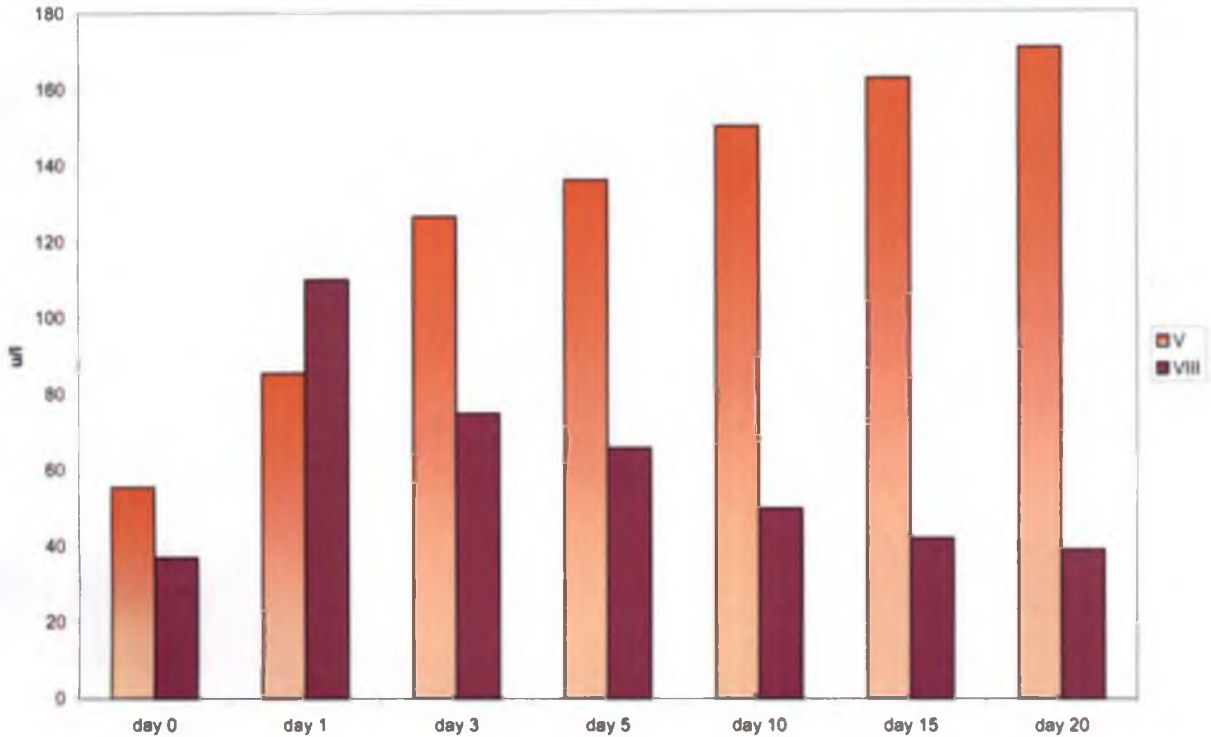
**P<0.01, *P<0.05

Table 24. Effect of decoction on serum GGT levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	5.5 ± 0.56	6.67 ± 0.42	8.17 ± 0.60**	9.83 ± 0.87**	10.67 ± 0.33**	11.5 ± 0.22**	11.0 ± 0.26**
VIII	4.83 ± 0.40	5.0 ± 0.52	5.0 ± 0.45	6.5 ± 0.34	6.17 ± 0.48	6.83 ± 0.31	4.5 ± 0.34

