HEPATOPROTECTIVE EFFECT OF *Azadirachta indica* (NEEM) AND *Tridax procumbens* (CHIRAVANAK) IN RATS

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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 the thesis entitled **"HEPATOPROTECTIVE EFFECT OF *Azadirachta indica* (NEEM) AND *Tridax procumbens* (CHIRAVANAK) IN RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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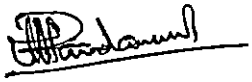
CERTIFICATE

Certified that this thesis, entitled **"HEPATOPROTECTIVE EFFECT OF *Azadirachta indica* (NEEM) AND *Tridax procumbens* (CHIRAVANAK) IN RATS"** is a record of research work done independently by **Ranjana Mooken.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.



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1. INTRODUCTION

The liver plays a major role in the metabolism of carbohydrate, protein and fat. It secretes bile and synthesizes clotting factors, Vitamin A and plasma proteins. It detoxifies and excretes both ingested and internally produced toxic substances including drugs, alcohol and toxins from intestinal microbes and tissue metabolism.

Hepatotoxicity implies chemically driven liver damage. The excessive use of certain drugs like paracetamol, carbon tetrachloride (CCl_4), heavy metals, antitubercular drugs like isoniazid and rifampicin, erythromycin and mycotoxins like aflatoxin B_1 can cause hepatotoxicity. Hepatotoxins damage liver cells mainly by lipid peroxidation and other oxidative damages which eventually lead to hepatitis and cirrhosis. When the liver cells are damaged, elevated levels of liver marker enzymes like serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) are noticed in blood.

Paracetamol (N-acetyl-p-amino phenol) is an extensively used analgesic and antipyretic drug and though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose (Ramsay *et al.*, 1989). Paracetamol is metabolized primarily in the liver into non-toxic products and a minor portion of the drug is metabolized by hepatic cytochrome p450 enzyme system forming an alkylating metabolite NAPQI (N-acetyl- p- benzo-quinone imine). NAPQI is primarily responsible for the toxic effects of paracetamol. In normal conditions NAPQI is detoxified by conjugating with glutathione. In paracetamol overdose large amount of NAPQI is generated which depletes glutathione, a natural antioxidant. This in combination with direct cellular injury by NAPQI leads to cell damage and death.

Liver diseases remain as one of the serious health problems and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. In India, about 40 polyherbal commercial formulations

reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, biodegradability, fewer side effects and relatively low cost (Mohan *et al.*, 2007).

Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety. The hepatoprotective plants include *Andrographis paniculata, Azadirachta indica, Cassia fistula, Elephantopus scaber, Hibiscus rosasinensis, Phyllanthus debilis, Picrorrhiza kurroo* and *Glycyrrhiza glabra* (Hemalatha 2008).

Azadirachta indica belongs to the family Meliaceae, commonly known as Neem. The Sanskrit name of Neem tree is 'Arishtha' meaning 'reliever of sickness'. It is a medium to large sized tree. Native to India, neem is widely planted and naturalized in semiarid areas throughout Asia and Africa. Neem has short straight trunk, furrowed dark brown to grey bark and dense rounded crowns of pinnate leaves.The leaflets are dark green, glossy, sickle shaped with toothed margin. Various medicinal properties of *Azadirachta indica* include hepatoprotective, hypoglycemic, anti-inflammatory, antifertility, antimalarial, anticarcinogenic, antifungal, antioxidant, antibacterial and antiviral effects. More than 135 compounds have been isolated from different parts of neem. These include proteins and carbohydrates, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, coumarins, tannins, aliphatic compounds etc. (Biswas *et al.*, 2002). Leaves are used in the treatment of neuromuscular pains, remove toxins and purify blood. Neem bark heals wounds and is also used in vomition, skin diseases, worm infestations and excessive thirst (Atawodi and Atawodi, 2009).

Tridax procumbens belongs to the family Asteraceae, commonly known as coat buttons (English), Kansari or Ghamra (Hindi), Chiravanak (Malayalam),

which is a hispid, procumbent herb. It is found as a weed in fields, meadows, crop lands, lawns and roadsides in areas with tropical or semi-tropical climates. Leaves are opposite, green, rough and scabrous. Base is wedge shaped and margin irregularly toothed. Shape ranges from lanceolate to ovate. It has got a characteristic odour and acrid taste. It has been extensively used in Indian traditional medicine as anti-coagulant, antifungal and insect repellent. It possesses wound healing activity and promotes hair growth. *Tridax procumbens* is also dispensed as 'Bhringraj', which is a well known Ayurvedic medicine for liver disorders. It is also used to treat diarrhea and dysentery (Bhagwat *et al.*, 2008). Earlier workers have already reported the presence of luteoline, glucotureolin, β-sitosterol, flavone, glycoside and quercetin in *Tridax procumbens*.

The present study was undertaken to assess the hepatoprotective activity of ethanolic extract of *Azadirachta indica* and *Tridax procumbens* leaves on paracetamol induced hepatic damage in rats and to compare their action.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PARACETAMOL

Dixon *et al.* (1975) studied serum transaminase levels after experimental paracetamol induced hepatic necrosis in rats and found that a dose of 4 g/kg paracetamol could induce hepatic necrosis and an increase in alanine amino transferase and aspartate amino transferase after 24 hours of administration. They reported that the serum enzyme levels give a reliable indication of the severity of necrosis of liver.

Mogre *et al.* (1980) stated that paracetamol at the dose rate of 500 mg/kg orally twice a day for 7 days inhibited the sodium pump by interfering with sodium dependent phosphokinase in the reaction sequence of ATPase and thereby caused liver injury in rats.

Makin and Williams (1993) studied the relationship between alcohol consumption and hepatotoxicity related to paracetamol ingestion both in cases of over dosage and in cases where paracetamol was apparently taken for therapeutic reasons and reported that heavy drinkers did not develop more severe hepatotoxicity following paracetamol overdose than non-drinkers.

Paracetamol at the dose rate of 2 g/kg orally in rats lowered bile flow, bile salts, total oxygen consumption and altered the biochemical parameters. The toxic metabolite of paracetamol (N-acetyl-p-benzoquinone imine-NAPQ1) covalently bound to the receptor of hepatocyte membrane and disturbed the metabolic activities thereby reducing the viability of hepatocytes (Visen *et al.,1993*).

Effect of acetaminophen on glutathione (GSH) S-transferase and related drug metabolizing enzymes was studied by Yonamine *et al.* (1996). They reported that acetaminophen caused oxidative stress and reduced GSH content in rat liver homogenates 1 hour after administration of acetaminophen and GSH content reached its minimum level at 3 hour.

Kaushal *et al. (1999)* evaluated the effects of paracetamol induced hepatotoxicity on microsomal functions and found that paracetamol at the dose of 650 mg/kg intraperitoneally in rats produced a six fold increase in AST and eight fold increase in ALT levels. It decreased the Na^+-K^+- ATPase activity and disturbed the Ca^{2+} homeostasis and caused microsomal dysfunctions by impairing phospholipids metabolism. They also reported that paracetamol hepatotoxicity did not affect the lipid peroxidation significantly.

Prescott (1999) observed that the liver damage caused by paracetamol is through conversion by hepatic cytochrome P 450 enzymes to a toxic intermediate metabolite N-acetyl-p- benzoquinone imine (NAPQ1) by a process called metabolic activation. Fasting increased the paracetamol hepatotoxicity in rats by decreasing glucuronide and sulphate conjugation so that the proportion converted to the toxic metabolite is significantly enhanced.

Udupa *et al.* (2000) reported that chronic administration of paracetamol at the dose rate of 500 and 1000 mg/kg for 4 weeks to rats produced a dose dependent increase in ALT and AST and reduction in liver Na^+ K^+-ATPase activity, glycogen and glutathione levels indicating the hepatocellular damage. Histological examination showed evidence of swelling, hydropic degeneration and necrosis of the hepatocytes.

Asha (2001) stated that paracetamol over dosage (2 g/kg) in rats damaged liver mainly by inducing lipid peroxidation directly or indirectly. It elevated the levels of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT).

Paracetamol at the dose rate of 5 mmol/kg intraperitoneally in rats caused increased plasma ALT and AST and glutamate dehydrogenase activities, without affecting bilirubin or creatinine concentration (Moore *et al.*, 2001).

Kurtovic and Riordan (2003) conducted studies on hepatotoxicity induced by paracetamol at recommended dosage and reported that nutritional impairment and

recent fasting have been identified as key precipitants in patients who developed liver damage following moderate paracetamol overdose.

Mc.Gregor *et al.* (2003) studied liver death and regeneration in paracetamol toxicity and found that paracetamol toxicity in murine liver cause hepatocyte apoptosis. Apoptosis can cause a significant loss of hepatic parenchyma.

Guzy *et al.* (2004) studied the effect of paracetamol on mitochondrial respiration, ATPase, glutathione peroxidase (GPX) and glutathione reductase (GR) activities in rats and found that paracetamol in toxic doses (500 mg/kg) exhibited unfavourable effects on mitochondrial respiration and on the activity of ATPase and showed no significant effects on GPX and GR activities.

Kumar *et al.* (2004) stated that paracetamol at the dose rate of 3 g/kg orally in rats produced increase in levels of SGOT, SGPT, ALP and bilirubin but a decrease in total protein level.

Dixon *et al.* (1975) examined the changes in the liver of rats sacrificed at various intervals after administration of large dose of paracetamol. It was found that the changes in liver were marked congestion, dilatation of central veins, disruption of surrounding sinusoids and frank necrosis of hepatocytes. He also observed that the histopathological changes of paracetamol over dosage were similar in man and rats.

Paracetamol at the dose rate of 3 g/kg orally for three days in rats produced elevations of serum marker enzymes like ALP, AST, ALT and lipid peroxides but caused a decrease in total protein and albumin. The haematological parameters were not much affected and on histological examination focal areas of necrosis were noted (Mathew, 2005).

Mohamed *et al.* (2005) reported that acetaminophen at the dose of 1 g/kg caused hepatotoxicity in mice and rats and the toxic pathway is a cytochrome P-450 dependent N- hydroxylation process in the liver and the hepatotoxicity of acetaminophen occurred when the liver enzymes catalyzing the normal conjugation reactions were saturated. Acetaminophen then metabolized and

produced a toxic metabolite N- acetyl- *p*- benzoquinone imine (NAPQ1-hydroxanic acid) which is then inactivated by conjugation with glutathione. A decrease in hepatic GSH to about 20 per cent of initial concentration lead to covalent binding of acetaminophen metabolite to nucleophilic macromolecules in the cell and the toxic metabolites of acetaminophen induced cell damage in liver and other tissues such as kidney tubules.

Paracetamol at the dose rate of 200 mg/kg orally produced the abnormally high levels of serum ALT, AST, ALP and bilirubin which denoted the damage to the hepatic cells and also fatal hepatic necrosis in rats, man and mice. They also reported a reduction in total serum protein and liver glycogen which is associated with a decrease in the number of hepatocytes and decreased capacity to synthesize proteins and glycogen and consequently decreased the liver weight. (Gupta and Misra, 2006).

Acetaminophen challenge (300 mg/kg, intraperitoneally) in mice for 7 days caused significant increase in the levels of bilirubin, liver enzymes, thio barbituric acid reactive substances (TBARS) and iron, while catalase activity and total protein levels were reduced significantly (Iwalokun *et al.*, 2006).

Studies conducted by Ojo *et al.* (2006) in some common Nigerian trees revealed that paracetamol at the dose rate of 2 g/kg produced increase in serum levels of ALT, AST and ALP in rats.

Studies conducted by Roy *et al.* (2006) revealed that paracetamol at the dose rate of 1 g/kg in three divided doses produced marked increase in serum levels of ALT,AST,ALP and bilirubin in rats and it also produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis in liver.

Dash *et al.* (2007) reported that paracetamol at the dose rate of 750 mg/kg resulted in a significant elevation of liver specific serum markers like ALT, AST, ALP, bilirubin and total protein and the levels of thiobarbituric acid reactive

substances (TBARS) in rats. A decrease in the activities of glutathione, SOD and catalase was also noted.

2.2 HEPATOPROTECTIVE AGENTS

2.2.1 *Azadirachta indica*

According to Chattopadhyay *et al.* (1992), water soluble portion of alcoholic extract of *Azadirachta indica* at the dose rate of 1 g/kg orally for seven days produced marked decrease in the levels of ALT, AST, ALP and acid phosphatase in paracetamol induced hepatotoxicity in rats. It also prevented the necrotic changes in the liver.

Tripathi *et al.* (1994) studied the hepatoprotective activity of *Azadirachta indica* against thioacetamide induced liver injury in albino rats by the administration of alcoholic extract of its leaves at a daily dose of 50 mg/kg for 7 days. A marked reduction in the elevated level of serum parameters like glutamate oxaloacetate transaminase, glutamate pyruate transaminase, acid phosphatase, alkaline phosphatase, and glutamate dehydrogenase was noticed.

Bhanwra *et al.* (2000) conducted studies on the effect of *Azadirachta indica* (Neem) leaf aqueous extract on paracetamol- induced liver damage in rats and found that aqueous *A. indica* leaf extract (500 mg/kg) significantly reduced the elevated levels of AST, ALT and gamma GT. Paracetamol induced liver necrosis was also found to be reduced as observed macroscopically and microscopically.

Biswas *et al.* (2002) conducted studies on the biological activities and medicinal properties of *Azadirachta indica* and reported the biological activities of some of the neem compounds isolated, pharmacological actions of the neem extracts, clinical studies and medicinal applications of neem along with their safety evaluation.

Chattopadhyay (2003) studied the possible mechanism of hepatoprotective activity of *A. indica* leaf extract in rats by investigating its effects on blood and liver glutathione, Na^+ K^+-ATPase activity and thiobarbituric acid reactive substances against paracetamol induced hepatic damage in rats. *A. indica* leaf

extract had reversal effects on the levels of above mentioned parameters in paracetamol hepatotoxicity.

Kale *et al.* (2003) studied the effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats and reported that aqueous leaf extract significantly prevented changes in the serum levels of bilirubin, protein, alanine amino transferase, aspartate amino transferase and alkaline phosphatase. It also significantly reversed the biochemical and histological changes.

Chattopadhyay and Bandyopadhyay (2005) studied the possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract against paracetamol induced hepatic damage in male albino Wistar rats and found that paracetamol over dosage caused oxidative stress and *Azadirachta indica* leaf extract significantly enhanced the hepatic level of glutathione dependent enzymes and superoxide dismutase activity, which were lowered due to oxidative stress.

Mathew (2005) studied the hepatoprotective effect of *Aegle marmelos* (Indian Bael) and *Azadirachta indica* (Neem) aqueous leaf extract on paracetamol induced toxicity in rats and found that *A. indica* leaf extract at the dose rate of 500 mg/kg caused significant reduction in the elevated levels of biochemical parameters like AST, ALT and bilirubin. The haematological parameters were not much affected and on histological examination focal areas of necrosis were noted.

Kalaivani *et al.* (2009) conducted studies on hepatoprotective properties of aqueous and ethanolic leaf extracts of *Azadirachta indica* in carbon tetrachloride induced liver damage in mice at the dose rates of 500 and 600 mg/kg Bwt. Analysis of SGOT, SGPT and ALP levels revealed that the extracts were moderately effective when compared to standard drug silymarin treatment.

Maruthappan and Shree (2009) conducted studies on hepatoprotective effect of *Azadirachta indica* (Neem) leaves against alcohol induced liver injury in albino rats. Liver damage was induced in Wistar rats by administering 30 per cent of

ethyl alcohol daily for 14 days. Silymarin was given as the reference drug. Elevated level of serum marker enzymes was significantly reduced.

