HEPATO PROTECTIVE EFFECT OF *Aegle marmelos* (INDIAN BAEL) AND *Azadirachta indica* (NEEM) AQUEOUS LEAF EXTRACT ON PARACETAMOL INDUCED TOXICITY 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 *Aegle marmelos* (Indian Bael) and *Azadirachta indica* (Neem) aqueous leaf extract on paracetamol induced toxicity in rats."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"Hepatoprotective effect of *Aegle marmelos* (Indian Bael) and *Azadirachta indica* (Neem) aqueous leaf extract on paracetamol induced toxicity in rats."** is a record of research work done independently by **Anu Mathew.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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Anu Mathew

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1. INTRODUCTION

Liver has a major role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Further more detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by liver has an important role in digestion.

Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons etc.), excess consumption of alcohol, infections and auto immune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver.

Liver damage and recovery from damage are assessed mainly by measuring serum marker enzymes, bilirubin, histopathological changes in the liver, biochemical changes in liver (superoxide dismutase, catalase etc.) and bile flow. When liver is damaged, liver enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase enter into circulation. An increase in levels of these enzymes in serum is an indication of liver damage (Khedun *et al.*, 1993).

Paracetamol (N- acetyl p- aminophenol / acetaminophen) is a common analgesic and antipyretic agent. At therapeutic dose paracetamol is a safe drug. However it can cause hepatic necrosis, extra hepatic lesions and even death on over dosage (Kumar *et al.*, 2004; Handa and Sharma, 1990). Symptoms of paracetamol over dosage in the first 24 hours are palor, nausea, vomiting, anorexia and abdominal pain. Liver damage may become apparent 12 to 24 hours after ingestion.

Paracetamol is metabolized in the liver primarily to glucuronide and sulphate conjugates. Hepatotoxicity has been attributed to the formation of toxic metabolites when a part of paracetamol is metabolized by cytochrome P_{450} pathway to an electrophilic metabolite N- acetyl P- benzoquinone imine (NAPQI). The therapeutic doses of this drug are safely biotransformed and eliminated as nontoxic conjugates in bile or urine. Overdoses of acetaminophen deplete glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and acute hepatic necrosis.

Also depletion of glutathione enhances expression of TNF alpha and it primes phagocytic NADPH oxidase to the enhanced production of oxygen free radicals and contributes liver damage (Ahmed and Khater, 2001).

Recently another pathway of paracetamol toxicity has proposed that potential of acetaminophen to influence the integrity of genomic DNA, may lead to cell death in the liver (Shon and Nan, 2004).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine (Gupta *et al.*, 2004). About 80 percentage of the world population rely on the use of traditional medicine, which is primarily based on plant materials. The traditional medicine refers to a broad range of ancient natural health care practices including folk or tribal practices as well as Ayurveda. These medical practices originated from time immemorial and developed gradually to a large extend by relying on practical experience. Although herbal medicines are effective in the treatment of various ailments, very often these drugs are unscientifically exploited and or improperly used. Therefore these plant drugs deserve detailed studies in the light of modern medicine (Subramoniam and Pushpangadan, 1999).

Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. Public, academic and government interest in traditional medicine is growing exponentially due to the increased incidence of adverse drug reactions and economic burden of modern system of medicine. The major hindrance in the amalgamation of medicine is the lack of scientific and clinical data and better understanding of efficacy and safety of herbal products. To ensure the quality and safety of its products and practices, standardization is of vital importance (Seth and Sharma, 2004).

Inspite of tremendous advances made in the allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are made to play a vital role in the management of liver diseases. There are numerous drugs and polyherbal formulations claimed to have hepatoprotective activities. Nearly 150

phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India more than 87 medicinal plants are used in different combinations to treat liver diseases. Most commonly used plants are *Andrographis paniculata, Boerhavia diffusa, Eclipta alba, Picrorrhiza kurroa, Oldenlandia corymbasa, Cichorium intybus, Tinospora cordifolia, Azadirachta indica, Ocimum sanctum* and *Emblica officinalis*.