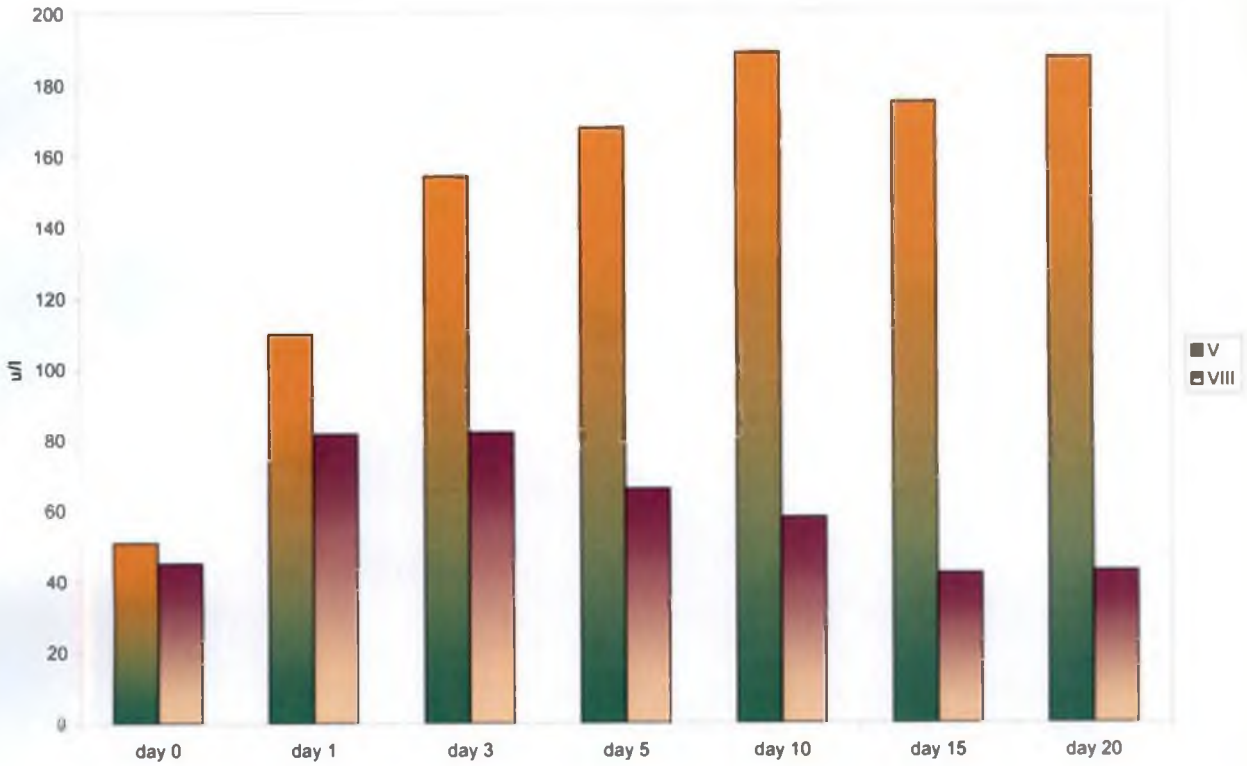
**P<0.01

Fig.35. Effect of decoction on serum ALT levels



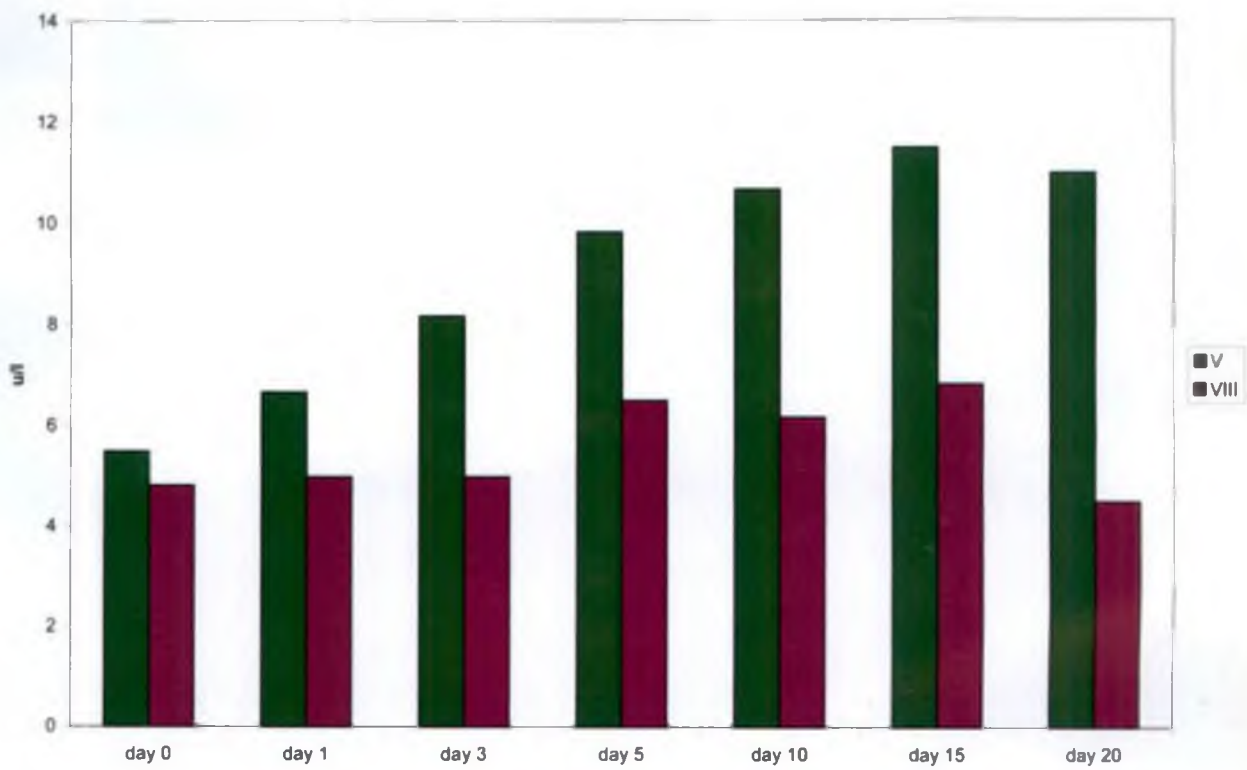
V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Fig. 36. Effect of decoction on serum AST levels



V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Fig.37. Effect of decoction on serum GGT levels



V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Table 25. Effect of decoction on serum Creatine kinase (u/l), Mean±S.E. (n=6).

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	133.83 ± 10.11	170.67 ± 15.6**	199.33 ± 13.76**	215.33 ± 15.81**	170.33 ± 6.98**	161.5 ± 3.28	140.5 ± 3.43
VIII	199.67 ± 6.04	211 ± 3.99	201.5 ± 4.2	204 ± 4.54	197.5 ± 4.9	200.5 ± 4.17	200.83 ± 5.67

**P<0.01

Table 26. Effect of decoction on serum Alkaline Phosphatase levels (u/l), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	42.83 ± 10.64	46.5 ± 11.05	62.17 ± 12.25*	79.67 ± 14.58*	63.67 ± 10.63*	64.5 ± 9.59*	53.67 ± 8.19
VIII	50.67 ± 5.18	84 ± 4.49*	53.83 ± 2.3	52.5 ± 2.05	53.5 ± 2.05	47.5 ± 2.26	47.17 ± 1.66

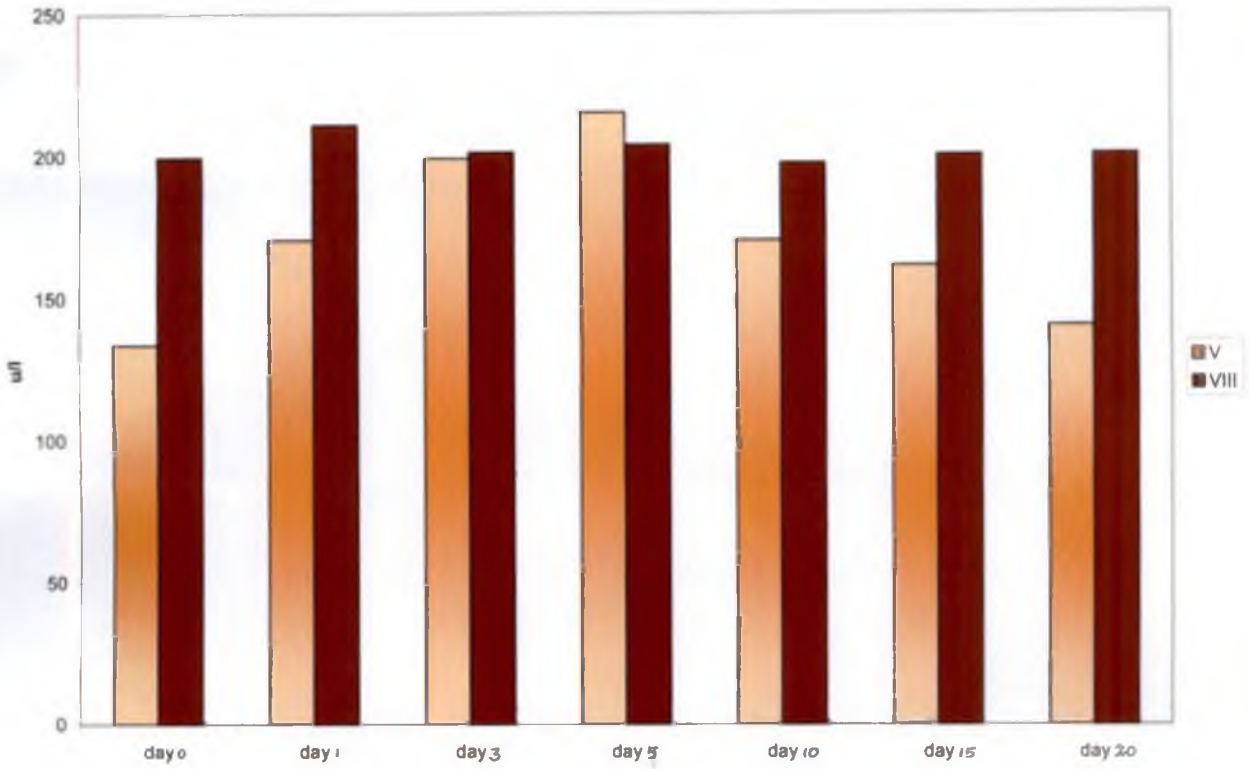
*P<0.05

Table 27. Effect of decoction on serum Creatinine levels (mg/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	2.17 ± 0.31	4.67 ± 0.33*	7.17 ± 0.40**	8.33 ± 0.49**	9.83 ± 0.31**	10.83 ± 0.40**	12.17 ± 0.31**
VIII	2.67 ± 0.21	4.5 ± 0.22*	5 ± 0.26*	5.5 ± 0.22*	4.83 ± 0.17*	4.67 ± 0.21*	2.5 ± 0.22