2.2.2 *Tridax procumbens*

Ravi kumar *et al.* (2005) conducted studies on the hepatoprotective activity of *Tridax procumbens* against D-galactosamine / lipopolysaccharide induced hepatitis in rats and found that pre-treatment of rats with a chloroform insoluble fraction from ethanolic extract of *Tridax procumbens* reversed the elevated levels of serum marker enzymes like aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase and bilirubin to normal levels. The biochemical observations were supplemented by histopathological examination of liver sections.

Hemalatha (2008) studied the anti hepatotoxic and anti oxidant defence potential of *Tridax procumbens* and found that the chloroform fraction of the ethanolic extract showed the maximum anti oxidant activity, and also reported the presence of anti oxidant minerals such as Fe, Mg, Cu and Zn. The results were confirmed by conducting studies on rodents on the anti oxidant potential of *T. procumbens* induced through LPS, CCl_4, alloxan and paracetamol intoxication induced hepatitis.

Nwanjo (2008) studied the effect of aqueous extract of *Tridax procumbens* leaves on lipid peroxidative stress and anti oxidant status in chloroquine induced hepatotoxicity in rats and found that *T. procumbens* leaf extract significantly reduced the elevated levels of malonaldehyde AST, ALT, ALP and increased superoxide dismutase and catalase levels.

2.2.3 Silymarin

Abrol *et al.* (2005) evaluated the different silymarin formulations both *in vivo* and *in vitro* and found that the hepatoprotective potential of silymarin in lipid microspheres was enhanced when tested in animal models like rat. It was also reported that phospholipids have a synergistic effect with silymarin.

Silymarin offered good protection in various toxic models of experimental liver diseases in laboratory animals and it acted by antioxidative, antilipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms (Pradhan and Girish, 2006).

Studies conducted by Dash *et al.* (2007) also revealed that silymarin is a flavolignan having hepatoprotective property. It is a mixture of three structural components viz silibinin, silydianine and silychristine. Silymarin has hepatoprotective effects in acute viral hepatitis and intoxication with paracetamol, carbon tetrachloride and ethanol in rats.

Silymarin, a flavolignan from *Silybum marianum* has excellent hepatoprotective action and its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on hepatocyte membrane, reduction of glutathione oxidation to enhance its level in the liver and intestine, antioxidant activity, stimulation of ribosomal RNA polymerase and subsequent protein synthesis leading to enhanced hepatocyte regeneration. The study was conducted in animal models like mice, rabbits and rats. (Dixit *et al., 2007).*

2.2.4 Other agents/drugs having hepatoprotective activity

Natu *et al.* (1977) studied the effect of *Ricinus communis* leaves in experimental liver injury in rats caused by carbon tetrachloride and found that whole leaves of *Ricinus communis* provided protection against liver necrosis as well as fatty changes induced by CCl_4, while the glycosidic and cold aqueous extract provided protection only against liver necrosis and fatty changes respectively. Because of the parasympathetic activity of these leaves, it increased the blood supply to the liver and thereby offered protection against the hepatotoxins.

Mogre *et al.* (1980) studied the effects of *Picrorhiza kurroo* and *Eclipta alba* on Na^+K^+ATPase in hepatic injury in rats by various hepatotoxic agents like carbon tetra chloride, paracetamol and aflatoxin. They reported that alcoholic

extract of *Picrorhiza kurroo* at the dose rate of 20 mg/kg once daily for seven days reduced the liver GOT and SGPT in all the three hepatic injuries and Na^+K^+ATPase levels were restored to normal. *Eclipta alba* at the dose rate of 50 mg/kg for seven days also reduced the liver GPT in all hepatic injuries. But GOT content was reduced only in CCl_4 and paracetamol hepatic injury. Na^+K^+ATPase activity was raised in all hepatic injuries.

Singh *et al.*,(1984) reported that *Tinospora cordifolia* provided protection to liver from the injurious effect of CCl_4 by the action of stabilizing the liver cell membrane and by protecting the enzyme related to rough endoplasmic reticulum. Administration of *T. cordifolia* before CCl_4 treatment significantly shortened the hexobarbitone and pentobarbitone induced sleeping time in CCl_4 treated rats.

Agrawal *et al.* (1986) conducted screening of *Phyllanthus niruri* and *Ricinus communis* on alcohol induced liver cell damage in non- hepatectomised and partially hepatectomised rats and found that 50 per cent alcoholic extract of roots and leaves of *Phyllanthus niruri* has got hepatoprotective effects on alcohol induced liver cell damage. The alcoholic extract of *Ricinus communis* did not show any significant hepatoprotective activity.

Gulati *et al.* (1991) reported that ethanolic extract of roots of *Boerhaavia diffusa* at the dose rate of 100 mg/kg body weight/day for 21 days in albino rats offered significant hepatoprotection in country made liquor hepatotoxicity. It normalized the level of ALT, triglycerides and cholesterol in extract treated animals.

Chattopadhyay *et al.* (1992) studied the effect of *Ocimum sanctum* leaf extract on paracetamol induced hepatic damage in rats and found that *Ocimum sanctum* protected the rats from the hepatotoxic action of paracetamol as evidenced by significant reduction in serum levels.

Murti and Srinivasan (1993) stated that *Tephrosia purpurea* at 500 mg/kg dose level offered hepatoprotection in D-galactosamine and CCl_4 induced toxicity in rats. It decreased the serum marker enzymes like SGPT, SGOT and bilirubin.

A synthetic agent 89/62 (3 cyano-4 –Methyl -5- Vinyl Pyridine) which is structurally similar to ricinine from the *Ricinus communis* produced a marked increase in bile flow, bile salts, bile acids like cholic acid and deoxycholic acid in rats. It also normalized the serum marker enzymes in various toxicities and thereby acting as hepatoprotective (Visen *et al.*, 1993).

Sultana *et al.* (1995) conducted studies on crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* in the reaction mixture containing calf thymus DNA which inhibit free radical mediated DNA damage and reported that this effect was dependent on the concentration of plant extracts. These studies suggested that the observed hepatoprotective effect of these crude plant extracts was due to their ability to suppress the oxidative degradation of DNA in the tissue debris.

Ursolic acid which is isolated from the leaves of *Eucalyptus tereticomis* have significant hepatoprotective activity in thioacetamide, galactosamine and CCl_4 induced hepatotoxicity in rats (Saraswat *et al.*, 1996). Pre-treatment with ursolic acid increased the viability of rat hepatocytes. It also showed anticholestatic activity in a dose dependent manner.

N Acetyl Cysteine (NAC) at the dose rate of 400 mg/kg showed significant hepatoprotective property in mice by its ability to enhance the glutathione synthesis there by providing more substrate for the detoxification of the hepatotoxic metabolite of paracetamol (Al-Mustafa *et al.*, 1997)

Jayasekhar *et al.* (1997) stated that at the dose rate of 250 mg/kg the ethyl acetate extract of *Acacia catechu* offered significant hepatoprotection by lowering the increased level of serum transaminase, serum ALP and serum bilirubin in CCl_4 treated rats.

Rao and Misra (1997) studied the effects of different parts of *Sida rhombifolia* on chemical and drug induced hepatotoxins like paracetamol, carbon tetrachloride, rifampicin and on carrageenan induced paw oedema in rats. The powdered roots, aerial parts and their aqueous extract showed significant

hepatoprotective activity while methanolic extract of aerial parts has significant oedema suppressant activity in rats.

Ahmad *et al.* (1999) studied the hepatoprotective potential of Jigrine, which is a polyherbal preparation containing 14 medicinal plants on thioacetamide induced hepatotoxicity in rats and reported that pre-treatment with Jigrine reduced the elevated levels of AST, ALT, Na^+ and K^+ levels in serum and TBARS. Its effects on tissues were similar to Silymarin which was recognized as a standard hepatoprotective drug.

Al- Sereiti *et al.* (1999) reported that the lyophilized ethanol and aqueous extracts of young sprouts of *Rosemarinus officinalis* (Rose Mary) has choleretic activity and offered protection against CCl_4 induced toxicity in rats. It caused a significant increase in bile flow and a significant reduction in elevated plasma liver enzymes.

Aqueous and alcoholic extract of *Cassia occidentalis* at the dose rate of 500 mg/kg orally for 7 days provided sufficient hepatoprotection in paracetamol and ethyl alcohol intoxication in rats (Jafri *et al.*, 1999).

Dahanukar *et al.* (2000) conducted phytochemical studies associated with the pharmacological activity of medicinal plants and natural products and provided a description on individual plants and polyherbal formulations. They also compiled the data generated through research studies done on a large number of medicinal plants.

Trivedi and Rawal (2000) conducted hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liver damage caused by BHC (hexachloro cyclohexane) in albino mice. They found that *Andrographis paniculata* at the dose of 12 mg/kg provided protection by decreasing the elevated levels of ALP, AST, ALT and gamma GPT and lowering the lipid peroxidase activity.

Asha (2001) studied the hepatoprotective activity of *Marmodica subangulata* and found that administration of tender leaves at the dose rate

of 50 mg/kg markedly prevented paracetamol induced elevation of serum enzymes such as ALT, AST and ALP in rats.

Ginkgo biloba at the dose rate of 50 mg/kg intraperitoneally for 7 days decreased the elevated levels of AST, ALT, ALP and increased the total protein and albumin levels in rats against CCl_4 induced toxicity and reported that *Ginkgo biloba* protected the liver from CCl_4 induced liver damage (Shenoy *et al.*, 2001).

Hewawasam *et al.* (2003) conducted studies on the protective effect of *Asteracantha longifolia* extract in mouse liver injury induced by carbon tetrachloride and paracetamol and reported that *A. longifolia* reduced the alanine amino transferase (ALT) level but increased the liver reduced glutathione level in the pre-treated group, 4 days after the administration of CCl_4. A similar pattern was observed in the pre treated group 4 hours after the administration of paracetamol with a reduction in serum levels of ALT, AST and ALP. Histopathological studies also provided supportive evidence for the results.

Ethanolic and butanolic extract of fruits of *Piper longum* at the dose rate of 300 mg/kg orally for 4 days offered hepatoprotection in CCl_4 induced hepatotoxicity in Wistar rats by decreasing the elevated SGPT and SGOT activity (Jalalpure *et al.*, 2003).

Aqueous extract of the seeds of *Nigella sativa* were tested for hepatoprotective activity on male Wistar rats against carbon tetrachloride induced hepatotoxicity. Aqueous extract showed significant hepatoprotective activity by lowering the elevated levels of serum marker enzymes like ALT and AST (Mohideen *et al.*, 2003).

According to the studies conducted by Gupta *et al.* (2004) the methanol extract of the plant *Bauhinia racemosa* at the dose rate of 50,100 and 200 mg/kg produced significant hepatoprotective effect on both paracetamol and CCl_4 toxicity by decreasing the activity of serum marker enzymes like ALP, AST, ALT and bilirubin and lipid peroxides. It significantly increased the levels

of GSH, SOD, CAT and protein in a dose dependent manner and it also had antioxidant effects on ferric chloride- ascorbate- induced lipid peroxidation in rat liver homogenate.

Guzy *et al.* (2004) reported that quarcetin (3, 3' 4', 5, 7 Penta hydroxy flavone) is the major bioflavonoid in tea, onion and red wine which offered a potential for ameliorating the hepatotoxicity of paracetamol in rats by its antioxidant effect and preventing the paracetamol induced reduction in oxygen consumption.

Hewawasam *et al.* (2004) found that pre-treatment of *Epaltes divaricata* extract at the dose rate of 0.9 g/kg orally for 7 days in mice significantly reduced the elevated serum levels of ALT, AST, ALP but significantly increased the liver reduced glutathione in CCl_4 induced hepatotoxicity.

Kumar *et al.* (2004) evaluated the effect of ethanolic extract of *Trianthema portulacastrum* against paracetamol induced hepatotoxicity in rats. Treatment of rats with a dose rate of 200 mg/kg orally for 10 days significantly reduced the increased levels of AST, AST, ALP and bilirubin. It significantly increased the total protein concentration.

According to Banerjee *et al.* (2005) the antioxidant property of fruit skin of *Eugenia jambolana* was due to the presence of anthocyanins, antioxidant vitamins, phenolics or tannins which can prevent lipid peroxidation.

Maheswari and Rao (2005) studied the effect of oral administration of grape seed oil (GSO) against CCl_4 induced hepatotoxicity in rats and found that oral administration of GSO (3.7 g/kg) for seven days resulted in a significant reduction in serum AST, ALT, ALP levels, liver MDA(Malondialdehyde) and hydroperoxidases. A significant improvement of glutathione, SOD, catalase and total protein were also noted. The histological changes caused by CCl_4 were also brought back to normalcy in drug co-administered rats.

Mangathayuru *et al.* (2005) studied the effect of *Leucas aspera* on CCl_4 induced hepatotoxicity in rats and reported that pre-treatment with the dose of

200 and 400 mg/kg significantly reduced the elevated liver enzymes, which showed its hepatoprotective action. They also stated that the antioxidant activity of flavonoids was responsible for the hepatoprotective action.

Mankani *et al.* (2005) reported the hepatoprotective activity of ethanolic extract of stem bark of *Pterocarpus marsupium* in rats and described that at a dose rate of 25 mg/kg /day orally for 14 days, the toxic effect of CCl_4 was controlled significantly as evidenced by restoration of the levels of serum bilirubin, proteins and enzymes to the normal level.

Mathew (2005) conducted studies on the hepatoprotective effects of aqueous leaf extracts of *Aegle marmelos* and *Azadirachta indica* in paracetamol toxicity in rats and found that *Azadirachta indica* at the dose rate of 500 mg/kg orally for 12 days and *Aegle marmelos* at the rate of 1 g/kg orally for 12 days offered good protection by reducing the liver serum marker enzymes.

The methanolic extract of the plant *Berberis tinctoria* at the dose rates of 150 and 300 mg/kg produced significant hepatoprotective effects on paracetamol induced hepatic damage in rats by decreasing the activities of serum marker enzymes like ALT, AST, ALP and levels of bilirubin and lipid peroxidase. But it significantly increased the levels of glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in a dose dependent manner because it possessed good antioxidant property. Its effects were comparable with the standard hepatoprotective drug silymarin (Murugesh *et al.*, 2005).

Oyejide and Olushola (2005) studied the hepatoprotective and antioxidant effects of extract of *Carmellia sinensis* (black tea) in rats against the sodium oxalate toxicity. They reported that at a dose rate of 100 mg/kg, the serum and tissue levels of malonaldehyde as well as AST and ALT activities were significantly lowered in a dose dependent manner. The results also indicated that prolonged tea administration at the dose rate of 200 mg/kg body weight for 20 days significantly increased serum vitamin C level and the activity of catalase in the serum, liver and kidney.

Studies conducted by Tabassum *et al.* (2005) revealed that *Phyllanthus niruri* extract at the dose rate of 100 mg/kg/day for five consecutive days offered sufficient hepatoprotection in paracetamol induced hepatotoxicity in mice as evidenced by the estimation of the liver marker enzymes.

Antony *et al.* (2006) studied the hepatoprotective effect of *Centella asiatica* against CCl_4 induced hepatotoxicity in rats and found that the alcohol extract orally in two doses 20 and 40 mg/kg/day significantly reduced the elevated levels of ALT, AST and ALP toxicity. They also reported that the administration of asiaticoside significantly increased the level of antioxidant enzymes like Super Oxide Dismutase, catalase and glutathione peroxidase.

Chattopadhyay *et al.* (2006) conducted studies on the liver regenerative effect of *Phyllanthus amarus* Linn. against alcohol induced liver cell injury in partially hepatectomised albino rats and found that oral administration of the extracts increased the activities of thymidine kinase in regenerating rat liver at 24 hr. The increase in thymidine kinase was caused by comparable increase in the DNA and protein content. These findings suggested that *Phyllanthus amarus* induced DNA synthesis by inducing the levels of nucleic acid synthesizing enzymes during liver regeneration.