Azadirachta indica (Meliaceae; Neem) is an indigenous plant commonly grown in India and Burma. It has great reputation in Ayurvedic medicine for treatment of liver disorders. Almost all the parts of this tree are used for medicinal purposes in India. It consists mainly of nimbin, nimbidin and nimbidiol. Water soluble portion of alcoholic extract of leaves of *Azadirachta indica* possesses significant hypoglycemic, anti ulcer, anti inflammatory, hypotensive, hypolipidemic, antibacterial, antifungal, antiviral, anticancer, astringent, emollient, antifertility and immunostimulant activities (Bajaj and Srinivasan, 1999; Kasturi *et al.*,1997; Bhanwra *et al.*, 2000).

Aegle marmelos (Rutaceae; Bael) indigenous to India, is grown through out the subcontinent as well as in Burma, Pakistan and Bangladesh. Indigenous people use both leaves and fruits of this plant to treat diabetes mellitus. The plant also possesses analgesic, anti spermatogenic, astringent, laxative, febrifuge activities and it is good for heart, brain and jaundice (Sabu and Kuttan, 2001; Kale *et al.*, 2003; Kirtikar *et al.*, 1975). The plant contains aegelin, alloimperatorin, imperatorin, marmelide, marmeline, marmelosin and psoralen (Kamalakkannan and Prince, 2003).

The present study was undertaken to evaluate the hepatotoxicity induced by paracetamol in rats and to assess the hepatoprotective effect of aqueous leaf extract of *Aegle marmelos* and *Azadirachta indica*.

2. REVIEW OF LITERATURE

2.1 PARACETAMOL

Chattopadhyay *et al.* (1992) reported that paracetamol (2g/kg) elevated serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and acid phosphatase in rats. It has also produced hepatocellular necrosis and dilated vasculature around sinusoids around the necrotic zones.

A significant increase in levels of aspartate aminotransferase, alanine aminotransferase, serum bilirubin, plasma prothombin time and tissue lipid peroxides were found due to paracetamol overdosage (750 mg/kg). Paracetamol overdosage caused necrosis of centrilobular hepatocytes and eosinophilic cytoplasm (Kapur *et al.*, 1994).

Rao and Mishra (1998) showed that a single oral dose of paracetamol 3 g/kg p.o. elevated the levels of serum enzymes like alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin in rats.

Bhanwra *et al.* (2000) showed that liver necrosis was produced by administering single dose of paracetamol (2g/kg p.o.) and was evidenced by elevated levels of aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transpeptidase.

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

Roberts and Morrow (2001) found that the most serious adverse effect of acute overdosage of acetaminophen is a dose dependent, potentially fatal hepatic necrosis.

Shenoy *et al.* (2002) confirmed that a dose of 2 g/kg paracetamol for three days induced liver damage in rats as indicated by a significant elevation of serum

marker enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and a significant reduction of serum total protein and albumin. Histopathological examination revealed extensive centrilobular necrosis extending to midzone with neutrophilic collection and there was chronic inflammatory cell infiltration in the portal tracts.

McGregor *et al.* (2003) concluded that paracetamol overdosage is the commonest cause of fulminant hepatic failure, while the main loss of liver mass occurs following hepatocyte necrosis.

Tripathi (2003) reported that a very large dose of paracetamol depleted the hepatic glutathionine. The highly reactive arylating metabolite, N-acetyl-p-benzoquinone imine (NAPQI) formed, binds covalently to proteins in the liver cells causing necrosis.

4.2 HEPATOPROTECTIVE AGENTS

Cherian (1970) showed that administration of *Phyllanthus niruri* and *Andrographis paniculata* decoction reduced the retention of bromsulphophthalein and the level of SGPT compared to control and histopathological examination revealed regeneration of hepatic lobules.

Kirtikar *et al.* (1975) reported the use of fresh juice of *Aegle marmelos* leaves in the treatment of jaundice.