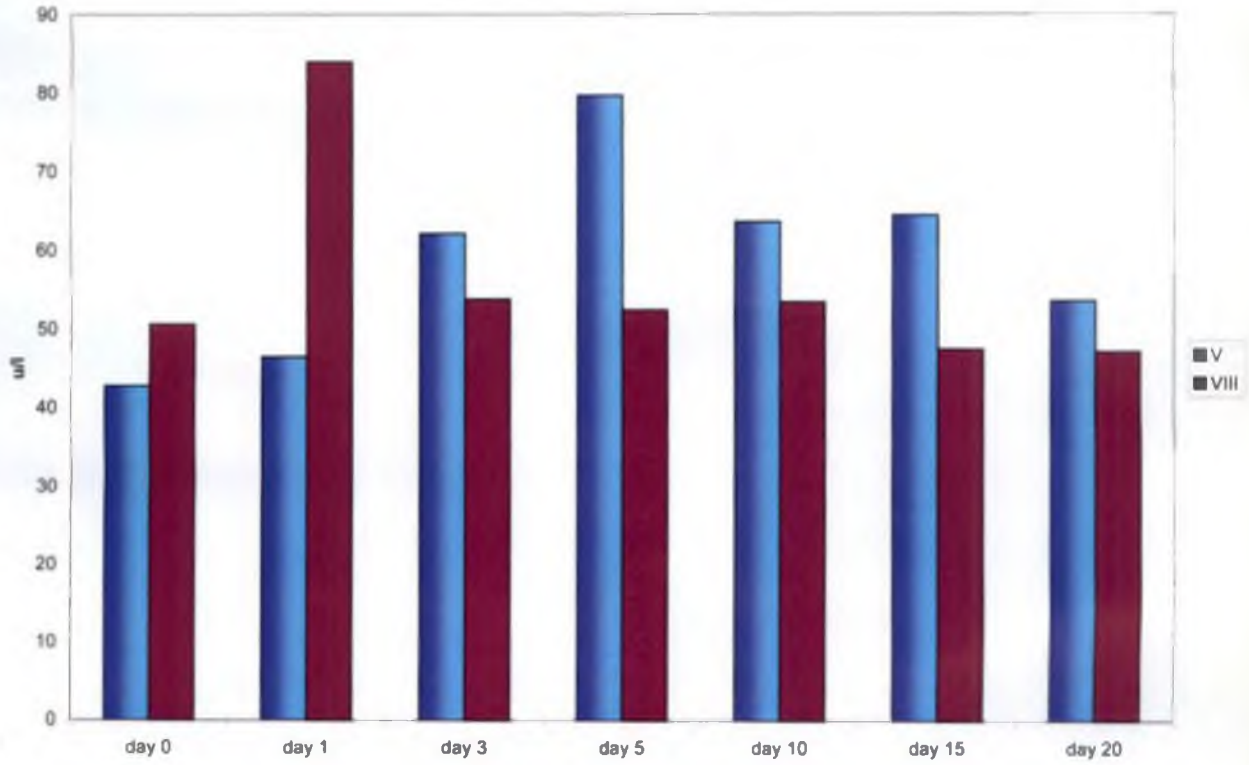
**P<0.01, *P<0.05

Fig.38. Effect of decoction on serum creatine kinase levels



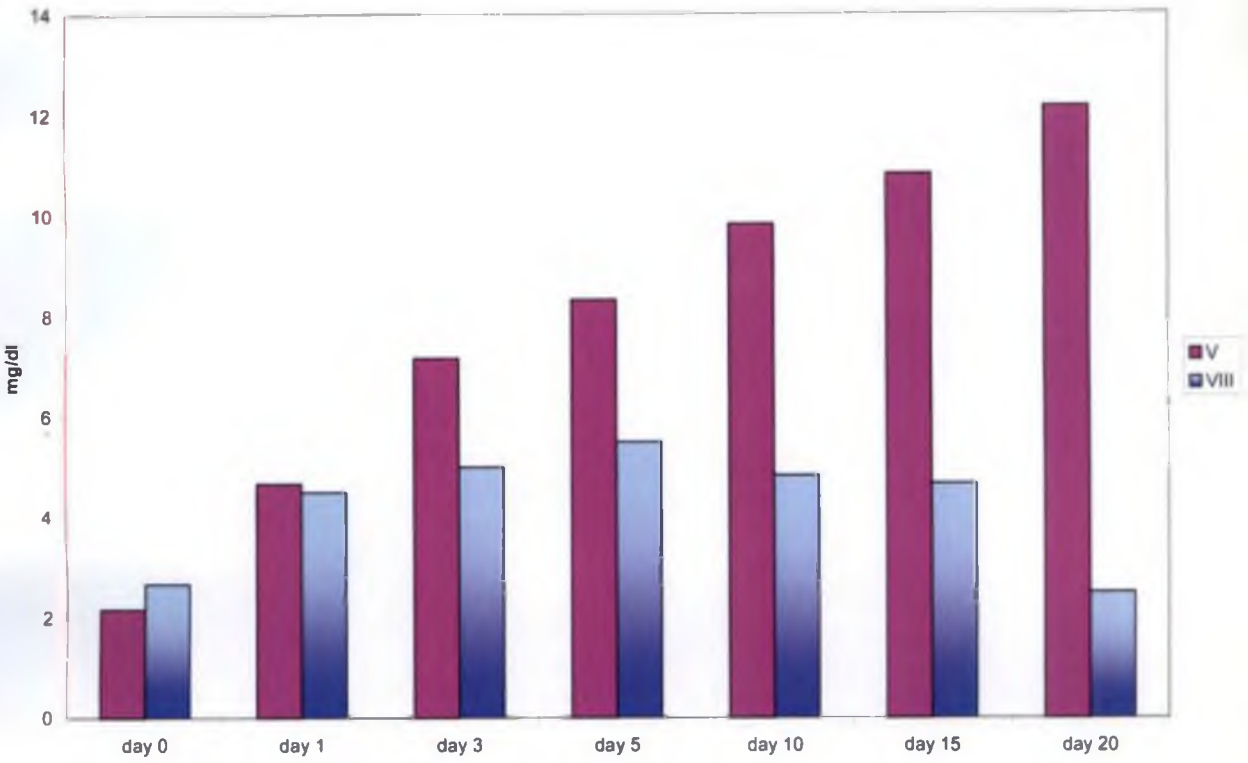
V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Fig.39. Effect of decoction on serum alkaline phosphatase levels



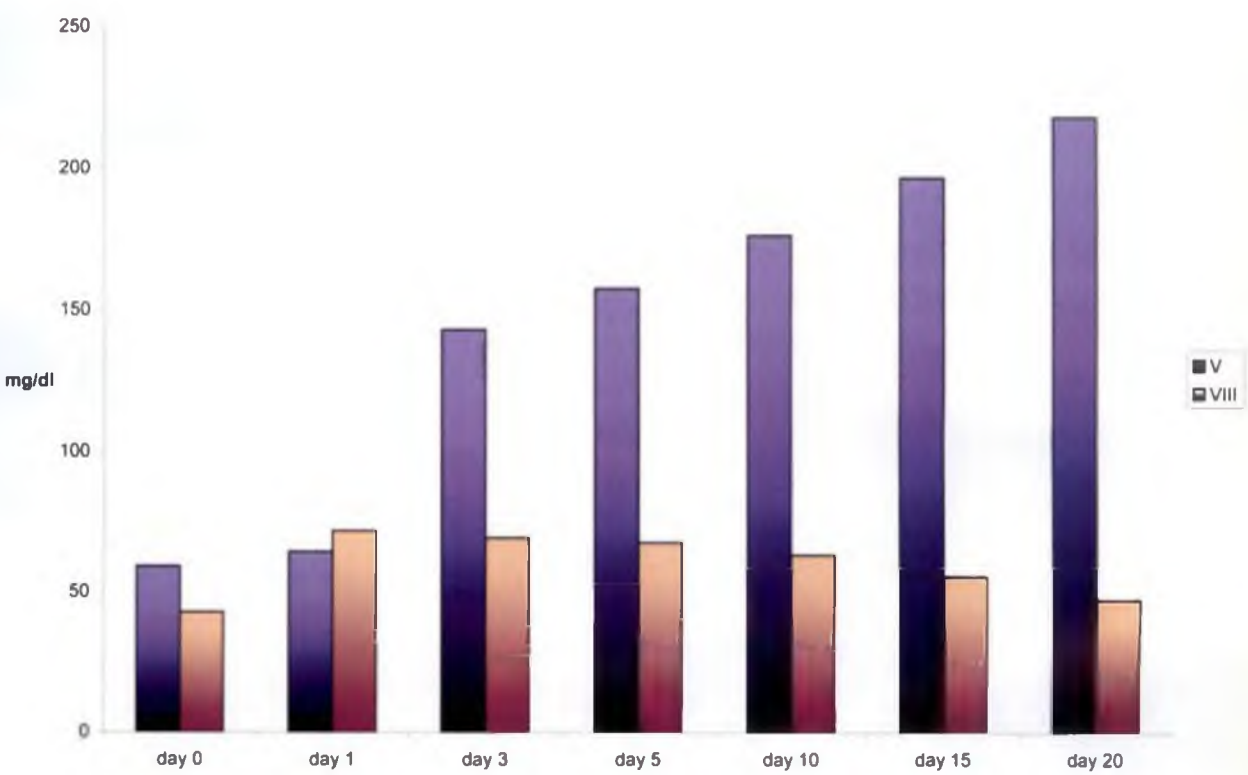
V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Fig.40. Effect of decoction on serum creatinine levels



V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Fig.41. Effect of decoction on serum urea levels