Gupta and Misra (2006) reported the hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxication in rats. Administration of the plant extract increased the concentration of glutathione and enhanced Na^+ K^+ ATPase activity. Treatment with aqueous ethanolic extract of *Chamomile recutita* reduced the enhanced level of serum ALT, AST, ALP and bilirubin. It acted as a free radical scavenger and increased the GSH in blood and by its antioxidant effects. They also reported that the hepatoprotective activity of the powdered drug and its extract was due to the stimulatory effects on hepatic regeneration or free radical scavenging effects.

Iwalokun *et al.* (2006) evaluated the hepatoprotective and antioxidant effects of an aqueous extract of *Vernonia amygdalina* leaves against acetaminophen induced hepatotoxicity and oxidative stress in mice *in vivo.* They reported that

pre administration of *Vernonia. amygdalina* resulted in a dose dependent (50-100 mg/kg) reversal of acetaminophen induced alterations of all the liver function parameters and suppression of acetaminophen induced lipid peroxidation and oxidative stress.

According to Jain *et al.* (2006) ethanolic extract of leaves of *Tephrosia purpurea* and flavonoid isolated from leaf extract offered sufficient hepatoprotection in CCl_4 induced hepatotoxicity in rats at the dose rate of 100 mg/ kg/day for 4 days. It caused a decrease in SGOT, SGPT, SALP and total bilirubin to normal level.

Kumar *et al.* (2006) evaluated the protective effect of root extract of *Operculina turpethum* against paracetamol induced hepatotoxicity in rats and found that the ethanolic extract obtained from the plant at a dose rate of 200 mg/kg has significant hepatoprotective effect by lowering serum levels of SGOT, SGPT, ALP and total bilirubin. It could prevent paracetamol induced hepatic necrosis also.

Olaleye *et al.* (2006) discovered that an African plant *Alchornea cordifolia* significantly protected against hepatotoxic, necrotic and peroxidative actions of acetaminophen and prevented the accumulation of toxins in liver and inhibited the formation of peroxides at a dosage between 300-500 mg/kg of the extract. It normalized the elevated serum enzymes in paracetamol toxicity in Wistar rats.

Ozbek *et al.* (2006) reported that the *Foeniculum vulgare* fixed oil (FFO) offered sufficient hepatoprotection in CCl_4 induced liver fibrosis in rats. The FFO at the dose rate of 0.2 mg/kg three times a week for 7 days reduced the elevated levels of ALT, AST, ALP and bilirubin.

Psidium guajava leaf extract at the dose level of 500 mg/kg orally significantly reduced the elevated serum levels of ALT, AST, ALP and bilirubin. It prevented the increase in liver weight in paracetamol toxicity in rats. It gave protection against CCl_4 and thioacetamide induced hepatotoxicity. This plant also showed

antibacterial, antidiarrhoeal, hyperglycaemic and antioxidant activities in addition to the hepatoprotective effect (Roy *et al.*, 2006).

Antioxidant and protective effect of an oleanolic acid enriched extract of *Actinidia deliciosa* (Chinese gooseberry) root on CCl_4 induced liver injury in rat was studied by Bai *et al.* (2007). They reported that at 120 mg/kg dose, ethanol water extract of this plant showed high antioxidant activity *in vitro* and hepatoprotective property *in vivo* when analyzed by ferric thiocyanate and thiobarbituric acid methods. It reduced the elevated serum marker enzymes and lipid peroxidase while GSH concentration was increased.

Bose *et al.* (2007) evaluated the antioxidant and hepatoprotective effects of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity in rats and reported that the methanol extract of the plant at the dose rate of 200 and 300 mg/kg produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, uric acid, lipid peroxides. It also significantly increased the levels of SOD, CAT, GSH and protein in a dose dependent manner.

Chloroformic and methanolic extract of the plant *Ichnocarpus frutescens* at a dose level of 250 mg/kg and 500 mg/kg offered significant hepatoprotection by decreasing the activity of serum enzymes, bilirubin and lipid peroxidase. These significantly increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in a dose dependent manner in paracetamol induced hepatotoxicity in rats and these results were comparable with those of the standard hepatoprotective drug silymarin. These have potent antioxidant effects also (Dash *et al.*, 2007).

Guirati *et al.* (2007) investigated the hepatoprotective activity of alcoholic aqueous extract of leaves of *Tylophora indica* against ethanol induced hepatotoxicity in rats. They found that pre-treatment with alcoholic or aqueous extract significantly prevented the physical, biochemical, histological and functional changes induced by ethanol in liver at the dose levels of 200 and 500 mg/kg alcoholic extract and 125 and 300 mg/kg aqueous extract.

Mir *et al.* (2007) reported that the alcoholic extract of *Embilica officinalis* at the dose rate of 100 mg/kg orally for 8 weeks reversed the CCl_4, paracetamol and thioacetamide induced abnormal histopathology in liver cells of rats by its ability to accelerate the regenerative activity.

The methanol extract of the leaves of *Ficus carica* was evaluated for its hepatoprotective activity in rats with liver damage induced by CCl_4. It was reported that the extract at an oral dose of 500 mg/kg exhibited a significant protective effect by lowering the increased serum levels of ALT, AST, total bilirubin and malonaldehyde equivalent and an index of lipid peroxidation in liver (Mohan *et al.*, 2007).

Pimple *et al.* (2007) reported the hepatoprotective effect of fruits and leaves of *Tamarindus indica* at the dose rate of 350 mg/kg. It reduced the serum enzyme levels of ALT, AST and ALP in paracetamol induced hepatotoxicity.

Sengottuvelu *et al.* (2007) conducted studies on the hepatoprotective activity of *Cleome viscosa* in CCl_4 induced hepatotoxicity in rats. They reported that aqueous seed extract of *Cleome viscosa* at the dose rate of 200 mg/kg administered orally for 7 days, reduced the activities of serum ALT, AST, ALP, Gamma- glutamyl transpeptidase and lipid peroxidase but increased the reduced glutathione (GSH) in the treated animals. The histopathological changes were also reversed by the extract.

Sundaram and Mitra (2007) reported on the antioxidant activity of ethyl acetate soluble fraction of *Acacia arabica* bark in rats and they reported that polyphenol rich active fractions of *Acacia arabica* as a potent free radical scavenger thereby showing its hepatoprotective effect at the dose rate of 150 mg/ kg for 19 days in carbon tetrachloride induced toxicity.

Usha *et al.* (2007) conducted studies on hepatoprotective effect of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in rats and found that the increased enzyme levels after liver damage were nearing to normal value when treated with aqueous extract of root samples.

Histopathological observation also proved the hepatoprotectivity of the root samples.

He *et al.* (2008) conducted studies on the hepatoprotective effects of *Gangoderma lucidum* peptides (GLP) against carbon tetrachloride induced liver damage in mice and revealed that the serum ALT and AST activities and necrotic and pathological hepatocytes were reduced in a dose dependent manner in GLP treated groups. CCl_4 induced liver damage was largely prevented as suggested by histopathological examination.

Kang *et al.* (2008) studied the effects of fucocidan extracts (FE) on CCl_4 - induced liver injury and found that female Sprague-Dawley rats which were administered FE (100 mg/kg) for 14 days and CCl_4 on the 15th day suppressed the increment levels of GOT, GPT, ALP, LDH and MDA as well as recovered the levels of SOD, CAT and GPX in CCl_4 treated rats. Moreover there was a significant decrease in the incidences of necrosis and cirrhosis in the liver tissues of FE treated rats.

Manoharan *et al.* (2008) conducted studies on the potential hepatoprotective effect of aqueous extract of *Gracilaria corticata* in AFB_1 induced hepatotoxicity in Wistar rats and found that elevated levels of total bilirubin, SGOT, SGPT, LDH and ALP in aflatoxin B_1 intoxicated rats were restored to normal level in the rats treated with aqueous extract (250 mg/kg Bwt) and aflatoxin B_1.

Akare *et al.* (2009) studied the hepatoprotective activity of *Acacia ferruginea* leaves against carbon tetrachloride induced liver damage in Wistar rats and revealed that the ethanol extract at the dose of 200 mg/kg significantly decreased the level of ALT, AST, total bilirubin and direct bilirubin in blood as compared to that of aqueous extract. The phytochemical screening revealed the presence of flavonoids and tannins which offer hepatoprotection.

Bhawna *et al.* (2009) conducted studies on the hepatoprotective activity of some indigenous plants and confirmed the efficacy of several plants in the

treatment of liver diseases. They also reported some indigenous plants which are used commonly for prevention and treatment of liver disorders.

Gupta *and* Kumar (2009) evaluated the hepatoprotective activity of *Cleome viscosa* Linn extract against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats and found that the elevated levels of SGOT, SGPT, ALP and bilirubin (total and direct) were significantly reduced after treatment with the ethanolic extract at doses (100 and 200 mg/kg Bwt).

The aqueous extract and steam distilled oil obtained from *Ocimum sanctum* Linn (OS) were evaluated for hepatoprotective activity against ethanol induced cell damage in human derived Hep G_2 cells. The results indicated that morphological changes in Hep G_2 cells induced by ethanol were restored to normal by the aqueous extract and steam distilled oil at different doses (3-100 µg/ ml).They also reduced the elevated enzyme levels of ALT and AST (Kaur *et al.*, 2009).

2..3 OTHER PHARMACOLOGICAL EFFECTS OF ***Tridax procumbens*** **(CHIRAVANAK)**

Diwan *et al.* (1989) conducted pharmacological and biochemical evaluation of *Tridax procumbens* on inflammation by evaluating its effects on various inflammatory models in rats and mice and reported that *T. procumbens* significantly reduced exudates volume, leukocyte migration, edema fluid, granuloma tissue and gamma glutamyl trans peptidase activity which indicated good anti inflammatory action.

Udupa *et al.* (1991) conducted studies on the influence of *Tridax procumbens* on lysyl oxidase activity and wound healing and reported that lysyl oxidase activity, protein content, specific activity and breaking strength were all increased in drug treated animals as compared to controls. A fall in the lysyl oxidase activity was observed in drug treated animals after day 8.

Devi *et al.* (1994) studied the cardiovascular effects of *Tridax procumbens* by subjecting the water extract of leaves of *T.procumbens* to pharmacological studies on cardiovascular system. The extract produced significant cardiac stimulation in isolated mammalian heart and perfused amphibian heart *in situ*. On anaesthetised dog the extract produced sharp but short lasting fall in BP which was not blocked by atropine and mepyramine.

Kanungo *et al.* (1994) conducted studies on the effects of *Tridax procumbens* Linn on normal and heparin induced prolongation of clotting time in rabbits by injecting normal saline i.p. and clotting time was recorded at prefixed time interval. Groups 2, 3 and 4 were injected *Tridax procumbens* extract (200 mg/kg, i.p), heparin (50 mg/kg i.v.) and extract with heparin respectively and clotting time was recorded. The extract significantly reduced the normal clotting time prolonged by heparin.

Yadava and Saurabh (1998) conducted studies on the isolation and identification of a new flavone glycoside, 5, 7, 4'- trihydroxy-6, 3'- dimethoxy – flavone 5-O- alpha – L – rhamnopyranoside 1, having antioxidant property from the leaves of *Tridax procumbens*.

Ali and Jahangir (2002) conducted studies on a bis – bithiophene from *Tridax procumbens* and found that the ethyl acetate soluble part of hexane extract of *T.procumbens* yielded a new bis – bithiophene named tridbisbithiophene along with four known terpenoids: taraxasteryl acetate, beta – amyrenone, lupeol and oleanolic acid. The structures of all the isolated constituents were elucidated with the aid of spectroscopic methods.

Tiwari *et al.* (2004) studied the immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals and reported that intraperitoneal administration of *T. procumbens* ethanol insoluble fraction (TPEIF) at doses of 0.25 and 0.5 mg/kg Bwt caused a significant increase in the phagocytic index, leukocyte count and spleenic antibody secreting cells. Stimulation of humoral immune response was further observed with elevation in the haemagglutinin

antibody titre. The results suggested that TPEIF influenced both humoral as well as cell mediated immune system.

Bhagwat *et al.* (2008) conducted studies on anti-diabetic activity of leaf extract of *Tridax procumbens* and concluded that the oral administration of dried aqueous, alcoholic and petroleum ether extracts at doses of 200 mg/kg led to a significant blood glucose reduction.

Agrawal *et al.* (2009) studied the antioxidant activity of fractions from *Tridax procumbens* and showed that the ethyl acetate and n-butanol fractions from methanolic extract had significant activity which is comparable to the activity of ascorbic acid. Fractionation of the parent extract reduced the complexity of the material and provided more accurate idea related to the phytochemicals responsible for antioxidant activity.

Pareek *et al.* (2009) conducted studies on the hypoglycaemic and anti-hyperglycaemic potential of *Tridax procumbens* by orally administrating the leaf extract at doses 250 and 500 mg/kg Bwt. which showed a significant reduction in fasting blood glucose levels in diabetic rats. The results of anti diabetic effect of *T. procumbens* were compared with the reference drug glibenclamide (10 mg/kg Bwt.).

2.4 OTHER PHARMACOLOGICAL EFFECTS OF *Azadirachta indica* (NEEM)

Chattopadhyay (1999) studied the possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract in rats and found that the extract significantly blocked the inhibitory effect of serotonin on insulin secretion mediated by glucose.

Siddiqui *et al.* (2002) isolated two new triterpenoids, 22, 23- dihydronimocinol (1) and desfurano-6 alpha-hydroxy azadirone (2) from a methanolic extract of fresh leaves of *Azadirachta indica* along with a known meliacin. Compounds 1 and 2 showed mortality for fourth instar larvae of the mosquito *Anopheles stephensi.*

Abudulai *et al.* (2004) studied the effects of Neem *(Azadirachta indica)* on predators of *Nezara viridula* by evaluating the effects of the commercial Neem formulation Neemix on predators of *N. viridula* eggs in the field like ants and coccinellids and suggested that Neem may not interfere with predation on *N. viridula* eggs.

Chattopadhyay *and* Bandyopadhyay (2005) conducted studies on the effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats and found that the extract significantly reduced the total cholesterol, LDL-VLDL cholesterol, triglycerides and total lipids of serum in streptozotocin induced diabetic rats but HDL cholesterol levels remained unchanged when compared with streptozotocin induced diabetic control rats.

Thakurta *et al.* (2007) conducted studies on antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica used* to treat cholera and diarrhoea in India and found that the methanol extract of neem leaf had significant antibacterial activity against the multidrug resistant *Vibrio cholerae* of various serotypes. Neem extract showed antisecretory activity on *V. cholerae* induced fluid secretion in mouse intestine. Oral administration of the extract inhibited haemorrhage induced by *V. cholerae* in mouse intestine.

Mossini *and* Kemmelmeier (2008) conducted studies on the inhibition of citrinin production in *Penicillium citrinum* cultures by Neem and found that mycotoxin production by the isolates was suppressed depending on the concentration of the plant extract added to the culture media at the time of spore inoculation. High performance liquid chromatography was performed to confirm the spectrophotometric results. Neem leaf extract showed inhibition of toxin production without retarding fungal mycelia growth.

Koul *et al.* (2009) conducted qualitative tests of *Azadirachta indica* leaf extracts for screening of various phytochemicals. Aqueous and petroleum ether *A. indica* leaf extracts reduced the inflammation caused by *S.typhimurium* and its outer membrane proteins as assessed by paw flicking response in rats. Petroleum

ether *A. indica* leaf extract was found to be more effective than aqueous leaf extract. Significantly lower levels of monokines were also observed in the presence of petroleum ether leaf extracts than aqueous extract. These observations may be due to the presence of steroids and triterpenoids observed in petroleum ether extract.