Dwivedi *et al.* (1990) showed that oral feeding of Picroliv to carbon tetrachloride treated rats reversed the changes induced in the activities of superoxide dismutase, 5^1 nucleosidase, succinate dehydrogenase, acid phosphatase, acid ribonuclease, glucose 6- phosphatase, total lipid level and cytochrome P_{450} in liver. It also reduced the level of serum alanine aminotransferase, aspartate aminotransferase and lipoprotein –X.

The alcoholic extract of *Azadirachta indica* at a fixed dose (1g/kg) offered protection from paracetamol induced liver damage. The values of serum enzymes glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline

phosphatase and acid phosphatase were much lower in case of animals receiving extract (Chattopadhyay *et al.*, 1992).

Sudhir and Budhiraja (1992) found that concomitant treatment of the rats with Withaferin'A' (withanolide from the leaves of *Withania somnifera*) and hydrocortisone protected the liver significantly by reducing the liver alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in carbon tetrachloride induced damage.

Kapur *et al.* (1994) reported that oral administration of Jigrine (a herbal drug containing *Rheum emodi, Solanum nigrum, Vitex negundo, Foenicum vulgare, Phyllanthus niruri, Cychorium intybus, Rubia cordifolia, Careya arborea, Plantago major* and *Rosa damascena)* exhibited a significant reduction in alcohol- carbon tetrachloride induced increase in levels of alanine aminotransferase, aspartate aminotransferase, bilirubin, lipid peroxides and plasma prothrombin time. Histopathological examination of liver showed normalcy of hepatic cells, central vein and portal triad.

Singh *et al.* (1995) reported that calcium channel blockers nitrendipine, nimodipine and nisoldipien (1mg/100g of rat) significantly reduced the elevated levels of alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase in carbon tetrachloride induced liver damage. The liver necrosis was also found to be reduced macroscopically and histologically.

Ursolic acid, isolated from the leaves of *Eucalyptus tereticonis* showed a dose dependant (4-20mg/kg) hepatoprotective activity in mice against thioacetamide, galactosamine and carbon tetrachloride induced liver damage in rats (Saraswat *et al.*,1996). Pretreatment with ursolic acid increased the viability of rat hepatocytes and showed a potent anticholestatic activity.

The anti hepatotoxic activity of different fractions of ethanolic extract of *Lagenaria siceraria* fruit administered orally to rats were evaluated in carbon tetrachloride induced hepatotoxicity by Shirwaikar and Sreenivasan (1996). Administration of the petroleum ether fraction to the carbon tetrachloride treated rats led to near normalization of the elevated serum transaminase levels and liver sections appeared normal on microscopical examination.

Rao and Mishra (1997a) reported that *Sida rhombifolia* showed significant hepatoprotective effect against carbon tetrachloride, paracetamol and rifampicin induced hepatotoxicity in rats. Pretreatment with powder and aqueous extract significantly reduced the elevated enzyme activities and bilirubin levels in serum.

Rao and Mishra (1997b) found that rats treated with *Fumaria indica* extract showed reduction in the elevated levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and direct bilirubin comparable to those of silymarin.

Rao and Mishra (1998) noted that the powdered aerial parts of *Sida acuta* showed significant reduction in the elevated levels of serum biochemical parameters like alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and direct bilirubin against paracetamol intoxication.

Saraswathy *et al.* (1998) evaluated the effect of Liv.100 against antitubercular drugs (isoniazid, rifampicin and pyrazinamide) induced hepatotoxicity in rats. Simultaneous adminstration of liv.100, showed near normal levels of marker enzymes, level of lipid peroxides and glutathione content on comparison with normal rats.

Zafar and Ali (1998) demonstrated the antihepatotoxic effect of root extract of *Cichorium intybus*. Treatment by root extract afforded good protection against carbon tetrachloride induced increase in liver enzymes, bilirubin levels and reduced total protein and albumin levels.