V - Pooled toxic fraction of *M.invisa* - 0.4g/kg
VIII - Pooled toxic fraction + decoction

Table 28. Effect of decoction on serum Urea levels (mg/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	59.17 ± 3.7	64.33 ± 3.90	142.83 ± 26.2**	157.33 ± 23.92**	175.67 ± 12.07**	195.83 ± 8.99**	217 ± 10.96**
VIII	42.83 ± 1.54	71.83 ± 4.71*	69.17 ± 2.12*	67.5 ± 2.36*	63 ± 1.61*	55.35 ± 3.48	47 ± 5.01

**P<0.01, *P<0.05

Table 29. Effect of decoction on serum Total Protein levels (g/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	5.6 ± 0.15	5.37 ± 0.15	5.95 ± 0.11	5.67 ± 0.29	5.45 ± 0.21	5.37 ± 0.22	5.4 ± 0.16
VIII	5.45 ± 0.15	5.42 ± 0.14	5.18 ± 0.04	5.22 ± 0.03	5.2 ± 0.03	5.12 ± 0.08	5.56 ± 0.14

Table 30. Effect of decoction on serum albumin levels (g/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	3.58 ± 0.14	3.47 ± 0.14	3.48 ± 0.17	3.5 ± 0.09	3.47 ± 0.06	3.73 ± 0.1	3.67 ± 0.12
VIII	3.55 ± 0.10	3.55 ± 0.10	3.53 ± 0.09	3.51 ± 0.09	3.5 ± 0.13	3.83 ± 0.11	3.67 ± 0.12

Table 31. Effect of decoction on serum globulin levels (g/dl), Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	1.97 ± 0.18	1.93 ± 0.22	1.57 ± 0.17	1.75 ± 0.23	1.87 ± 0.19	1.64 ± 0.22	1.73 ± 0.20
VIII	1.93 ± 0.34	1.95 ± 0.30	1.65 ± 0.14	1.71 ± 0.23	1.7 ± 0.24	1.29 ± 0.28	1.89 ± 0.12

Table 32. Effect of decoction on Albumin-Globulin ratio , Mean±S.E. (n=6)

Group	Day 0	Day 1	Day 3	Day 5	Day 10	Day 15	Day 20
V	1.92 ± 0.19	1.9 ± 0.5	2.37 ± 0.19	2.18 v 0.29	1.95 ± 0.21	1.70 ± 0.15	1.92 ± 0.19
VIII	2.02 ± 0.23	1.93 ± 0.20	1.65 ± 0.07	1.58 ± 0.12	1.7 ± 0.12	1.28 ± 0.11	1.9 ± 0.09

Table 33. Effect of decoction on VPRC (%) , Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	51.17 ± 1.14	46.5 ± 1.23**	43.83 ± 1.54**	43 ± 1.50**	41.83 ± 2.07**
VIII	49 ± 1.23	49.17 ± 1.45	47.5 ± 1.38	48.33 ± 1.50	49 ± 1.21

**P<0.01

Table 34. Effect of decoction on Haemoglobin Concentration (g/dl), Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	15.08 ± 0.32	14.25 ± 0.288*	13.42 ± 0.15**	12.58 ± 0.20**	11.82 ± 0.15**
VIII	14.33 ± 0.72	14.25 ± 0.57	14.33 ± 0.67	14 ± 0.45	14.08 ± 0.60

**P<0.01, *P<0.05

Table 35. Effect of decoction on Total Erythrocyte Count (millions/cumm), Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	10.99 ± 0.39	10.83 ± 0.28	9.5 ± 0.19**	9.63 ± 0.34*	10.5 ± 0.52
VIII	8.36 ± 0.34	8.49 ± 0.45	8.53 ± 0.35	8.38 ± 0.32	8.39 ± 0.28

**P<0.01, *P<0.05

Table 36. Effect of decoction on MCV (μm^3) , Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	46.87 ± 2.13	42.98 ± 1.28	46.4 ± 1.59	43.4 ± 1.93	38.33 ± 2.38
VIII	58.8 ± 1.09	58.37 ± 1.88	58.87 ± 0.96	57.77 ± 0.86	58.58 ± 1.42

Table 37. Effect of decoction on MCH (µg), Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	14.03 ± 0.62	13.05 ± 0.31	14.15 ± 0.30	12.78 ± 0.41	11.37 ± 0.78
VIII	16.87 ± 0.44	16.87 ± 0.42	16.77 ± 0.39	16.73 ± 0.25	16.78 ± 0.16

Table 38. Effect of decoction on MCHC (g%) , Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	29.38 ± 0.47	30.35 ± 0.59	30.75 ± 0.91	29.58 ± 0.85	29.22 ± 0.58
VIII	29.17 ± 0.80	28.97 ± 0.80	30.13 ± 0.71	28.98 ± 0.43	28.73 ± 0.82

Table 39. Effect of decoction on Total leucocyte count (numbers/cumm) , Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	7484.67 ± 613.78	7891.67 ± 486.38	8416.67 ± 766.12*	8408.33 ± 467.87*	8708.33 ± 482.77*
VIII	6316.67 ± 453.26	7233.33 ± 378.3*	6666.67 ± 372.53	6683.33 ± 486.26	6475 ± 483

*P<0.05

Table 40. Effect of decoction on Lymphocyte count (%), Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	56.67 ± 1.23	67.83 ± 1.49*	68.83 ± 1.14*	68.0 ± 1.03*	65.5 ± 1.31*
VIII	53.83 ± 1.25	57.00 ± 0.82	57.83 ± 0.60	57.5 ± 1.57	58.5 ± 1.20

*P<0.05

Table 41. Effect of decoction on Neutrophil Count (%) , Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	42.33 ± 1.15	31.5 ± 1.31*	30.5 ± 1.17*	31.33 ± 0.95*	33.83 ± 1.19*
VIII	45.67 ± 1.30	42.17 ± 1.01	41.67 ± 0.61	41.83 ± 1.62	40.83 ± 1.01

*P<0.05

Table 42. Effect of decoction on Eosinophil count (%), Mean±S.E. (n=6)

Group	Day 0	Day 5	Day 10	Day 15	Day 20
V	1.00 ± 0.26	0.67 ± 0.33	0.67 ± 0.21	0.67 ± 0.21	0.67 ± 0.26
VIII	0.5 ± 0.22	0.83 ± 0.31	0.50 ± 0.22	0.5 ± 0.22	0.5 ± 0.22

Discussion

5. DISCUSSION

The main objective of the present study was to identify the toxic fractions present in the alcoholic extract of *Mimosa invisa*, which is a toxic weed causing toxicity in cattle and goat. A toxicity study was also conducted using rabbit as models. In the same model system a treatment schedule utilizing indigenous medicinal plant decoction was also conducted with the objective of generating baseline information, which could be utilized in extrapolating the schedule in large animals.

5.1. EXTRACTS AND FRACTIONS

Preliminary studies in our laboratory indicated that oral administration of alcoholic extract prepared from dried plant material was ineffective in producing any toxicity in rabbits. However fresh juice could produce toxicity. This revealed that drying and heating may destroy the toxic principle present in *Mimosa invisa*. Hence for further studies fresh juice, alcoholic extract and pooled toxic fractions were utilized.

5.2. CLINICAL OBSERVATION

The animals dosed with 15 g/kg of fresh juice and 0.5 g/kg of alcoholic extract failed to produce toxicity indicating that the dose was insufficient to elicit toxicity. But surprisingly these animals developed resistance on continued administration of higher doses. The main clinical symptoms observed in group II (*M. invisa* fresh juice, 25 g/kg), III (*M. invisa* alcoholic extract, 1 g/kg) and V (pooled toxic fraction of *M. invisa*, 0.4 g/kg) were similar. Inappetence, dullness, reluctant to move and persistent recumbency were the symptoms, which could be attributed lack of feed and water intake (Rankins *et al.*, 1993). Similar observations were reported by Alex *et al.* (1991) in heifers. Alikutty and Pillai (1992) also reported reduced appetite in a clinical case of mimosa poisoning in a buffalo. Inappetence, loss of condition and recumbency were reported by

Barni *et al.* (1990) in *Abrus precatoris* poisoning. Group VI (pooled toxic fraction of *M. invisa*, 0.2 g/kg) did not show any symptoms of toxicity since the dose rate was too low to produce toxicity.