Olabinri *et al.* (2009) studied the antioxidant capacity of the leaf, stem and root barks of *Mangifera indica* and *Azadirachta indica* and reported that the leaf of *A. indica* belonged to good ferric reducing antioxidant power (FRAP). Both the stem and root bark of *A. indica* and all the parts of *M. indica* investigated belonged to high FRAP. Antioxidant activity depends on total phenolic concentration for stem bark and leaf of *M. indica* and for the root bark of *A. indica.*

Wankar *et al.* (2009) evaluated the effect of Neem *(Azadirachta indica)* leaf powder supplementation on growth in broilers, and found that all the treatment groups recorded significantly higher means for live body weight than that of control group. All the treatment groups showed non- significant increase in weekly gain in weight, feed consumption and feed efficiency as compared to that of the control group.

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The study was conducted in 40 adult male Wistar rats weighing 150-200 g procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The animals were housed in appropriate cages in a well ventilated room with a 12-hour light: 12-hour dark cycle. All the animals received standard diet and water *ad libitum*. They were maintained under identical feeding and management practices in the laboratory. An acclimatization period of seven days was allowed before the commencement of the experiment. The experiment was conducted for a period of 10 days.

3.2 PLANT MATERIALS

The leaves of the plants *Azadirachta indica* (Fig.1) and *Tridax procumbens* (Fig.2) were collected from the campus of College of Veterinary and Animal Sciences, Mannuthy, Thrissur district, Kerala.

3.2.1 Preparation of alcoholic extract of *Azadirachta indica* and *Tridax procumbens*

The leaves of *Azadirachta indica* and *Tridax procumbens* were air-dried under shade and coarsely powdered using an electrical pulverizer. The powder obtained was extracted using a Soxhlet apparatus with 95 per cent of ethanol. The ethanolic extracts were then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55°C) and kept under refrigeration for the complete evaporation of the solvent.

3.3 PHYTOCHEMICAL SCREENING

The ethanolic extract of leaves of *Azadirachta indica* and *Tridax procumbens* was tested for the presence of various active chemical constituents namely steroids, alkaloids, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure described by Harborne (1991).

Fig. 1. *Azadirachta indica* leaves

Fig. 2. *Tridax procumbens* leaves

3.3.1 Tests for Detection of Steroids

3.3.1.1 *Salkowski test*

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

3.3.1.2 *Lieberman Burchardt test*

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

3.3.2 Tests for Detection of Alkaloids

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

3.3.2.1 *Mayer's test (Potassium mercuric iodide)*

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

3.3.2.2 *Wagner's test*

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

3.3.2.3 *Hager's test (saturated solution of picric acid)*

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

3.3.2.4 *Dragendroff's test*

A few drops of Dragendroff's reagent Stock solution (1) 0.6 grams of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid and 8 ml of water was added. Stock Solution (2) six grams of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 ml) and were mixed with 1 ml of acid extract. Development of a reddish brown precipitate indicates the presence of alkaloids.

3.3.3 Test for Detection of Phenolic compounds

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

3.3.4 Tests for Detection of Tannins

3.3.4.1 *Ferric chloride test*

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

3.3.4.2 *Gelatin test*

About 0.5 g of the extract was mixed with a 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.

3.3.5 Tests for Detection of Flavonoids

3.3.5.1 *Ferric chloride test*

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

3.3.5.2 *Lead acetate test*

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

3.3.6 Tests for Detection of Glycosides

3.3.6.1 *Sodium hydroxide test*

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

3.3.6.2 *Benedict's test*

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

3.3.7 Test for Detection of Diterpenes

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

3.3.8 Tests for Detection of Triterpenes

3.3.8.1 *Salkowski test*

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

3.3.8.2 *Lieberman Burchardt test*

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

3.3.9 Test for the detection of Saponins

Foam test

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

3.4 EXPERIMENTAL DESIGN

Forty adult male rats were randomly divided into 5 groups comprising eight animals each. The experiment was conducted for a period of 10 days.

Group I – Healthy control was administered with vehicle (3 per cent gum acacia in distilled water at the rate of 5 ml/kg/day) orally for 10 days.

Group II – Rats were administered with 3 per cent gum acacia for 10 days and on 8th day paracetamol 2 g/kg was given orally in distilled water.

Group III – Ethanolic extract of leaves of *Azadirachta indica* was administered at a dose rate of 300 mg/kg orally in 3 per cent gum acacia at the rate of 5 ml/kg/day for 10 days and paracetamol 2 g/kg was given on 8th day in distilled water.

Group IV – Ethanolic extract of leaves of *Tridax procumbens* was administered at a dose rate of 300 mg/kg orally in 3 per cent gum acacia at the rate of 5 ml/kg/day for 10 days and paracetamol 2 g/kg was given on 8th day in distilled water.

Group V – Rats were administered with silymarin 100 mg/kg/day in gum acacia for 10 days orally and on day 8, 2 g/kg paracetamol was administered orally in distilled water.

Blood was collected from all the animals on the day of sacrifice. The serum was separated and used for the estimation of biochemical parameters such as serum alanine amino transferase, aspartate amino transferase, albumin, total protein and bilirubin. Disodium salt of ethylene diamine tetra acetic acid (EDTA Sodium) was added at the rate of 1 mg/ml of blood for studying the hematological parameters like total leukocyte count (TLC), differential leukocyte count (DLC) and hemoglobin. On day 10th, all the rats were sacrificed and liver was collected for conducting histopathological studies.

3.5 COLLECTION OF BIOLOGICAL SAMPLES

3.5.1 Collection of blood and separation of serum

Blood was collected from the retro orbital plexus under mild ether anaesthesia, with heparinized capillary tubes, into sterile centrifuge tubes without adding any anticoagulant. It was kept at refrigeration temperature for half an hour, taken out and kept at room temperature for another half an hour. It was then centrifuged at 3200 rpm for 10 minutes and the clear serum obtained was pipetted out.

3.5.2 Liver

The animals were euthanized and dissected upon and the liver was collected and washed in running tap water to remove the blood clots. The liver was preserved in 10 per cent formalin for conducting histopathological studies.

3.6 ESTIMATION OF SERUM PARAMETERS

The serum parameters were estimated colorimetrically in semiautomatic blood analyzer (Mircolab 200, MERCK) by using the analytical kits supplied by Agappe Diagnostics Pvt. Ltd., Ernakulam, Kerala, India.

3.6.1 Alanine amino transferase (ALT)

UV Kinetic test (Reitman and Frankel, 1957)

Principle

Serum ALT catalyses the transfer of amino group from L-alanine to L-oxoglutarate with formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH. The oxidation of NADH is measured as the decrease in the absorbance of NADH at 340 nm, which is proportional to ALT activity.

Procedure

Pipetted out 100 µl of sample into 1000 µl working reagent, mixed well and measured the absorbance at 340 nm in an auto analyzer.

3.6.2 Aspartate amino transferase (AST)

UV Kinetic test (Reitman and Frankel, 1957)

Aspartate amino transferase catalyses transfer of amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. The rate of this reaction

is monitored by an indicator reaction coupled with malate dehydrogenase in the presence of NADH. The oxidation of NADH at 340 nm is proportional to AST activity.

Procedure

Mixed well 100 µl of sample and 1000 µl of working reagent and measured the absorbance at 340 nm in an auto analyzer.

3.6.3 Estimation of serum total protein

Biuret method (Gornall *et al.*, 1949)

Principle

Protein in plasma or serum sample forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration and is measured at 540 nm.

Procedure

Sample, standard and blank were prepared as follows

	Sample	Standard	Blank
Serum	20 µl		
Standard		20 µl	
Reagent	1000 µl	1000 µl	1000 µl

Mixed the solutions and incubated for ten minutes at 37°C. Read the absorbance of standard and sample against blank at 546 nm

$$\text{Serum total protein (g/d l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6$$

3.6.4 Estimation of serum albumin (Bromocresol Green Dye Method, Doumas, 1971)

Principle

The reaction between albumin from serum or plasma and the dye bromocresol-green forms a blue green complex at slightly acidic pH that is proportional to the albumin concentration. This is measured photometrically.

Sample, standard and blank were prepared as follows

	Blank	Standard	Sample
Reagent	1000 µl	1000 µl	1000 µl
Standard	--	100 µl	--
Sample	--	--	10 µl

Mixed the solutions and incubated for one minute at 37°C. Read the absorbance of standard and sample against blank at 630 nm.

$$\text{Serum albumin (g/d l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3$$

3.6.5 Estimation of bilirubin (Modified DMSO method)

Principle

Sulfanilic acid reacts with sodium nitrite in the presence of diazotized sulfanilic acid to form azobilirubin. In the absence of dimethyl sulfoxide, only the direct bilirubin reacts to give azobilirubin.

Procedure

Sample, blank and test were prepared as follows

Total bilirubin reagent	Sample blank 1000 µl	Test 1000 µl
Activator	--	20 µl
Serum	50 µl	50 µl

Mixed well and incubated for exactly 5 minutes. Measured the absorbance of the sample against respective sample blank at 546 nm.

Total bilirubin concentration in mg/dl = OD of sample – OD of sample blank x 20.5 (multiplication factor)

3.7 HAEMATOLOGICAL PARAMETERS

3.7.1 Haemoglobin concentration

Haemoglobin was estimated by acid Haematin method (Benjamin, 1985).

3.7.2 Total leukocyte count

Leukocytes were counted by standard dilution technique using Thomas fluid. Counting of leukocytes was done in the zone for leukocytes in the haemocytometer placed under low power of microscope (Benjamin, 1985).

3.7.3 Differential leukocyte count

Blood smears were prepared from freshly drawn blood without anticoagulant by using slide technique. After staining with Wrights stain counting was done under oil immersion (Benjamin, 1985).

3.8 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

The gross and histopathological lesions in liver were studied by collecting representative samples of liver obtained from the dissected animals on the 10^{th} day.

3.8.1 Gross lesions

The gross lesions in liver of treated groups were compared with control groups.

3.8.2 Histopathological examination

The animals were sacrificed on the 10^{th} day, and liver was taken for histopathological examination for assessing the hepatotoxicity. Three mm thick, pieces of liver were selected randomly from both control and experimental groups of rats and were fixed in 10 per cent formalin. They were then processed and paraffin embedded as described by Sheehan and Hrapchak (1980). The sections were stained with haematoxylin and eosin as per the technique followed by Bancroft and Cook (1984). The sections were examined in detail under light microscope.

3.9 STATISTICAL ANALYSIS OF DATA

Results are analyzed by using analysis of variance (ANOVA) technique for comparison between groups and paired t test (Snedecor and Cochran, 1985). Results are expressed as mean ± standard error.

4. RESULTS

4.1 PHYTOCHEMICAL SCREENING

4.1.1 Steroids

In the Salkowski test red color was obtained and Lieberman Burchadt's test gave a reddish ring at the junction for *Tridax procumbens* extract and not for *Azadirachta indica* extract. Thus it could be concluded that steroids were present in the ethanolic extract of *Tridax procumbens* and no detectable level of steroids could be obtained in *Azadirachta indica* extract.

4.1.2 Alkaloids

A creamy white precipitate in Mayer's test and a yellow colored precipitate in Hager's test were obtained for *Azadirachta indica* and *Tridax procumbens* extracts. Dragendroff's test yielded a reddish brown precipitate for both extracts. With Wagner's reagent a reddish brown precipitate was obtained. Thus the test revealed detectable levels of alkaloids in the extracts of *A.indica* and *T.procumbens*.

4.1.3 Tannins

Brownish green color was obtained in ferric chloride test for *A.indica* and *T.procumbens* and white precipitate in gelatin test. It revealed the presence of tannins in the ethanolic extract of *A.indica* and *T.procumbens*.

4.1.4 Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in the lead acetate test indicated the presence of flavonoids in *A.indica* and *T. procumbens* extracts.

4.1.5 Glycosides

A red colour obtained in the Benedict's test indicated the presence of glycosides in the extracts. A yellow color was obtained by mixing the extracts

with sodium hydroxide reagent, which also indicated the presence of glycosides in both extracts.

4.1.6 Phenolic compounds

The extracts mixed with 10 per cent ferric chloride produced dark blue color, for *A.indica* extract and not for *T. procumbens* extract. Thus it could be concluded that phenolic compounds were present in the ethanolic extract of *A.indica* and no detectable level of phenolic compounds were present in the *T.procumbens* extract.

4.1.7 Diterpenes

Diterpenes were detected in *A.indica* extract as indicated by the green color when mixed with copper acetate solution and no such green color was obtained in the *T.procumbens* extract which indicated the absence of diterpenes.

4.1.8 Triterpenes

For *A.indica* extract lower layer turned to yellow on standing as per Salkowski test, and with Liebermann Burchadt's test, a deep red ring appeared at the junction of the two layers which indicated the presence of triterpenes. But no such changes were seen in *T.procumbens* extract.

4.1.9 Saponins

In the foam test, foam persisted for 10 minutes in the case of *A.indica* and *T. procumbens* extracts, which indicated the presence of saponins in the samples.

The results obtained are summarized in the Table 1.

4.2 PHYSIOLOGICAL PARAMETERS

4.2.1. Body weight

The individual and mean body weights of rats (group I, group II, group III, group IV and group V) were recorded on the days 0 and 10 of the experiment and are presented in the Table 2. The body weights recorded on the day 0 of groups I to V were 174.38±3.59, 180±4.63, 182.50±3.13, 183.13±5.08 and 177.50±4.23 g respectively. After treatment on the 10^{th} day, the mean body weights recorded

were 180.50±3.68, 175±5.43, 186.25±3.24, 182.88±5.04 and 181.88±4.11 g respectively. A reduction in body weight was shown by group II animals and not much change was shown by group IV animals. A gradual increase in weight was noticed in the groups I, III and V.

4.3 SERUM PARAMETERS

4.3.1 Alanine amino transferase (ALT)

The results obtained are presented in the Tables 3, 4 and Figure 3. The serum ALT levels before treatment were 54.25±2.63, 56±3.70, 55.38±2.49, 54.88±3.07 and 57±2.93 U/L, respectively in groups I to V. The highest level of ALT was noticed in the paracetamol treated group, where the value increased from 56.00±3.70 to 138.75±6.62 U/L. After ten days of experiment, the level of ALT was 54.38±2.62 in normal animals and in the extract treated animals the values became 93.13±4.12 and 101.50±5.00 U/L at 300 mg/kg for *Azadirachta indica* and *Tridax procumbens* respectively. In the silymarin treated group the value was near to those of normal animals (62.75±2.72 U/L). Group I and V and group III and IV animals did not differ significantly ($p<0.05$).

4.3.2 Aspartate amino transferase (AST)

The results obtained are presented in the Tables 5, 6 and Figure 4. The AST levels before treatment were 151.50±7.32, 139.75±7.41, 133.63±6.18, 145.38±5.49 and 150.13±5.23 U/L respectively in groups I, II, III, IV and V animals. After treatment, the value was highest in the paracetamol treated animals. Here the value increased from 139.75±7.41 to 275±5.82 U/L. After treatment the values were 150.75±7.02, 191.38±5.35, 192.13±3.86, and 161.13±4.63 U/L respectively in groups 1, III, IV and V animals. Group I and V and Group III and IV animals did not differ significantly ($p<0.05$).

4.3.3 Serum total protein

The values of serum total protein obtained before and after treatment are presented in tables 7 and 8 and Figure 5. The means on the day 0 were 6.73±0.14, 6.66±0.13, 6.56±0.14, 6.34±0.15, 6.31±0.13 g/dl respectively, for groups 1 to V.

After treatment with ethanolic extract of *Azadirachta indica* and *Tridax procumbens* at the dose of 300 mg/kg, the level changed to 6.60±0.11 and 6.49±0.20 g/dl respectively. The value of group I animals was almost same before and after the treatment. The total protein level of Group II animals decreased from 6.66±0.13 to 6.41±0.14 g/dl. The values of groups I and V were 6.75±0.16 and 6.50±0.16 g/dl respectively.

4.3.4 Serum albumin

The data obtained before and after the treatment are shown in tables 9, 10 and Figure 6. On the day 0, the mean serum albumin levels of Groups I to V were 2.38±0.09, 2.41±0.10, 2.69±0.09, 2.39±0.10 and 2.51±0.05 g/dl respectively. After treatment with *Azadirachta indica* and *Tridax procumbens* extracts at 300 mg/kg doses, the values changed to 2.79±0.05 and 2.56±0.07 g/dl respectively in Groups III and IV animals. The mean serum albumin levels of Groups I and V were 2.36±0.09 and 2.53±0.06 g/dl respectively. A reduction in value was noticed in the paracetamol treated group. Here the value was reduced to 2.08±0.5 g/dl. There was no significant difference between the groups IV and V.

4.3.5 Serum total bilirubin

The results obtained before and after the treatment are shown in tables 11, 12 and Figure 7. The serum total bilirubin values before the treatment were 0.33±0.01, 0.30±0.01, 0.33±0.01, 0.36±0.03 and 0.33±0.02 mg/dl respectively for the groups I to V. A high level of bilirubin 1.01±0.06 mg/dl was noticed in the group II animals. The level of total bilirubin in the other groups were 0.31±0.01, 0.40±0.02, 0.41±0.04 and 0.35±0.02 mg/dl for the groups I, III, IV and V respectively. Groups I, III, IV and V did not differ significantly ($P<0.05$).

4.4 HAEMATOLOGICAL PARAMETERS

4.4.1 Haemoglobin concentration

The mean values are presented in the Tables 13 and 14. The haemoglobin concentration of groups I, II, III, IV and group V animals before treatment were 10.26±0.15, 10.19±0.10, 10.03±0.15, and 9.97±0.14 and 10.09±0.13 g/dl. After

treatment the concentrations were 10.36±0.15, 10.18±0.10, 10.04±0.15, 10.04±0.17 and 10.08±0.18 g/dl respectively for the groups I to V. The values were in normal range on both the occasions in all the groups.

4.4.2 Total Leukocyte count (TLC)

The values before and after the treatment are presented in Tables 13 and 14. All the values were in normal range before and after the treatment. On the day 0 of the experiment the values were 11.48±0.29, 11.51±0.32, 11.47±0.30, 11.92±0.28 and 11.59± 0.28 × 10^3/µl respectively for the groups I to V. After the treatment the values were 11.54± 0.22, 11.49± 0.38, 11.57±0.23, 11.46±0.39 and 11.85±0.35 ×10^3/µl respectively for the groups I to V.

4.4.3 Differential leukocyte count (DLC)

4.4.3.1 *Lymphocytes*

The results obtained are presented in the tables 13 and 14. Before the treatment the values were 79.38±0.25, 79.02±0.28, 78.86±0.21, 79.03±0.20 and 78.61±0.16 per cent respectively for the groups I to V. After the treatment with the plant extracts the values were 78.55±0.14 and 78.57±0.13 per cent respectively for groups III and IV. In the group II animals the mean value was 78.41±0.13 per cent. For the groups I and V the mean values noticed were 78.62±0.20 and 78.66±0.10 per cent.

4.4.3.2 *Neutrophils*

The data is presented in the tables 13 and 14. On the day 0 of the experiment the values were 18.42±0.13, 18.45±0.13, 18.57±0.15, 18.44±0.13 and 18.57±0.17 per cent respectively for the groups I to V. After the experiment on the 10th day the values obtained were 18.32±0.12, 18.71±0.13, 18.68±0.14, 18.44±0.12 and 18.59±0.11 per cent respectively for the groups I to V.

4.4.3.3 *Eosinophils*

The results obtained are presented in the tables 13 and 14. Before the treatment the values were 0.61±0.06, 0.60±0.06, 0.61±0.05, 0.60±0.03 and 0.60±0.03 per

cent respectively for the groups I to V. After treatment with the plant extracts the values were recorded as 0.61±0.05 and 0.61±0.05 per cent respectively for groups III and IV. In the group II animals the mean value was 0.61±0.08 per cent. For group I and V animals the values noticed were 0.60±0.05 and 0.61±0.05 per cent. No significiant change was noticed in values before and after the treatment.

4.4.3.4 *Monocytes*

The data is presented in the tables 13 and 14. Before treatment the values were 0.14±0.05, 0.13±0.05, 0.14±0.05, 0.13±0.06 and 0.14±0.06 and after the treatment the values were 0.14±0.08, 0.14±0.04, 0.14±0.05, 0.14±0.06 and 0.14±0.04 per cent respectively for the groups I to V. No significiant change was noticed in values before and after the treatment.

4.4.3.5 *Basophils*

Basophils were not observed before and after the treatment.

4.5 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

With the exception of the paracetamol treated group, the liver from all groups were normal in terms of appearance and color. (Fig 8) reveals the appearance of normal liver. Paracetamol treated group showed marked greyish white areas of necrosis and multiple petechiae (Fig 9).

Histopathological examination of the liver from the normal control group revealed normal hepatic architecture (Fig 10). In the paracetamol treated group there were extensive areas of centrilobular coagulation necrosis. The hepatocytes showed deeply eosinophilic cytoplasm with pyknotic nuclei and infiltration of massive number of inflammatory cells (Fig 11). In the 300 mg/kg *Azadirachta indica* extract treated animals, liver showed abundance of binucleate hepatocytes indicating areas of regeneration (Fig 12). In the 300 mg/kg *Tridax procumbens* extract treated group the binucleate cells were scanty and vacuolation of cytoplasm was noticed. All the blood vessels were congested (Fig 13). Silymarin treated group showed apparently normal hepatic architecture (Fig 14).

Table1. Results of Phytochemical screening of ethanolic extract of *Azadirachta indica* and *Tridax procumbens*

Sl.No	Active principle	*Azadirachta indica*	*Tridax procumbens*
1	Steroids	Absent	Present
2	Alkaloids	Present	Present
3	Tannins	Present	Present
4	Flavonoids	Present	Present
5	Glycosides	Present	Present
6	Phenolic compounds	Present	Absent
7	Diterpenes	Present	Absent
8	Triterpenes	Present	Absent
9	Saponins	Present	Present

Table 2. Effect of treatment on body weights (g) of rats (n=8)

Animal No	Group 1		Group II		Group III		Group IV		Group V	
	Day 0	10th day	Day 0	10th day	Day 0	10th day	Day 0	10th day	Day 0	10th day
1	160	166	180	160	190	190	170	170	175	185
2	180	188	160	155	180	190	175	175	170	170
3	165	170	200	200	170	170	160	160	200	200
4	175	180	170	170	180	190	180	180	165	165
5	185	180	190	180	200	200	190	190	180	190
6	190	200	190	190	180	180	200	200	170	175
7	170	180	170	180	180	180	200	200	190	190
8	170	180	180	165	180	190	190	188	170	180
Mean ±SE	174.38 ±3.59	180.50 ±3.68	180 ±4.63	175 ±5.43	182.50 ±3.13	186.25 ±3.24	183.13 ±5.08	182.88 ±5.04	177.50 ±4.23	181.88 ±4.11

Table 3. Serum ALT level (U/L) before treatment in rats (n=8) on day 0

Animal No	Group I	Group II	Group III	Group IV	Group V
1	54	48	60	55	55
2	58	65	45	62	54
3	54	56	62	55	45
4	48	54	54	64	48
5	64	35	60	50	70
6	48	65	55	65	58
7	64	65	45	48	62
8	44	60	62	40	64
Mean± SE	54.25± 2.63	56.00± 3.70	55.38± 2.49	54.88± 3.07	57.00± 2.93

Table 4. Effect of *Azadirachta indica* and *Tridax procumbens* on ALT (U/L) in paracetamol induced hepatotoxicity in rats (n=8) on day 10

Animal No	Normal Control (Group I)	Paracetamol treated (Group II)	Para + *A.indica* extract (Group III)	Para + *T.procumbens* extract (Group IV)	Silymarin treated (Group V)
1	55	130	100	110	62
2	60	114	88	106	60
3	52	175	90	98	48
4	46	150	92	108	60
5	62	145	94	96	74
6	50	126	95	92	62
7	45	128	100	100	68
8	65	142	86	102	68
Mean ±SE	54.38^{a} ±2.62	138.75^{c} ±6.62	93.13^{b} ±4.12	101.50^{b} ±5.00	62.75^{a} ±2.72

Means bearing the same superscripts do not differ significantly at $P<0.05$

Table.5 Serum AST level (U/L) before treatment in rats (n=8) on day 0

Animal No	Group I	Group II	Group III	Group IV	Group V
1	124	105	106	161	175
2	140	124	145	162	155
3	186	135	123	154	160
4	134	161	124	125	154
5	152	138	136	128	130
6	156	165	165	135	153
7	175	160	130	138	134
8	145	130	140	160	140
Mean ±SE	151.50 ±7.32	139.75 ±7.41	133.63 ±6.18	145.38 ±5.49	150.13 ±5.23

Table.6 Effect of *Azadirachta indica* and *Tridax procumbens* on AST (U/L) in paracetamol induced hepatotoxicity in rats (n=8) on day 10

Animal No	Normal Control (Group I)	Paracetamol treated (Group II)	Para + *A.indica* extract (Group III)	Para + *T.procumbens* extract (Group IV)	Silymarin treated (Group V)
1	122	276	185	198	182
2	150	284	218	204	165
3	180	270	170	185	162
4	130	296	208	210	140
5	158	242	182	180	148
6	148	270	186	192	172
7	174	290	192	182	162
8	144	272	190	186	158
Mean ±SE	150.75^{a} ±7.02	275.00^{c} ±5.82	191.38^{b} ±5.35	192.13^{b} ±3.86	161.13^{a} ±4.63

Means bearing the same superscripts do not differ significantly at $P<0.05$

Table 7. Serum total Protein level (g/dl) before treatment in rats (n=8) on day 0

Animal No	Group I	Group II	Group III	Group IV	Group V
1	6.2	6.3	6.2	7.0	6.5
2	7.0	6.4	6.5	6.9	6.9
3	6.7	6.2	7.0	6.4	6.5
4	6.3	7.2	6.7	6.1	5.9
5	7.2	7.0	6.5	6.2	6.3
6	7.1	6.7	7.2	5.9	6.2
7	6.4	7.0	6.1	5.8	5.8
8	6.9	6.5	6.3	6.4	6.4
Mean ±SE	6.73 ±0.14	6.66 ±0.13	6.56 ±0.14	6.34 ±0.15	6.31 ±0.13

Table 8. Effect of *Azadirachta indica* and *Tridax procumbens* on total protein level in paracetamol induced hepatotoxicity in rats (n=8) on day 10

Animal No	Normal Control (Group I)	Paracetamol treated (Group II)	Para + *A.indica* extract (Group III)	Para + *T.procumbens* extract (Group IV)	Silymarin treated (Group V)
1	6.2	6.2	6.7	7.1	7.0
2	6.8	6.3	5.9	7.3	7.1
3	7.1	7.0	6.5	6.6	6.0
4	6.5	6.1	6.9	6.9	6.6
5	7.4	6.2	6.7	5.9	6.5
6	6.1	6.3	6.5	6.0	6.8
7	6.8	6.1	6.8	6.1	6.0
8	7.1	7.1	6.8	6.0	6.0
Mean ±SE	6.75 ±0.16	6.41 ±0.14	6.60 ±0.11	6.49 ±0.20	6.50 ±0.16

Table 9. Serum albumin level (g/dl) before treatment in rats (n=8) on day 0

Group I	Group I	Group II	Group III	Group IV	Group V
1	2.7	2.8	2.8	2.8	2.5
2	2.2	2.4	2.3	2.5	2.8
3	2.2	2.2	2.8	2.2	2.5
4	2.3	2.4	2.5	2.2	2.3
5	2.5	2.2	2.8	2.2	2.2
6	2.4	2.7	2.9	2.2	2.5
7	2.6	2.4	2.6	2.7	2.4
8	2.1	2.2	2.8	2.3	2.9
Mean ±SE	2.38 ±0.09	2.41 ±0.10	2.69 ±0.09	2.39 ±0.10	2.51 ±0.05

Table 10. Effect of *Azadirachta indica* and *Tridax procumbens* on serum albumin (g/dl) in paracetamol induced hepatotoxicity in rats (n=8) on day 10

Animal No	Normal Control (Group I)	Paracetamol treated (Group II)	Para + *A.indica* extract (Group III)	Para + *T.procumbens* extract (Group IV)	Silymarin treated (Group V)
1	2.6	2.4	2.8	2.9	3.0
2	2.2	2.0	3.0	2.9	2.5
3	2.3	1.8	3.1	3.2	2.6
4	2.1	1.9	3.0	2.9	2.3
5	2.6	2.1	2.8	2.4	2.2
6	2.4	2.2	2.7	2.2	2.4
7	2.5	2.0	2.9	2.0	2.6
8	2.2	2.2	2.0	2.0	2.6
Mean ±SE	2.36^{ab} ±0.09	2.08^{a} ±0.5	2.79^{c} ±0.05	2.56^{bc} ±0.07	2.53^{bc} ±0.06

Means bearing the same superscripts do not differ significantly at $P<0.05$

Table 11.Serum total bilirubin (mg/dl) before treatment in rats (n=8) on day 0

Animal No	Group I	Group II	Group III	Group IV	Group V
1	0.3	0.3	0.3	0.4	0.2
2	0.4	0.3	0.3	0.4	0.3
3	0.4	0.2	0.4	0.3	0.4
4	0.3	0.3	0.3	0.4	0.3
5	0.3	0.4	0.3	0.5	0.3
6	0.3	0.3	0.4	0.2	0.3
7	0.3	0.3	0.3	0.3	0.4
8	0.3	0.3	0.3	0.4	0.4
Mean ±SE	0.33 ±0.01	0.30 ±0.01	0.33 ±0.01	0.36 ±0.03	0.33 ±0.02

Table 12. Effect of *Azadirachta indica* and *Tridax procumbens* on serum total bilirubin (mg/dl) in paracetamol induced hepatotoxicity in rats (n=8) on day 10

Animal No	Normal Control (Group I)	Paracetamol treated (Group II)	Para + *A.indica* extract (Group III)	Para + *T.procumbens* extract (Group IV)	Silymarin treated (Group V)
1	0.3	1.2	0.4	0.4	0.3
2	0.3	1.1	0.4	0.5	0.2
3	0.4	0.8	0.5	0.4	0.4
4	0.3	1.0	0.3	0.4	0.4
5	0.3	0.9	0.5	0.2	0.4
6	0.3	0.8	0.2	0.5	0.3
7	0.3	1.1	0.4	0.5	0.4
8	0.3	1.2	0.5	0.4	0.4
Mean ±SE	0.31^{a} ±0.01	1.01^{b} ±0.06	0.40^{a} ±0.02	0.41^{a} ±0.04	0.35^{a} ±0.02