5.3. BIOCHEMICAL PARAMETERS

5.3.1. Serum ALT

The serum ALT levels were significantly increased and peak values were 212.5 ± 33.56, 185.33 ± 10.22, and 170.5 ± 5.54 u/l in Groups II, III and V respectively. Significant increase in ALT levels clearly indicated hepatotoxicity and damage to hepatocytes. The results of the present study also agree with the observation made by Flaoyen *et al.* (1997) in which they observed increase in ALT levels after feeding flower stem of *Narthecium ossifragum* which indicated the intrinsic hepatotoxicity of the plant. Kako, *et al.* (1993) observed increase in serum ALT levels after feeding chopped *Hypericum perforatum* plants to sheep for one week. Adams *et al.* (2000) also noticed increase in ALT levels after oral administration of *citrullus colocynthis* and *Rhazya stricta* in Najdi sheep.

The serum levels of ALT and AST became elevated whenever disease process affect cell integrity and ALT is more liver specific. Serum elevations of ALT activity is seen in parenchymal liver diseases (Burtis and Ashwood, 1996). Hence increase in serum ALT levels in the present study may be due damage to the liver parenchymal cells.

5.3.2. Serum AST

A significant increase in serum AST levels was noted in Group II (*Mimosa invisa* fresh juice), III (*Mimosa invisa* alcoholic extract) and V (pooled toxic fraction of *Mimosa invisa*). Highly significant changes ($P<0.01$) were noted in group V. The peak values observed were141.5±15.11, 123.67±7.97 and 188.5±11.65 u/l for groups II, III and V respectively. This also reflects hepatotoxicity. The ALT and AST are generally elevated in acute hepatic injury

(Kaneko *et al.*, 1997). Simliar observations were made by Loung and Mecoy (1992) who observed increased level of AST activity after experimentally induced C*assia obtusifolia* poisoning and Lugt *et al.* (1992) in experimentally induced *Cestrum laevigatum* poisoning in sheep. An increase in aspartate aminotransferase activity was reported by Flaoyen *et al.* (1995) after feeding a mixture of bog plants containing *Narthecium ossifragum* for two consecutive days in sheep. According to Burtis and Ashwood (1996) in liver diseases associated with hepatic necrosis, the levels of ALT and AST rises even before the start of clinical symptoms. An increased level of AST appears in serum after myocardial infarction also. In the present study also the ALT and AST levels increased which indicates liver damage.

5.3.3. Serum GGT

In Group II (*M. invisa* fresh juice), III (*M. invisa* alcoholic extract) and V (Pooled toxic fraction of *M. invisa*) GGT values reached the peak on 3rd day whereas for Group V the GGT values continued to increase till the 20th day. Craig *et al.* (1991) reported a continuous increase in GGT levels above control levels in toxicity due to pyrolizzidine alkaloids. Flaoyen *et al.* (1995) also noted an increase in serum GGT activity indicating hepatic dysfunction after feeding *Narthecium ossifragum* plants. GGT present in the serum appears to originate from hepatobiliary system and it is elevated in all forms of liver diseases (Burtis and Ashwood, 1996). The sensitivity and specificity of GGT as a screening test for liver disease was described by Curran *et al.* (1996).

5.3.4 Serum Creatine Kinase

The serum creatine kinase levels were found to be increased significantly on 3rd day of the experiment in the Group II and III, but the group V showed peak value on 5th day then regained to normal values by 20th day. Rankins *et al.* (1993) observed a similar increase in creatine kinase levels in Sachuiste *(Nolina microcarpa)* toxicity. A substantial increase in activity of creatine kinase in all

types of muscular dystrophies was reported by Burtis and Ashwood (1996). Creatine kinase activity is greatest in striated muscles, heart tissues, kidneys and diaphragm. Group VI did not show any variation in creatine kinase activity. The changes in creatine kinase levels could not be observed in group VI (half the toxic dose of pooled toxic fraction) because the dosage of pooled toxic fraction of *Mimosa invisa* is too low to produce any changes in creatine kinase activity.

5.3.5. Serum ALP

The serum ALP showed a significant increase ($P<0.05$) on 1st day of the experiment in Group II whereas in group III and V significant increase from 3rd day onwards. In all the above groups the values regained to normal by 20th day. An increase in serum ALP after ingestion of *Cistus salvifolius* in beef cattle was reported by Yeruham *et al.* (2002). Fan *et al.* (2005) also noticed increase in alkaline phosphatase when they studied the toxic effect of *Oxytropis glacialis* in goats. Group VI (half the toxic dose of pooled toxic fraction) did not show any changes, as the dosage administered is very low.

5.3.6. Serum Creatinine

The serum creatinine values of Group II (*M. invisa* fresh juice) and III (*M. invisa* alcoholic extract) exhibited maximum increase on 3rd day whereas for the group V (pooled toxic fraction) the values continued to increase till 20th day of the experiment. The group VI failed to produce any change in serum creatinine as the dosage of pooled toxic fraction of *Mimosa invisa* is only half the toxic dose. The results of the present study is in agreement with the work done by Ferriera *et al.* (1991) in which they reported increased levels of urea and creatinine in two outbreaks of amaranthus species poisoning in cattle. Flaoyen *et al.* (1994) found an increase in creatinine values after a single dose of *Narthecium ossifragum* in cattle and sheep. Flaoyen *et al.* (2001) reported tolerance to nephrotoxic component of *Narthecium ossifragum* in sheep. This finding is in agreement with the present study in which repeated administration

produced initial increase followed by decrease in creatinine value. Flaoyen *et al.* (1997) suggested that serum creatinine was more sensitive and reliable indicator of kidney function than serum urea values. Therefore the present finding of increased levels of creatinine indicate kidney damage. This also evident from the histopathological findings.

5.3.7. Serum Urea

The levels of urea significantly increased ($P<0.01$) throughout the experiment for Group II, III and V, even though there were decrease in values from 5th day onwards in Group II and III. Group V exhibited a continuous increase in serum urea levels, which indicate that the pooled toxic fraction of *Mimosa invisa* has no tendency for development of tolerance. However the rapid increase in both creatinine and urea level indicate typical impairment of kidney function (Flaoyen *et al.*, 2001). Yeruham *et al.* (2002) also reported increase blood urea levels after ingestion of *Cistus salvifolius* in beef cattle. Wisloff *et al.* (2003) reported elevated serum urea concentration in goats dosed with *Narthecium ossifragum*. The usefulness of urea as an independent indication of renal failure is limited because of the involvement of non-renal factors. Thus clinical utility is there, when it is measured along with creatinine (Burtis and Ashwood, 1996).

5.3.8. Serum Total Protein

There were no significant changes in serum protein levels when compared with zero day values and control values, even though a slight decrease is seen in Group II, III and V throughout the experiment. This observation was supported by the research done by Barni *et al.* (1990) who noticed decrease in serum protein level in *Abrus precatoris* toxicity in Nubian goats. Al-khafagi (1994) observed decrease in serum protein levels after administration of *Hypericum perforatum* in rabbits. This can be attributed to functional disturbances caused by damage to hepatacytes.

5.3.9. Serum Albumin

There were no significant differences in serum albumin levels after administration of *Mimosa invisa* fresh juice, alcoholic extract and various fractions. But a slight decrease was noticed in Group II, III, and slight increase was noted in group V. The decrease may be due to either albumin loss or failure of albumin synthesis. The liver is the only site of albumin synthesis and hypoalbuminaemia is an important feature of chronic liver disease (Kaneko *et al.*, 1997). The hypoalbuminemia in the present study may be interpreted as the injury to the liver due to toxin from *Mimosa invisa.*

5.3.10. Serum Globulin

The serum globulin levels did not show significant variations even though there were slight decrease noticed in groups II, III and V. Since half the toxic dose is administered in Group VI, no variations in serum globulin could be noticed.

5.3.11. Albumin-Globulin Rratio

There were no significant variations in Albumin-Globulin ratio in groups II, III, V and VI.