Means bearing the same superscripts do not differ significantly at $P<0.05$

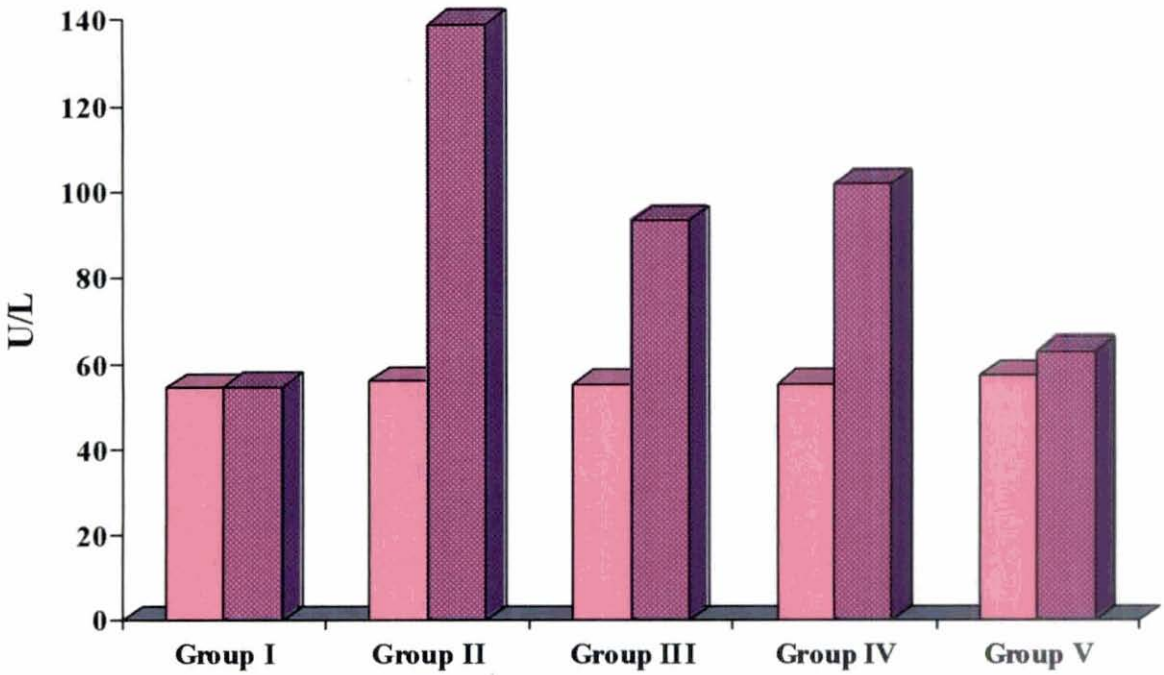
Table 13. Haematological values before treatment in rats on day 0

		Haemoglobin (g/dl)	Leukocyte Count ($10^3/\mu l$)	Differential leukocyte count (%)				
				Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
Group I	Mean ±SE	10.26 ±0.16	11.48 ±0.29	79.38 ±0.25	18.42 ±0.13	0.61 ±0.06	0.14 ±0.05	0.00 ±0.00
Group II	Mean ±SE	10.19 ±0.10	11.51 ±0.32	79.02 ±0.28	18.45 ±0.13	0.60 ±0.06	0.13 ±0.05	0.00 ±0.00
Group III	Mean ±SE	10.03 ±0.15	11.47 ±0.30	78.86 ±0.21	18.57 ±0.15	0.61 ±0.05	0.14 ±0.05	0.00 ±0.00
Group IV	Mean ±SE	9.97 ±0.14	11.92 ±0.28	79.03 ±0.20	18.44 ±0.13	0.60 ±0.03	0.13 ±0.06	0.00 ±0.00
Group V	Mean ±SE	10.09 ±0.13	11.59 ±0.28	78.61 ±0.16	18.57 ±0.17	0.60 ±0.03	0.14 ±0.06	0.00 ±0.00

Table 14. Effect of *Azadirachta indica* and *Tridax procumbens* on hematological parameters in paracetamol induced hepatotoxicity in rats (n=8) on day 10

		Haemoglobin (g/dl)	Leukocyte Count ($10^3/\mu l$)	Differential leukocyte count (%)				
				Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
Group I	Mean ±SE	10.36 ±0.15	11.54 ±0.22	78.62 ±0.20	18.32 ±0.12	0.60 ±0.05	0.14 ±0.08	0.00 ±0.00
Group II	Mean ±SE	10.18 ±0.10	11.49 ±0.38	78.41 ±0.13	18.71 ±0.13	0.61 ±0.08	0.14 ±0.04	0.00 ±0.00
Group III	Mean ±SE	10.04 ±0.15	11.57 ±0.23	78.55 ±0.14	18.68 ±0.14	0.61 ±0.05	0.14 ±0.05	0.00 ±0.00
Group IV	Mean ±SE	10.04 ±0.17	11.46 ±0.39	78.57 ±0.13	18.44 ±0.12	0.61 ±0.05	0.14 ±0.06	0.00 ±0.00
Group V	Mean ±SE	10.08 ±0.18	11.85 ±0.35	78.66 ±0.10	18.59 ±0.11	0.61 ±0.05	0.14 ±0.04	0.00 ±0.00

Fig.3. Effect of treatment on ALT level (U/L)



Dotted bars indicate levels after treatment

Fig.4. Effect of treatment on AST level (U/L)

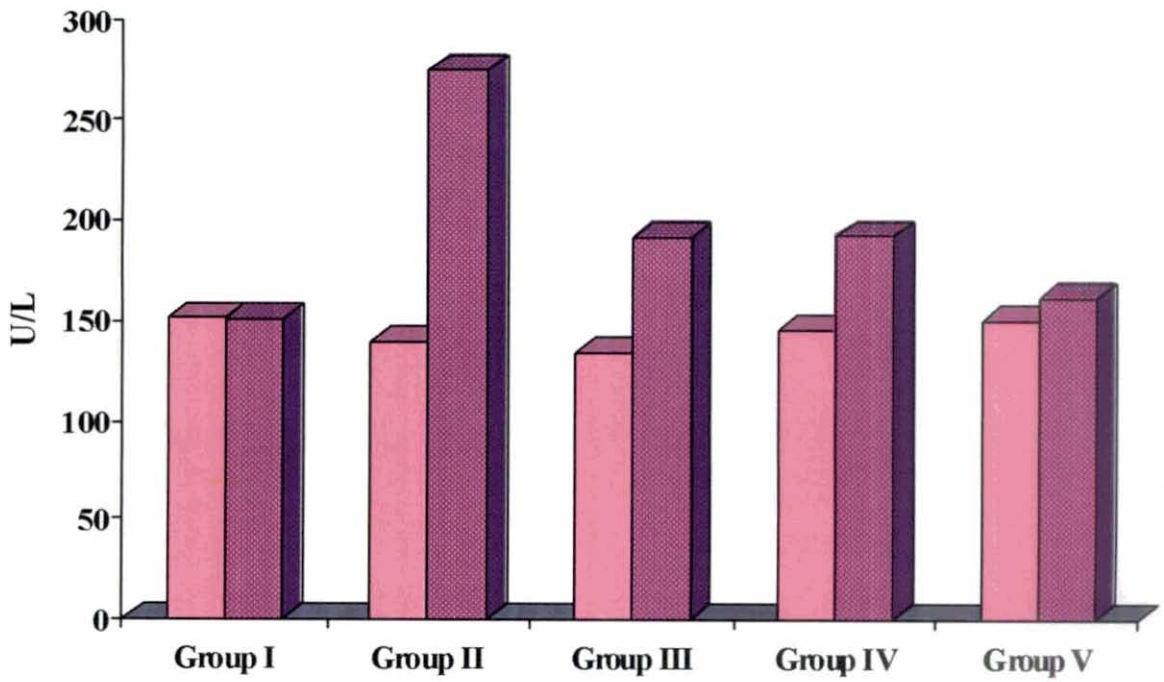
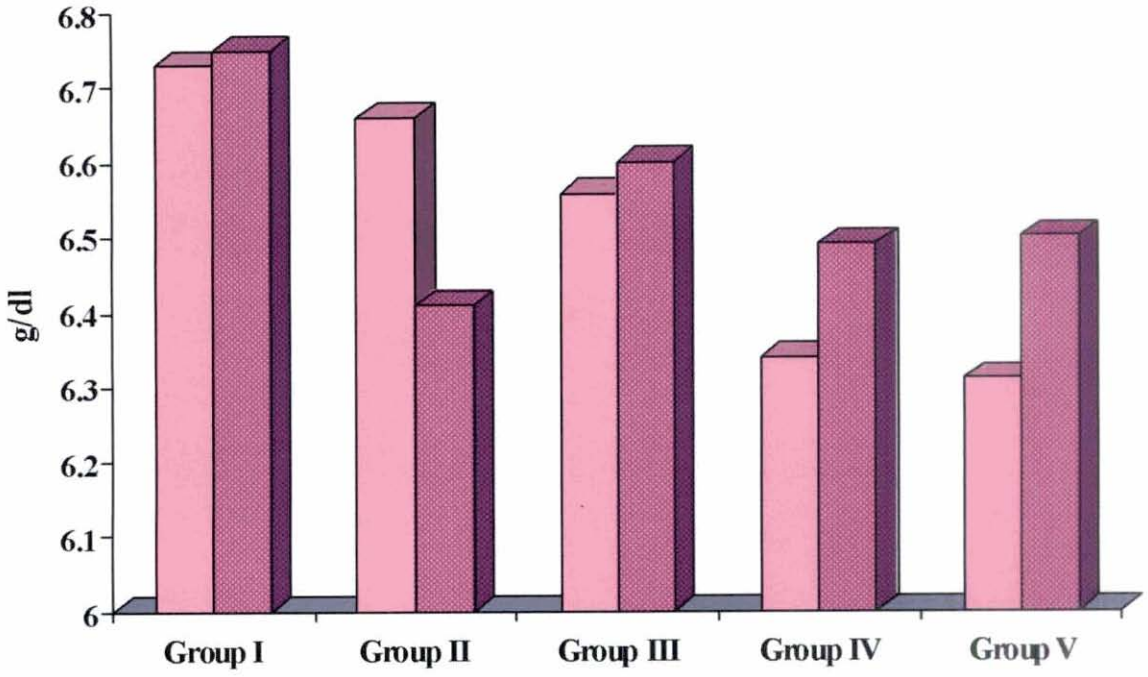


Fig.5. Effect of treatment on total protein level (g/dl)



Dotted bars indicate levels after treatment

Fig.6. Effect of treatment on albumin level (g/dl)

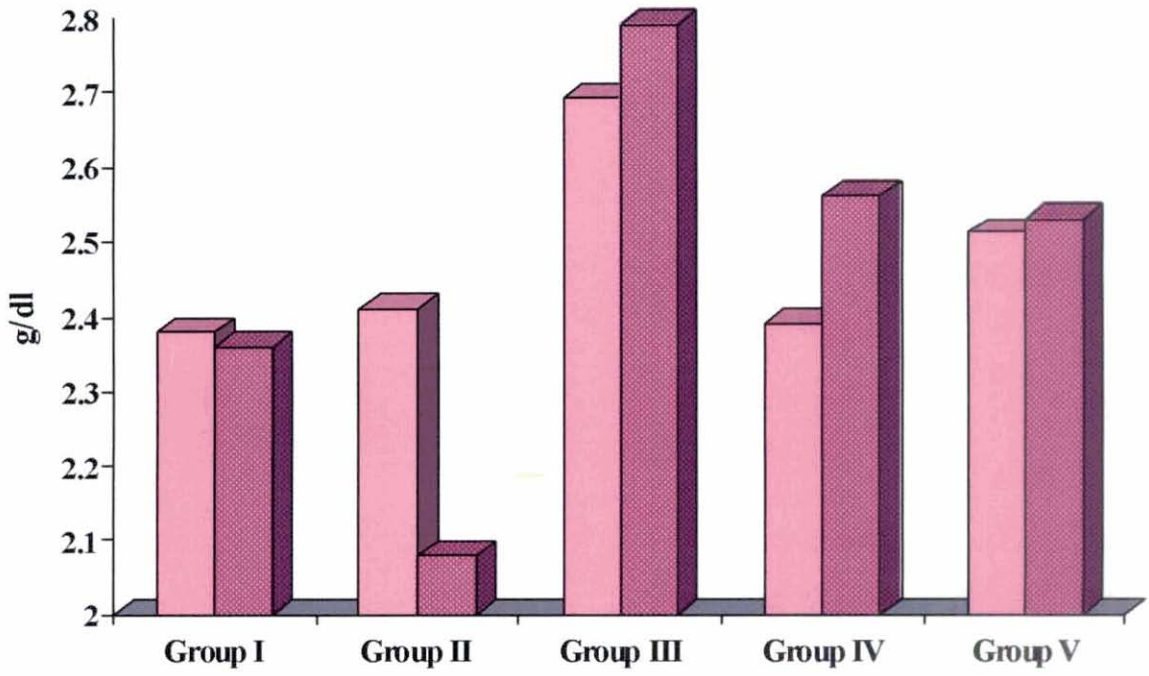
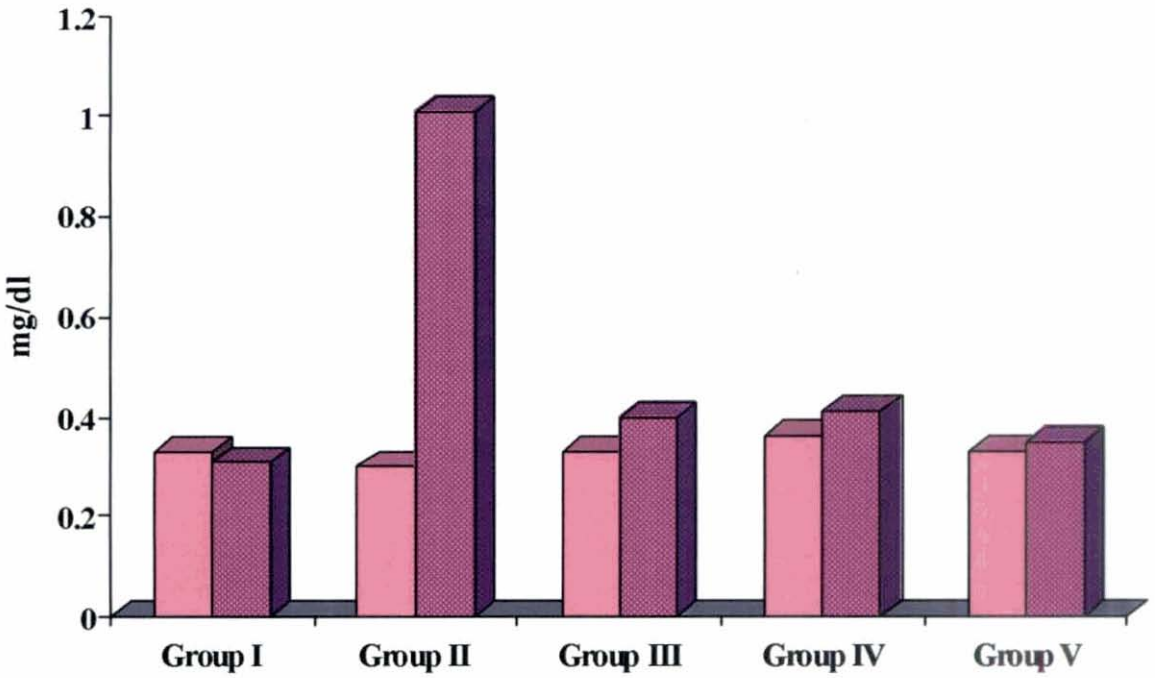


Fig.7. Effect of treatment on total bilirubin level (mg/dl)