5.4. HAEMOGRAM

5.4.1. Volume of Packed Red Cells

The volume of packed red cells significantly decreased ($P<0.01$) in all mimosa treated groups except in Group VI, which showed normal values. This is in agreement with the findings of Fan *et al.* (2005) who observed decrease in PCV when goats are fed with *Oxytropis glacialis.* Low PCV levels are observed in liver damage, which is accompanied by anaemia. Sharma and Somvanshi (2003) also noted significantly lowered PCV levels while studying

clinicopathological effects of green and shade dried *Christella dentata* (Forssk) fern toxicity in guinea pigs.

5.4.2. Haemoglobin Concentration

A significant decrease ($P<0.01$) in haemoglobin concentration was observed in group V (pooled toxic fraction, 0.4 g/kg) whereas group II (fresh juice) showed significant decrease at 5 per cent level. The group III (alcoholic extract) and VI (pooled toxic fraction, 0.2 g/kg) did not show any variation. Fan *et al.* (2005) noticed decrease in haemoglobin concentration in *Oxytropis glacialis* poisoning in goats. Rajan *et al.* (1986) also observed reduction in haemoglobin in 11^{th} and 21^{st} day after administration of *Mimosa invisa* in cattle. Reduction in haemoglobin was observed by Garg *et al.* (1992) in Oak *(Quercus incana)* leaf poisoning in cattle.

5.4.3. Red Blood Cell (RBC) Count

The group II and III showed significant decrease ($P<0.05$) in RBC count whereas group V exhibited significant decrease at one per cent level on 10^{th} day of the experiment. This indicated the suppressive effect of the toxin on the bone marrow. The decrease in RBC count may be due to decreased production or increased destruction of erythrocyte. Sarathchandra *et al.* (1996) observed decrease in erythrocyte count during the acute toxicity studies with *Cleisanthus collinus,* an indigenous plant. Lincoln *et al.* (1992) also noted decrease in number of erythrocytes in cattle fed with cull domestic onions (*Allium cepa*). The chronic renal disease causes sufficient renal damage so that erythropoietin production decreased and there is inadequate stimulation of erythrocyte production (Stockham and Scott, 2002). The renal damage caused by the toxin might have reduced erythrocyte production due to inadequate stimulation of erythropoiesis.

5.4.4. MCV

There were no significant differences in MCV levels noticed in any of the groups.

5.4.5. MCH

There were no significant differences in MCH levels in any of the groups

5.4.6. MCHC

No significant variations in MCHC could be noticed in the present study. Decrease in MCHC values were noted by Fan *et al.* (2005) after administration of *Oxytropis glacialis* in goats. Decreased MCHC indicates hypochromic anaemia.

5.4.7. Total Leucocyte Count (TLC)

There were significant increase ($P<0.05$) in total leucocyte count in group II, III and V which could be due to intoxication and tissue necrosis which in turn cause release of leucosis (Benjamin, 1985). In the present study the leucocytosis may be due to the toxins present in *Mimosa invisa*.

5.4.8. Differential Leucocyte Count (DLC)

Lymphocytosis was observed in group II, III and V but VI did not show any change in leucocyte count. This is in agreement with results of the work conducted by Al-Khafaji (1994). He observed significantly increased lymphocyte count while studying the toxicity of aran (*Hypericum perforatum*) at different stages of growth in rabbits.

Neutropenia could be observed in Group II, III and V. There was no significant change in neutrophil count in group VI. The neutropenia may be due to reduction of granulopoiesis in the bone marrow (Benjamin.1985). The neutropenia occurs as migration of neutrophils to inflammed tissue exeeds the release of neutrophils from bone marrow (Stockham and Scott, 2002).

The eosinophil count remained within normal range. But slight decreasing trend was noticed in group II, III and V which can be attributed to the toxin stress (Benjamin, 1985).

5.5. POST MORTEM EXAMINATION

The animals under group II, III and VI, which was sacrificed on 20th day of the experiment, did not show any gross lesions. But animals died during trial experiments with higher dose of the same treatment showed lesions in the liver and kidney. Heart did not show any gross lesions. The urinary bladder was full and the lesions observed were necrotic patches and petechial haemorrhages in the kidney and severe congestion and necrosis of the liver. Rajan *et al.* (1986) observed greyish white streaks of necrosis in the renal cortices, and hepatosis in mimosa poisoning in cattle. Dilatation of ventricles of heart were also observed. Sadekar (1995) noticed petechial haemorrhages in the kidney and distended urinary bladder in *Anagallis arvensis* poisoning in cattle and buffaloes.

5.6. HISTOPATHOLOGICAL CHANGES

The liver, kidney and heart of animals under group II and III sacrificed on 20th day of the experiment did not show any pathological changes but regenerative foci could be observed in liver and kidney, which indicated repair process. Biochemical changes also correlated with this observation. Though rise in the level of all parameters were observed, the levels came to normal on continued administration of the dose levels used. This also indicates the tolerance developed by the animals and a critical toxicity can be expected if higher doses are given for short periods. But the animals died of higher doses during trial experiments and animals under group V (pooled toxic fraction, 0.4 g/kg) showed lesions in liver, kidney and heart. The degenerative and necrotic lesions observed proved that the toxins present in the plant are hepatotoxic, nephrotoxic and cardiotoxic at higher dose levels. This has to be proved by conducting controlled experiments in calves and goats as they have more access to this plant toxicity.

Similar histopathological changes were observed by Rajan *et al.* (1986) after administration of *M.invisa* in calves. They noticed degenerative, necrotising changes in kidney, liver and heart. Shridhar *et al.* (2007) observed renal damage suggestive of tubular interstitial nephritis due to feeding of *M. invisa* in calves. Jayasree *et al.* (2007) also described necrosis of the kidneys and vacuolations of hepatocytes after incorporation of more than 50 per cent *M. invisa* along with fodder in rabbits.

5.7. ACTIVE PRINCIPLES OF *Mimosa invisa*

The alcoholic extract, n-butanol fraction and aqueous fractions were screened for various active principles and it was found that these fractions contained steroids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins. Abdullah *et al.* (1992) detected saponigenins identified as 3-spirostanols in *Brachiaria decumbens* toxicity in sheep. These isomeric steroid saponigenins are believed to be the toxic principle, that cause poisoning in sheep feeding *B. decumbens*. In the present study also saponins could be detected which may be responsible for the toxicity of *Mimosa invisa*. Another report by Garg *et al.* (1992) indicates that tannins and simple phenols may be causing nephrotoxicity and hepatotoxicity in Oak leaf poisoning in cattle. The tannins and phenolic compounds could be detected in the extracts of *Mimosa invisa* also. Since the chloroform fraction contained only flavonoids, this fraction was not toxic in rabbits. The water insoluble fraction was also not toxic since it contained only traces of tannins, triterpenes and saponins.

5.8. EFFECT OF DECOCTION OF *Hygrophila auriculata*, *Boerhavia diffusa* AND *Tribulus terrestris* ON *Mimosa invisa* TOXICITY

The protective effect of the decoction of the combination of these three plants was studied after administration of 0.4 g/kg pooled toxic fraction of *Mimosa invisa*. The biochemical and haematological parameters were studied to assess the effect of decoction.

5.8.1. Biochemical Parameters

5.8.1.1. Serum ALT

A significant increase ($P<0.01$) in serum ALT levels was observed on first day of Mimosa administration. Thereafter the ALT levels decreased and reached normal value by 20th day. A decoction of the root of *Hygrophila spinosa* is used in genitourinary diseases and in hepatic obstruction with dropsy (Nadkarni, 1976). Hence the decrease in ALT levels may be due to the protective effect of the decoction on liver and kidney. Hewawasam *et al.* (2003) observed decrease in serum ALT levels while studying the protective effect of *Asteracantha longifolia* (*Hygrophila spinosa*) extract in mouse liver injury induced by carbon tetrachloride and paracetamol. They suggested that this might be due to reduced leakage of enzymes from the hepatocytes.

5.8.1.2. Serum AST

The peak levels of AST were observed on1st and 3rd day after administration of *Mimosa invisa.* The values came back to normal by 20th day. Significant reduction in serum AST indicates reduced leakage of enzymes from hepatocytes (Hewawasam *et al.*, 2003). The protective effect of asteracantha (hygrophila) against hepatotoxin may be attributed to the presence of flavonoids, lupenoids, sterols, betulin and aliphatic esters among plant constituents (Misra *et al.*, 2001).

5.8.1.3. Serum GGT

The serum GGT levels showed a significant increase ($P<0.05$) on 5th, 10th and 15th day, but the values attained normalcy by 20th day. When group VIII (Mimosa pooled toxic fraction+decoction) was compared with the group which was administered *Mimosa invisa* alone (GroupV), it was observed that a significant decrease in GGT levels in Group VIII. These results indicate that there

is considerable improvement in serum GGT levels after administration of the decoction.

5.8.1.4. Serum Creatin Kinase

The serum creatine kinase levels did not show much variations when compared with zero day values. But the group V (pooled toxic fraction of Mimosa alone) exhibited a significant rise in serum creatine kinase when compared to zero day values. This indicates that the muscular damage produced by *M.invisa* may be reversed by the administration of decoction.

5.8.1.5. Serum Alkaline Phosphatase

A significant rise in serum ALP was observed on 1st day after administration of decoction and the pooled toxic fraction of mimosa. Third day onwards the values came back to normal. But the group administered with toxic fraction alone showed a significant elevation of serum ALP from 3rd to 15th day. The results showed the efficacy of the decoction to reverse the toxicity produced by pooled toxic fraction of *Mimosa invisa*. Hewawasam *et al.* (2003) observed reduction in serum ALP after administration of *Asteracantha longifolia* in carbon tetrachloride induced hepatotoxicity.

5.8.1.6. Serum Creatinine

The serum creatinine values showed a significant increase ($P<0.05$) in decoction administered group whereas the group administered with pooled toxic fraction of *Mimosa invisa* alone, a highly significant ($P<0.01$) increase in serum creatinine values were observed. These indicate that the decoction has got some capacity to reverse the toxic effect produced by toxic fraction of *Mimosa invisa*. Meher *et al.* (2000) observed decrease in serum creatinine levels after administration of *Tribulus terrestris* and *Crataevia nurvala* in albino rats.

5.8.1.7. Serum Urea

A significant elevation ($P<0.05$) in serum urea levels was observed from 1st day to 15th day in decoction administered group but the values reached to normal on 20th day. In Group V (pooled toxic fraction of Mimosa) a highly significant ($P<0.01$) elevation in serum urea was observed up to 20th day. These observations indicate that the decoction administered group showed a significant decrease in urea levels when compared to the group administered toxin alone. This may be due to nephroprotective effect of *Hygrophila auriculata, Tribulus terrestris* and *Boerhavia diffusa.* A decrease in serum urea levels after administration of *Tribulus terrestris* and *Crataeva nirvala* was observed by Meher *et al.* (2000).

5.8.1.8. Serum Total Protein

No significant changes in serum protein could be detected in group V (pooled toxic fraction of *Mimosa invisa* alone) and group VIII (pooled toxic fraction + decoction). This indicates that neither the toxin nor the decoction had influence on serum protein level.

5.8.1.9. Serum Albumin

All the values of serum albumin fall within normal range. There were no significant difference between Group V (pooled toxic fraction of *Mimosa invisa* alone) and group VIII (pooled toxic fraction + decoction).

5.8.1.10. Serum Globulin

There were no significant changes in serum globulin levels in Group VIII when compared to zero day values and also with the group administered with toxin alone.

5.8.1.11. Albumin-Globulin Ratio

There were no significant variations in albumin-globulin values.

5.8.2. Haemogram

In Group VIII (pooled toxic fraction + decoction) the VPRC (volume of packed red cells) values remained within normal range, but in Group V (pooled toxic fraction of Mimosa alone) a significant decrease in VPRC could be detected. These indicate that the effect of toxic fraction of Mimosa could be reversed by the administration of decoction.

The haemoglobin level also decreased in group V (pooled toxic fraction alone), but in Group VIII (pooled toxic fraction + decoction) no significant variations could be detected. This finding suggests the ameliorating effect of decoction in mimosa toxicity

The RBC count also decreased in group V, whereas the decoction administered group reversed the action.

No significant difference in erythrocyte indices could be observed in group V as well as in group VIII.

The total leucocyte count significantly increased ($P<0.05$) in group V whereas no changes could be detected in group VIII. The decoction reversed the effect of toxic fraction of *Mimosa invisa* by protecting liver and kidney.

Differential leucocyte count indicated lymphocytosis with neutropenia in group V whereas there was no change in neutrophil and lymphocyte count in Group VIII.

5.8.3. Post Mortem Examination

On twentieth day of the experiment all the animals in group VIII were sacrificed. A detailed post mortem examination was conducted. There were no gross lesions in liver, kidney and heart. These tissues were collected and fixed in 10 per cent formalin for histopathological examination

5.8.4 Histopathological Examination

On histopathological examination no appreciable lesions could be identified in liver, kidney and heart except regenerative changes. This reveals the protective effect of the decoction on the toxicity produced by *Mimosa invisa*.

From the results of the present study, it is concluded that

- the fresh juice, alcoholic extract, aqueous fraction and n-butanol fractions of *Mimosa invisa* could produce toxicity in rabbits.
- the chloroform fraction and water insoluble residue failed to produce toxicity.
- the biochemical and histopathological studies revealed nephrotoxic and hepatotoxic effect of the plant.
- except pooled toxic fraction, others showed tolerance to repeated administration.
- lower doses failed to produce toxicity, but developed tolerance to higher doses.
- the decoction of *Hygrophila auriculata, Tribulus terrestris* and *Boerhavia diffusa* at a rate of 5 g/kg could protect liver and kidney as evidenced by biochemical parameters.
- the active principles that could be detected through qualitative tests were steroids, phenolic compounds, toxins, flavonoids, glycosides, diterpenes, triterpenes and saponins in alcoholic extract, butanol fraction and aqueous fraction. Cyanide and nitrate could be detected in fresh plant.

The results of the present study confirmed the nephrotoxic and hepatotoxic effect of *M. invisa*. This plant produced acute toxicity if consumed in large quantities. A decoction of a mixture of *Boerhavia diffusa,*

Tribulus terrestris and *Hygrophila auriculata* could protect the animal from the toxic effect of *M. invisa*. But this treatment may not be effective in acute cases of poisoning. Hence further study is needed to isolate the phytotoxin present in *M. invisa* so that, specific antidote can be developed which will be effective even in acute cases of poisoning.

Summary

6. SUMMARY

Poisoning due to *Mimosa invisa* among animals is very common in Kerala during rainy season when there is lots of lush green growth of this plant. This study was undertaken therefore to evaluate the toxic effect of *Mimosa invisa* and also to identify the toxic fraction in rabbits. The study was conducted in two phases. During the first phase, the toxicity of fresh juice and alcoholic extract of *Mimosa invisa* was studied. The second phase of the study involved toxicity evaluation of various fractions and a treatment schedule has also been tested in rabbits.

The preliminary studies were conducted to find out the toxic dose during each phase. Out of the four doses tested, 25 g/kg of the *Mimosa invisa* fresh juice was found to be toxic. The toxic dose for alcoholic extract was 1 g/kg. The group I served as control animals, which received the normal diet alone. The group II received the fresh juice equivalent to 25 g/kg of *Mimosa invisa* and group III, 1 g/kg of *Mimosa invisa* alcoholic extract. The clinical symptoms, biochemical and haematological parameters were studied in the above groups. The clinical symptoms noticed were inappetance, dullness and lethargy. The biochemical parameters were evaluated on day 0, 1, 3, 5, 10, 15 and day 20 and haematological parameters on day 0, 5, 10, 15 and day 20. There was significant increase in serum ALT, AST and GGT levels up to 10 days after administration of Mimosa, thereafter the values returned to normal. The serum creatine kinase values showed significant increase up to third day, then the values decreased to normal. Similar changes are observed when compared to control (Group I). The serum creatinine and urea levels showed significant increase up to 10^{th} day even though the values gradually reduced from 5^{th} day onwards. There were no significant changes in serum protein, albumin, globulin and albumin-globulin ratio. The VPRC levels showed significant decrease in group II and III when compared to Group I. The haemoglobin concentration also significantly

decreased when compared to zero day values but there were no changes when compared to control. Significant reduction in RBC count could also be detected. There were no changes in erythrocyte indices in group II and III. The total leucocyte count showed significant increase on 5th and 10th day in group III while in group II, a significant increase was observed throughout the experiment. The differential leucocyte count showed Lymphocytosis with neutropinia.