Dotted bars indicate levels after treatment

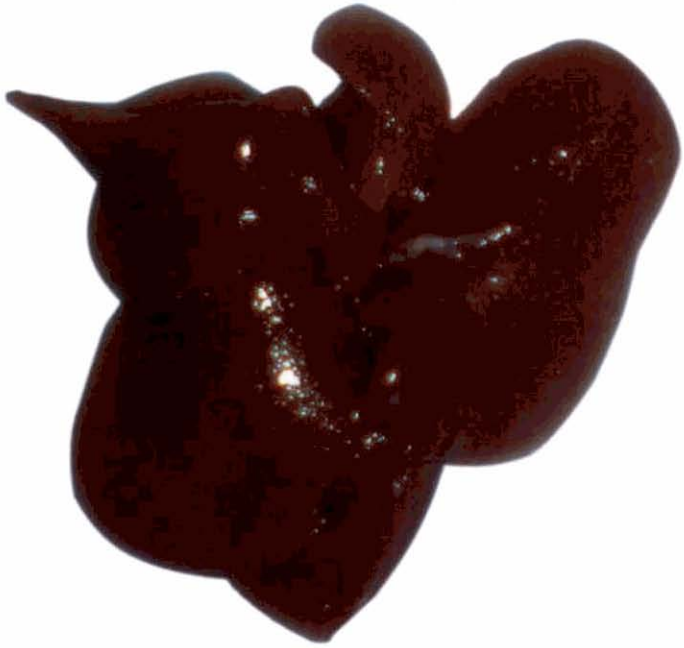


Fig.8. Normal Liver

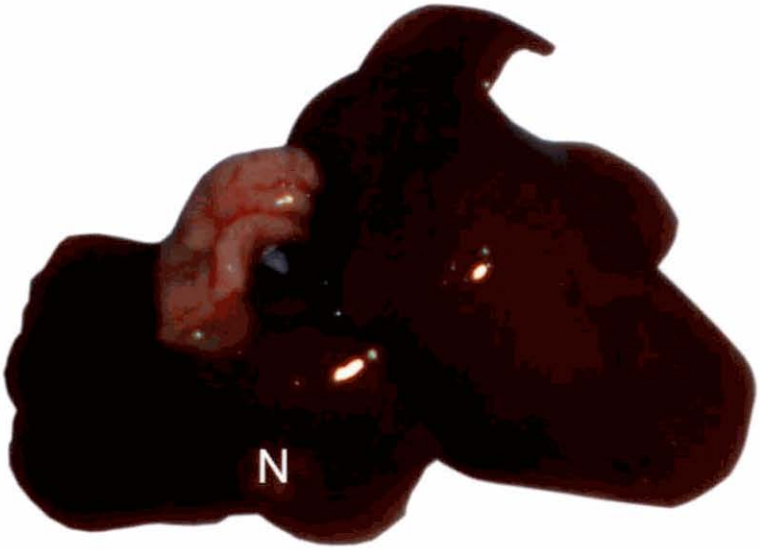


Fig. 9. Liver of paracetamol treated group

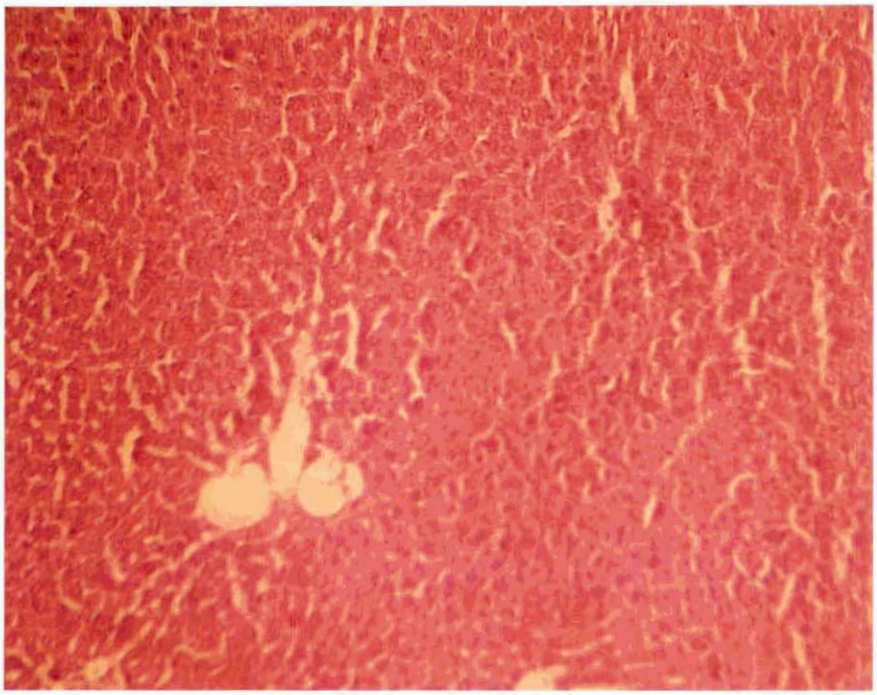
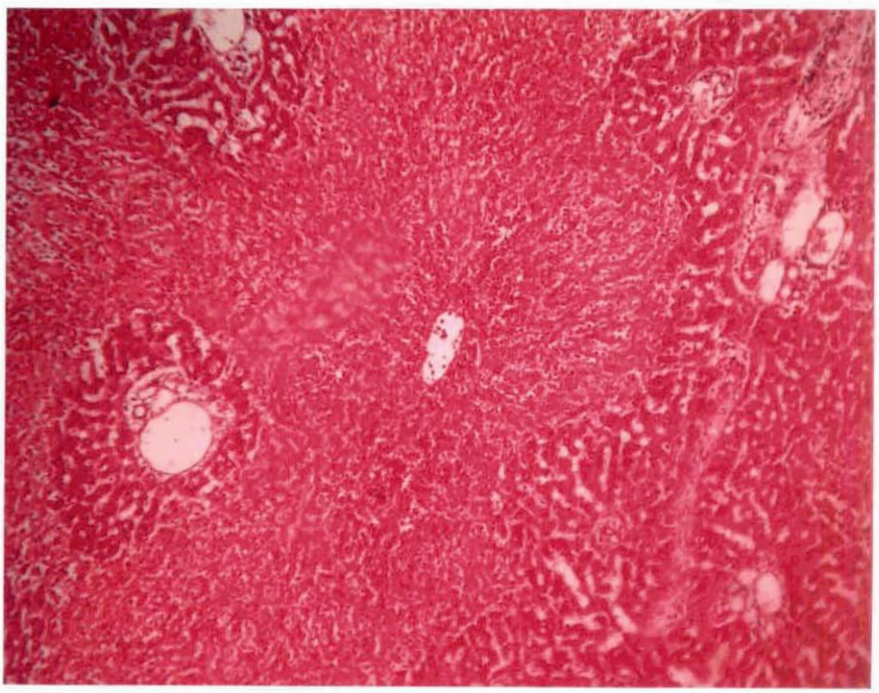


Fig.10. Liver - healthy control
Normal arrangement of hepatocytes (H & E X 100)



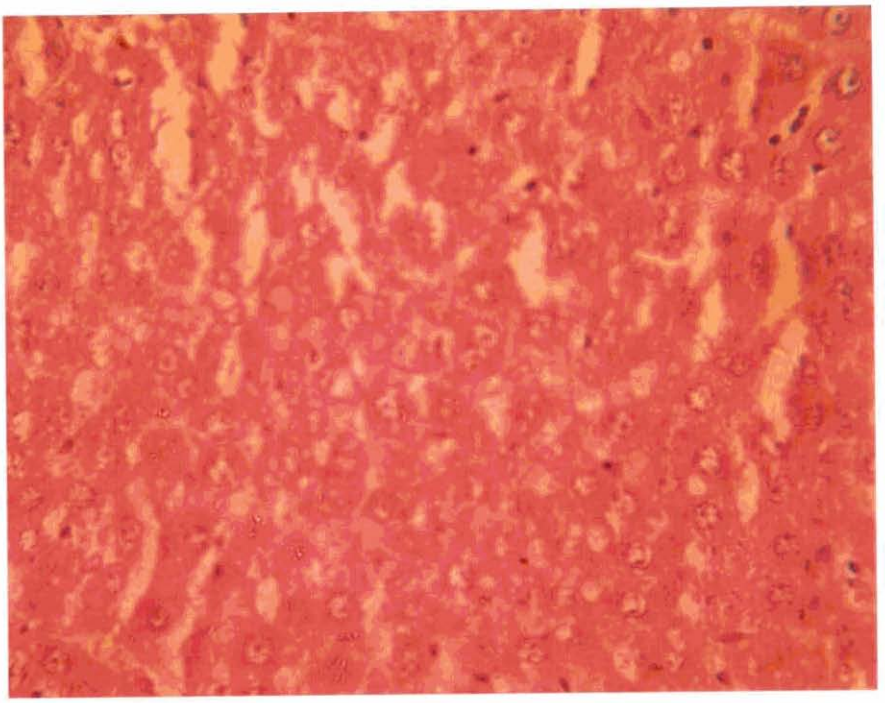


Fig.12. Liver - *Azadirachta indica* 300 mg/kg
Abundance of binucleate cells indicating areas of regeneration (H & E x 1000)

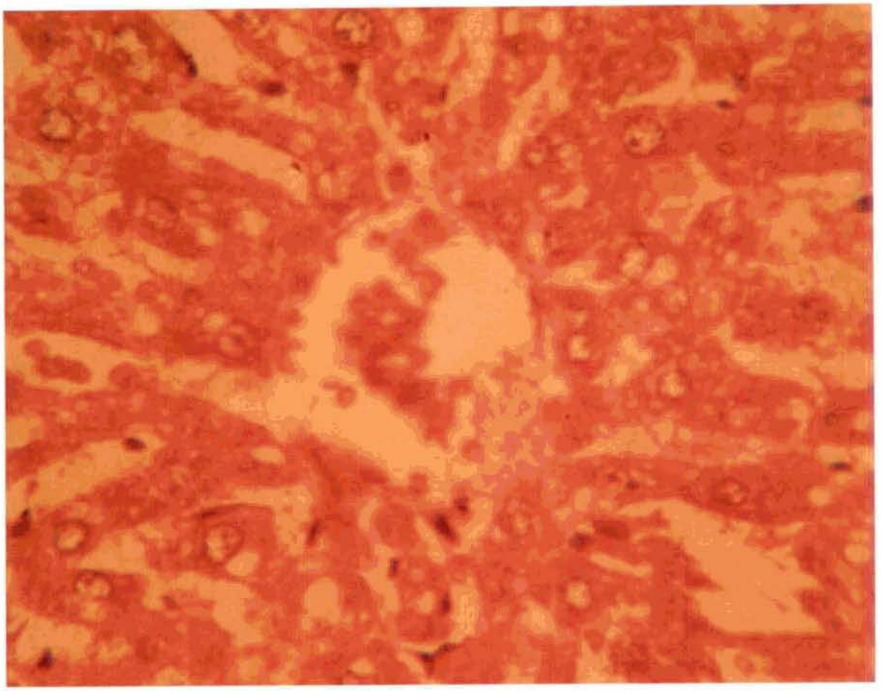


Fig. 13. Liver - *Tridax procumbens* 300 mg/kg

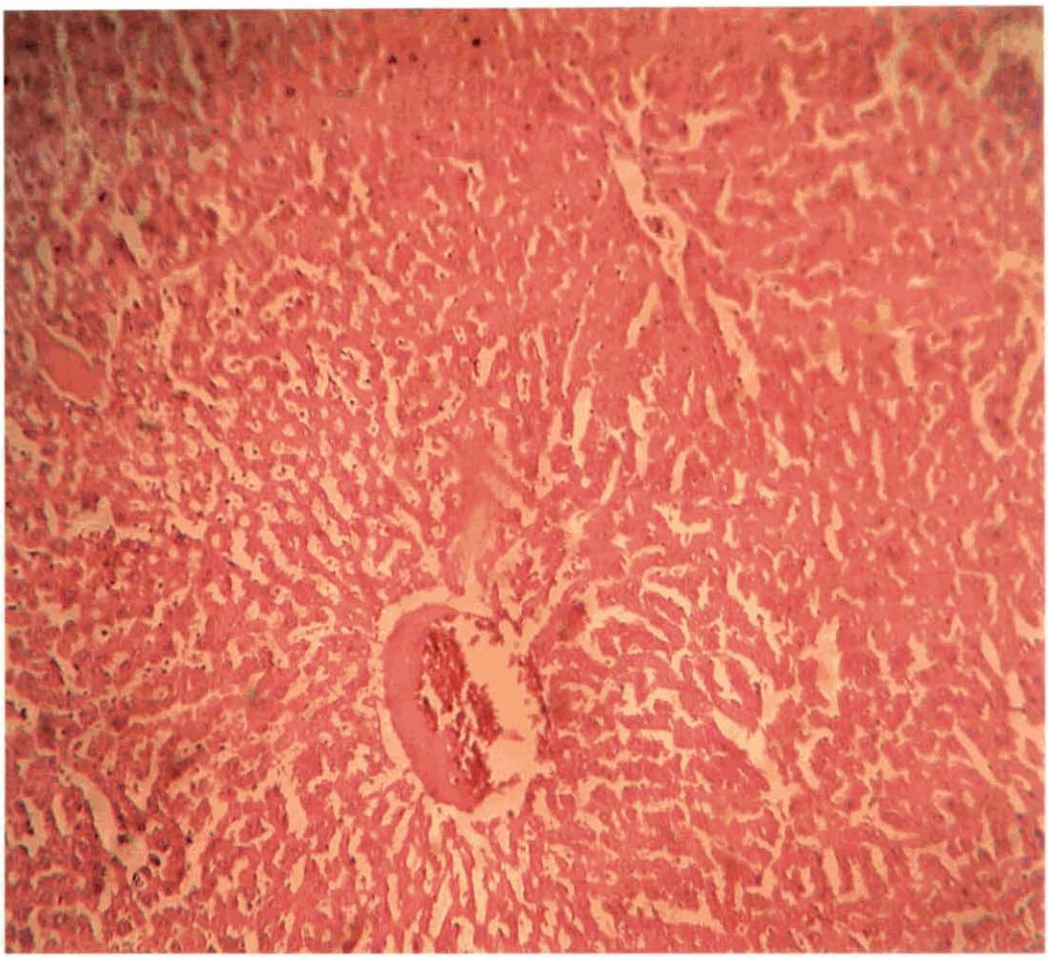


Fig. 14. Liver - silymarin 100 mg/kg on day 10
Apparently normal hepatic architecture (H & E X 100)

Discussion

5. DISCUSSION

Nowadays only a few hepatoprotective drugs are available for the treatment of liver disorders. There are herbal drugs like *Phyllanthus niruri,* which are used traditionally for the treatment of jaundice. Studies have been conducted in various herbal plants to identify the phytoconstituents responsible for hepatoprotective activity. The objectives of the present study are to evaluate the hepatoprotective effect of ethanolic extract of *Azadirachta indica* and *Tridax procumbens* in paracetamol induced hepatotoxicity in rats and to compare their efficacy.

5.1 PHYTOCHEMICAL SCREENING

In the present study phytochemical screening of ethanolic extract of *Azadirachta indica* revealed the presence of alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins and that of *Tridax procumbens* extract revealed the presence of alkaloids, steroids, flavonoids, glycosides, saponins and tannins.

A similar study conducted by Bhanwra *et al.* (2000) revealed the presence of flavonoids in neem leaf extract and these have got antioxidant property. Flavonoids are hepatoprotective agents since they act as antioxidants, free radical scavengers and antiperoxidants (Chakraborthy *et* al., 1989; Hewawasam *et al.*, 2003). Vallachira (1998) reported that the dried bark and of *Melia azadirachta* contained a bitter amorphous resin alkaloid margosine and margosic acid. The present study also revealed the presence of alkaloids in ne leaves em leaf extract.

The anti-oxidant activity of *Tridax procumbens* was studied *in vitro* through 2,2-diphenyl-picrylhydrazyl hydrate (DDPH) assays by Hemalatha (2008) in which the presence of flavonoids and alkaloids was identified in the ethanolic extract of *T. procumbens*. These observations are in agreement with the present study. In a similar study conducted by Jude *et al.* (2009) revealed the presence of alkaloids, carotenoids, flavonoids, saponins and tannins in the acid extract leaves of *T. procumbens.*

alkaloids, carotenoids, flavonoids, saponins and tannins in the acid extract leaves of *T. procumbens.*

The results of the present study are in agreement with that of the study conducted by (Nwanjo *et al.,* 2008) in which they reported the presence of alkaloids, steroids, flavonoids, saponins and tannins in the aqueous extract of *Tridax procumbens.*

5.2. PHYSIOLOGICAL PARAMETERS

5.2.1 Body weight

In the present study, all the groups except the paracetamol treated group showed a gradual increase in weight. The reduction in weight exhibited by the paracetamol treated group may be due to reduced feed intake and the stress of the hepatotoxic agent. In a similar study conducted by Mathew (2005) observed weight loss in rats, which were treated with paracetamol at the dose of 3 g/kg orally for three days.

5.3. SERUM PARAMETERS

The present study revealed the significance of liver specific serum markers such as alanine amino transferase (ALT), aspartate amino transferase (AST), bilirubin, albumin and total protein in assessing clinical and experimental liver damage. Paracetamol induced hepatotoxicity caused a significant elevation in the levels of serum markers like ALT, AST and bilirubin, while the levels of serum albumin and total protein were reduced after its administration. These observations are in agreement with the results of the study conducted by Dash *et al.* (2007) in which there is a significant elevation of hepatospecific serum markers like ALT, AST and bilirubin and a reduction in serum albumin and total protein in paracetamol – treated group, in comparison with the normal control group.

5.3.1 Alanine amino transferase (ALT)

An increased level of ALT was observed in the present study in the paracetamol treated rats than the other groups, which indicates the ability of

paracetamol to induce hepatotoxicity. These results are in agreement with the results of the study conducted by (Bose *et al.*, 2007) which indicated that hepatic damage releases the enzyme into circulation and ALT is more specific to the liver and is a better parameter for detecting liver injury. A study conducted by (Dixon *et al.*, 1975) assessed the serum transaminase levels after paracetamol induced hepatic necrosis in rats and reported that the serum enzyme levels give a reliable indication of the severity of necrosis of liver. These observations are in agreement with the present study.

The ethanolic extracts of both the plants reduced the elevated enzyme levels and there was no significant difference in enzyme levels between the normal animals and the silymarin treated animals. In a similar study conducted by (Bhanwra *et al.*, 2000) reported that the hepatic necrosis caused by paracetamol at a dose rate of 2 g/kg Bwt elevated the level of serum alanine amino transferase and administration of *Azadirachta indica* leaf extract at a dose rate of 500 mg/kg caused a marked reduction in the level of ALT.

5.3.2 Aspartate amino transferase (AST)

In the present study it was noticed that the elevated levels of AST caused by paracetamol intoxication were significantly reduced by the administration of *A. indica* and *T. procumbens* ethanolic extracts both at the doses 300 mg/kg Bwt. There was no significant difference in enzyme levels between the normal animals and the silymarin treated animals. An elevated level of SGOT is always noted along with an increased level of SGPT (Sallie *et al.*, 1999). These observations are in agreement with the present study.

In a similar study conducted by (Chattopadhyay *et al.*, 1992) they indicated that elevated serum levels of ALT and AST were much lowered in the group receiving the *A. indica* leaf extract than that of the paracetamol treated group. The results of the present study are in agreement with the study conducted by (Kumar *et al.*, 2004) in which they reported that the structural integrity of the hepatocellular membrane was preserved in a dose dependent manner by the administration of the ethanolic extract of roots of *Operculina turpethum* in

paracetamol induced hepatotoxicity as evident from the reduction in levels of elevated serum enzymes ALT, AST and ALP.

5.3.3 Total protein

In the present study a reduction in the level of total protein was noticed in the paracetamol treated group. These observations are in agreement with the results of the study conducted by (Venukumar and Latha, 2001) in which they indicated that hypoalbuminaemia is more frequent in advanced chronic liver disorders. Hence decline in total protein content has been deemed as a useful index of the severity of cellular dysfunction in liver disorders. Administration of the extracts elevated the levels of total protein but not much significant effect was noticed. In a study conducted by Mathew (2005) also noticed that paracetamol induced hepatotoxicity caused a marked reduction in the level of total protein and administration of *A. indica* extract at a dose of 500 mg/kg elevated the level of total protein.

5.3.4 Serum albumin

Results of the present study revealed that the paracetamol treated group produced liver damage in rats as indicated by significant reduction of total protein and albumin. Rats treated with the extracts have increased the levels of albumin in serum when compared to that of paracetamol alone treated group, which indicated its hepatoprotective activity. This observation can be compared with the findings of Shenoy *et al.*(2002) in which they reported that serum albumin is a marker of synthetic function of the liver and is a valuable guide to assess the severity of chronic disease. Albumin was significantly decreased in the paracetamol alone treated groups, while the decrease observed in the extract treated groups was lesser than the former group.

5.3.5 Serum total bilirubin

The rise in the levels of serum bilirubin in the present study confirms the hepatotoxicity induced by the paracetamol. These results are in agreement with that of the study conducted by (Bose *et al.*, 2007) in which they reported that

the bilirubin value to normal thus indicating their hepatoprotective effect. In a similar study conducted by (Gupta and Misra, 2006) also revealed that the elevated level of serum total bilirubin was brought back to normal by the administration of *Chamomile capitula* ethanolic extract at a dose of 400 mg/kg.

5.4 HAEMATOLOGICAL PARAMETERS

Haematological parameters like haemoglobin concentration, total leucocyte counts (TLC) and differential leucocyte count was studied before and after the treatment. In the case of haemoglobin concentration, total leucocyte count and differential leucocyte count, no change was noticed before and after the treatment.

5.5 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

After ten days of treatment all the animals were sacrificed and liver was subjected to gross examination. Gross examination of the liver from all the groups except the paracetamol treated group showed almost normal appearance and color. Liver of paracetamol treated group showed greyish white areas of necrosis. In a similar study conducted by Bhanwra *et al.* (2000) reported that liver of most of the paracetamol treated rats were covered with white slough and multiple white patches indicating necrosis.

On histopathological examination the control group showed portal triad, rows of hepatocytes and normal arrangement of hepatocytes. The paracetamol treated group showed areas of centrilobular coagulation necrosis. The hepatocytes showed deeply eosinophilic cytoplasm with pyknotic nuclei and infiltration of massive number of inflammatory cells. These observations are in agreement with that of the study conducted by Hewawasam *et al.* (2003) in which the liver showed confluent necrosis with vacuolation and ballooning degeneration in the surviving hepatocytes after treatment with paracetamol. A similar study conducted by Mathew (2005) reported that the paracetamol at the dose rate of 3 g/ kg for three days resulted in centrilobular coagulative necrosis of the liver. The covalent binding of the oxidation products of the paracetamol to the sulphydryl groups of the protein resulted in the cell damage and thereby necrosis of the liver.

covalent binding of the oxidation products of the paracetamol to the sulphydryl groups of the protein resulted in the cell damage and thereby necrosis of the liver.

In the 300 mg/kg *Azadirachta indica* extract treated animals, liver showed abundance of binucleate hepatocytes indicating areas of regeneration. In the 300 mg/kg *Tridax procumbens* extract treated group the binucleate cells were scanty and vacuolation of cytoplasm and fatty changes were noticed. All the blood vessels were congested. The areas of regeneration noticed were lower in this group compared to that of previous one. It was also noticed that the pre-treatment with the extracts reduced the damage induced by paracetamol proving their hepatoprotective action. It may be due to the presence of phenolic components, flavonoids and tannins present in the leaf extracts. It was also evident from the histopathological sections that, pre-treatment with *A. indica* caused better hepatoprotective effect than that of *T. procumbens*. In a similar study conducted by Chattopadhyay (2003) revealed that *A. indica* leaf extract itself could act as free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes and also reduced the elevated levels of serum enzymes by its membrane stabilizing action. (Nwanjo *et al.*, 2008) reported that the antioxidant properties and free radical scavenging activity of *T. procumbens* leaf extract protected the liver from damage associated with chloroquine administration.

The results of the present study revealed that the ethanolic extracts of leaves of *Azadirachta indica* and *Tridax procumbens* have hepatoprotective activity in paracetamol induced hepatotoxicity in rats and treatment with *A. indica* extract proved better hepatoprotective action than that of *T.procumbens*.

6. SUMMARY

The present study was undertaken to assess the hepatoprotective activity of ethanolic extract of *Azadirachta indica* and *Tridax procumbens* leaves in paracetamol induced hepatotoxicity in rats and to compare their efficacy.

Forty adult male wistar rats weighing 150-200 g, divided into five groups comprising eight animals in each group, were used for the study. Groups I and II animals were administered with 3 per cent gum acacia suspension in distilled water at the dose rate of 5 ml/kg/day for ten days. Groups III and IV animals received ethanolic extract of *Azadirachta indica* and *Tridax procumbens* leaves at the dose of 300 mg/kg and Group V animals received reference drug silymarin at the dose of 100 mg/kg/day in 3 per cent gum acacia for ten days. All the groups except the group I, received paracetamol orally on the eighth day at the dose rate of 2 g/kg in distilled water.

Blood was collected from all the groups prior to the experiment and forty eight hours after the paracetamol administration for hematological examination and serum was separated for biochemical studies. All the animals were sacrificed on 10^{th} day and liver was taken for histopathological examination. Body weight was recorded on day 0 and 10^{th} day. Serum was used for the estimation of ALT, AST, total protein, albumin and bilirubin.

Phytochemical analysis of the *Azadirachta indica* (Neem) leaf extract revealed the presence of alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins and that of *Tridax procumbens* (chiravanak) showed the presence of alkaloids, steroids, glycosides, flavonoids, saponins and tannins.

Paracetamol administered group showed a decrease in body weight while all others showed an increase in body weight except the 300 mg/kg *Tridax procumbens* extract treated animals which did not show much difference.

Liver marker enzymes like ALT and AST levels were used for the assessment of the intensity of hepatotoxicity. The levels of both these enzymes were highest in paracetamol treated groups. The elevated levels of these enzymes were decreased by the herbal extracts thereby proving their hepatoprotective activity.

A reduction in the total protein was observed in the paracetamol treated group. Both the herbal extracts elevated the total protein level to normal levels. The decrease in the level observed in paracetamol treated group was associated with a decrease in the number of hepatocytes thereby decreasing the hepatic capacity to synthesize protein. Serum albumin level was also lowest in the paracetamol treated group because of the decrease in the total protein level. Serum albumin level is a marker of synthetic function of the liver and is a valuable guide to assess the severity of chronic diseases. Both the extracts increased the serum albumin levels towards normal values.

Serum bilirubin level was also highest in the paracetamol treated group because of the liver damage caused by the paracetamol. *Azadirachta indica* and *Tridax procumbens* leaf extract at the dose rate of 300 mg/kg significantly decreased the elevated level of bilirubin.

From the biochemical studies, it was noted that the administration of *Azadirachta indica* (Neem) leaf extract caused better reduction in serum parameters like AST, ALT, serum albumin, total protein and serum bilirubin than that of *Tridax procumbens* extract.

The study of hematological parameters like hemoglobin concentration, total leukocyte count and differential leukocyte count revealed that all these parameters were not much affected with the treatment of extract of both plants.

Gross examination of the liver showed normal appearance in all the four groups except the paracetamol treated group in which the liver showed areas of coagulative necrosis and congestion. On histopathological examination the paracetamol treated group showed extensive areas of centrilobular coagulation necrosis. The *Azadirachta indica* treated animals showed diffuse necrotic areas and fatty changes in certain lobules. The areas of regeneration with binucleate hepatocytes were abundant in this group. The *Tridax procumbens* treated group showed hypertrophied hepatocytes with vacuolation of cytoplasm and presence of fat droplets. The areas of regeneration with binucleate hepatocytes were scanty in this group. Silymarin treated animals have almost normal liver architecture like the control group.

From the present study it can be concluded that the ethanolic extracts of leaves of *A.indica* and *T.procumbens* have significant hepatoprotective activities in paracetamol induced hepatotoxicity in rats and *A.indica* has a better hepatoprotective action compared to *T.procumbens.*

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HEPATOPROTECTIVE EFFECT OF *Azadirachta indica* (NEEM) AND *Tridax procumbens* (CHIRAVANAK) IN RATS

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ABSTRACT

The objectives of the present study were to evaluate the hepatoprotective activity of ethanolic extract of *Azadirachta indica* and *Tridax procumbens* leaves in paracetamol induced hepatic damage in rats and to compare their action.

Forty adult male wistar rats weighing 150-200 g, divided into five groups comprising eight animals in each group, were used for the study. Groups I and II animals were administered with 3 per cent gum acacia suspension in distilled water at the dose rate of 5 ml/kg/day for ten days. Groups III and IV animals received ethanolic extract of *Azadirachta indica* and *Tridax procumbens* leaves at the dose of 300 mg/kg and Group V animals received reference drug silymarin at the dose of 100 mg/kg/day in 3 per cent gum acacia for ten days. All the groups except the group I, received paracetamol orally on the eighth day at the dose rate of 2 g/kg in distilled water. Blood was collected from all the groups before and after the experiment for various biochemical and haematological parameters. Body weight was recorded on day 0 and 10^{th} day. All the animals were sacrificed on 10^{th} day and liver was taken for histopathological examination.

Phytochemical analysis of the *Azadirachta indica* (Neem) leaf extract revealed the presence of alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins and that of *Tridax procumbens* (chiravanak) showed the presence of alkaloids, steroids, glycosides, flavonoids, saponins and tannins.

Paracetamol administered group showed a decrease in body weight. Liver marker enzymes like ALT and AST were highest in paracetamol treated groups. The elevated levels of these enzymes were decreased by the herbal extracts thereby proving their hepatoprotective activity.

A reduction in the total protein was observed in the paracetamol treated group. Both the herbal extracts elevated the total protein level to normal levels. Serum albumin level was also lowest in the paracetamol treated group because of

the decrease in the total protein level. Both the extracts increased the serum albumin levels towards normal values.

Serum bilirubin level was also highest in the paracetamol treated group *Azadirachta indica* and *Tridax procumbens* leaf extract at the dose rate of 300 mg/kg decreased the elevated level of bilirubin. The haematological parameters showed not much significant change with the treatment.

From the biochemical studies it was noted that the administration of *Azadirachta indica* (Neem) leaf extract caused better reduction in serum parameters than that of *Tridax procumbens* extract.

Gross examination of the liver showed normal appearance in all the four groups except the paracetamol treated group in which the liver showed areas of coagulative necrosis and congestion. On histopathological examination the paracetamol treated group showed extensive areas of centrilobular coagulative necrosis. The *Azadirachta indica* treated animals showed diffuse necrotic areas and fatty changes in certain lobules. The areas of regeneration with binucleate hepatocytes were abundant in this group. The *Tridax procumbens* treated group showed hypertrophied hepatocytes with vacuolation of cytoplasm and presence of fat droplets. The areas of regeneration with binucleate hepatocytes were scanty in this group. Silymarin treated animals have almost normal liver architecture like the control group.

From the present study it can be concluded that the ethanolic extracts of leaves of *A.indica* and *T.procumbens* have significant hepatoprotective activities in paracetamol induced hepatotoxicity in rats and *A.indica* has a better hepatoprotective action compared to *T.procumbens.*