The second phase of the study involved identification of toxic dose of each fraction of alcoholic extract of *Mimosa invisa*. From the pilot studies, it was evident that the aqueous fraction, 0.4 g/kg (Fraction III) and butanol fraction 0.5 g/kg (Fraction II) were found to be toxic whereas chloroform fraction (Fraction I) and water insoluble residues (Fraction IV) were nontoxic. Hence the fraction II and fraction III were pooled together and tested in rabbits for toxicity.

The pilot studies revealed that the toxic dose of pooled toxic fraction was 0.4 g/kg. This toxic dose was administered in rabbits (group V) to study the toxic effects. The clinical symptoms observed were inappetance, dullness, lethargy and sternal recumbent posture. The hair coat was rough and gradual reduction in body weight noticed. The schedule of blood collection was same as that of first phase of the experiment. The biochemical parameters and haemogram were studied to assess the toxicity. The serum ALT, AST and GGT levels significantly increased when compared to zero day values control group (group IV). The serum creatinine levels showed maximum increase on 5th day and then gradually decreased and reached the normal value by 20th day. There was significant increase in serum ALP levels on 3rd, 5th, 10th and 15th day. A significant increase in serum creatinine and serum urea levels could be observed throughout the experiment. There were no significant alterations in total protein albumin, globulin and albumin-globulin ratio. The VPRC, RBC and haemoglobin significantly decreased. But the calculated values of erythrocyte indices remained within normal range. The leucocyte count significantly increased from 10th day onwards. The differential leucocyte count showed lymphocytosis with neutropenia. The animals under group VI received half the toxic dose of pooled

toxic fraction. This dose failed to produce toxicity in rabbits as evidenced by biochemical and haematological parameters. The group VII animals were administered with double the toxic dose pooled toxic fraction of *Mimosa invisa.* All the animals under this group died within 12-24 hours of administration of this dose.

The toxicity produced by the pooled toxic fraction was treated with a decoction prepared from equal quantity of *Boerhavia diffusa, Hygrophila auriculata(Asteracantha longifolia)* and *Tribulus terrestris*. The response to the treatment was assessed by observing clinical symptoms and by evaluation biochemical parameters and haemogram. The symptom of inappetance was observed on 1st and 2nd day of the experiment. Then the animal started taking feed and water normally. The serum ALT, AST and GGT levels significantly increased from 1st day to 5th day, then the values returned to normal level. The serum creatine kinase and ALP levels did not show significant changes. The serum creatinine and urea levels showed significant increase followed by decrease. There were no changes in serum protein, albumin, globulin and albumin-globulin ratio.

The screening of alcoholic extract and various fractions revealed that the alcoholic extract, butanol fraction and aqueous fraction contained steroids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins. The chloroform fraction was positive for flavonoids only whereas water insoluble fraction contained flavonoids and traces of tannins, triterpenes and saponins.

The results of the present study indicate that the fresh juice of *Mimosa invisa*, cold alcoholic extract and fractions (butanol and aqueous) were capable of inducing toxicity in rabbits. The treatment of toxicity using a decoction of equal quantities of *Boerhavia diffusa, Hygrophila auriculata* and *Tribulus terrestris* was found to reverse nephrotoxicity and hepatotoxicity as indicated by biochemical parameters and haemogram.

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IDENTIFICATION OF TOXIC FRACTIONS OF *Mimosa invisa* (ANATHOTTAVADI) AND ITS TOXICITY IN RABBITS

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ABSTRACT

The present study was undertaken to identify the toxic fraction of *Mimosa invisa* in rabbits and to assess to toxicity of *Mimosa invisa* fresh juice, cold alcoholic extract and various fractions of alcoholic extract utilizing rabbit as a model along with treatment study.

The experiment was conducted in two phases. The first part of the study involved assessment of toxicity of fresh juice of *Mimosa invisa* (group II) and cold alcoholic extract (group III). Group I served as control. The preliminary tests were conducted to derive the toxic dose of fresh juice and alcoholic extract of *Mimosa invisa*. The toxic doses were 25 g/kg and 1 g/kg body weight for *Mimosa invisa* fresh juice and alcoholic extract respectively. The clinical symptoms, biochemical parameters and haemogram were observed to assess the toxicity. The serum ALT, AST and GGT levels showed significant increase in both the groups. The serum creatine kinase levels exhibited an increase followed by a decrease. There was a significant increase in serum creatinine and urea levels. There were no changes in serum total protein, albumin, globulin and albumin-globulin ratio. Significant decrease in VPRC, haemoglobin and RBC count could be noticed. The erythrocyte indices did not show any variations. The leucocytosis was observed in group II and III when compared to control (group I). Lymphocytosis with neutropenia were also observed in both the groups.

The second phase of the study involved identification of toxic dose of each fraction of *Mimosa invisa*. The preliminary studies revealed that chloroform fraction (Fraction I) and water insoluble residue (fraction IV) were not toxic to rabbits while the fraction II (n-butanol fraction) and fraction III (aqueous fraction) were toxic to rabbits. Hence the two toxic fractions were pooled and used for further studies. It was found that 0.4 g/kg of pooled toxic fraction was toxic in rabbits. The toxicity was assessed by the evaluation of clinical

symptoms, biochemical parameters and haemogram. The group V (pooled toxic fraction) showed inappetence, dullness, lethargy and reluctant to move. A significant increase in serum ALT, AST and GGT levels were observed. The serum ALP levels showed an increase followed by a decrease. The serum creatine kinase also showed similar increase followed by decrease. The creatinine and urea levels exhibited a continuous increase in group V. There were no changes in total protein, albumin, globulin and albumin-globulin ratio. The VPRC, RBC and haemoglobin showed significant decrease but there were no changes in erythrocyte indices. A significant leucocytosis was observed in group V. The differential leucocyte count showed lymphocytosis with neutropenia. The group VI (Half the toxic dose of pooled toxic fraction) failed to produce toxicity as evidenced by biochemical parameters and haemogram. The group VII (Double the toxic dose of pooled toxic fraction), all the animals died within 12-24 hours of administration of the dose.

The group VIII animals were treated with a decoction prepared from equal quantities of *Boerhvia diffusa, Hygrophila auriculata* and *Tribulus terrestris* along with pooled toxic fraction of *Mimosa invisa*. The prominent symptom of inappetence was only for a short period of time (1-2 day). Then the animals started taking normal feed and water. The serum ALT, AST and GGT levels were significantly increased during the first five days, then the values returned to normal level. The serum creatine kinase and ALP levels did not show significant changes. The serum creatinine and urea levels showed significant increase followed by decrease. All the parameters showed significant improvement when compared with group V (pooled toxic fraction alone). There were no changes in serum protein, albumin, globulin and albumin-globulin ratio.

The screening of alcoholic extract and various fractions revealed that the alcoholic extract n-butanol fraction and aqueous fraction contained steroids phenolic compounds, tannins, flavonoids, glycosides, diterpenes triterpenes and saponins. The chloroform fraction was positive for flavonoids only, whereas

water insoluble fraction contained flavonoids and traces of tannins, triterpenes and saponins.

From the results of the present study, it is concluded that the phytotoxin present in *M.invisa* is nephrotoxic and hepatotoxic. The treatment schedule tried using a decoction of *Boerhavia diffusa, Tribulus terrestris and Hygrophila auriculata* could protect kidney and liver from the phytotoxin present in *M.invisa*. Further study is needed to isolate the phytotoxin present in *M.invisa* so that a specific antidote can be developed.