After carbon tetrachloride treatment liver total lipids, triglycerols and microsomal thiobarbituric acid reactive substances (TBARS) were significantly lowered in rats fed on a diet containing spirulina than in rats without spirulina in their diet and showed an increase in the percentage of HDL values (Duran *et al.*, 1999).

Grange *et al.* (1999) reported that administration of the compound sylimarin phytosome could reduce the elevated levels of serum gamma-glutamyl transpeptidase in ethanol induced toxicity of foetal rat liver.

Siddiqui *et al.* (1999) found that melatonin reversed the depletion of glutathione and increase in lipid peroxidation products in liver and aspartate aminotransferase and alanine aminotransferase activities in serum in sodium valproate induced hepatotoxicity in mice.

Bhanwra *et al.* (2000) reported that aqueous extract of *Azadirachta indica* (500 mg/kg p.o) significantly reduced the elevated levels of serum aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transpeptidase in paracetamol induced liver damage.

Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash extracts were studied using carbon tetrachloride induced liver injury models in rats (Jose and Kuttan, 2000). Both the extracts significantly decreased the elevated levels of serum and liver lipid peroxides, alanine aminotransferase, alkaline phosphatase and collagen –hydroxyproline.

Lim *et al.* (2000) evaluated the hepatoprotective effect of bergenin, a major constituent of *Mallotus japonicus* on carbon tetrachloride intoxicated rats. The substantially elevated serum enzyme activities of alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, gamma-glutamyl transpeptidase due to carbon tetrachloride treatment were dose dependently restored towards normalization and prevented the elevation of hepatic malonaldehyde formation and depletion of reduced glutathione content.

Mitra *et al.* (2000) studied the hepatoprotective effect of herbal formulation HD-03, containing *Solanum nigrum, Cichorium intybus, picrorrhiza kurroa, Tephrosia purpurea and Andrographis paniculata.* Pretreatment with HD-03 750 mg/kg for 15 days prevented elevation of serum alanine aminotransferase, aspartate aminotransferase levels and significant increase in liver glycogen levels.

Trivedi and Rawal (2000) showed that aqueous extract of *Andrographis paniculata* at the rate of 12 mg/kg body weight orally reduced the elevated serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase and lipid peroxidase and increased the glutathione level in mice treated with hexachlorocyclohexane (BHC).

Ubaid *et al.* (2000) found that co-administration of *Ocimum sanctum* leaf extract along with antitubercular drugs significantly prevented all the biochemical and histological alternations. The leaf extract significantly reduced the levels of hepatic lipid peroxidase and increased the levels of superoxide dismutase and catalase.

Ahmed *et al.* (2001) confirmed that administration of petroleum ether, acetone and methanolic extracts of *Luffa echinata* fruits at the dose rate of 250 mg/kg markedly prevented carbon tetrachloride induced elevation of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total albumin and diminution of total protein. *Luffa echinata* extracts showed significant hepatoprotective activity comparable with those of silymarin.

Ahmed and Khater (2001) observed that administration of *Ambrosia maritima* aqueous-methanolic extract (100 and 200 mg/kg) significantly lowered the serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in acetaminophen induced liver damage and also attenuated the increase in hepatic malonaldehyde formation and decreased the activities of reduced glutathione, glutathione reductase, total glutathione peroxidase and glutathione -s-transferase to normal levels.

Asha (2001) studied the hepatoprotective activity of *Marmodica subangulata* and found that administration of tender leaves at a dose of 500 mg/kg remarkedly prevented paracetamol induced elevation of serum enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase.

Ilavarasan *et al.* (2001) reported that pretreatment with alcoholic extract of *Cassia angustifolia* leaves resulted in significant protection against the increase of total bilirubin, alanine aminotransferase and aspartate aminotransferase. Markedly increased the level of reduced glutathione and reduced lipid peroxidase activity in liver.

Mahendran and Devi (2001) confirmed that treatment of rats with *Garcinia cambogia* significantly inhibited the rise in lipid levels and also the peroxidative damage caused by ethanol. The levels of serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were maintained at near normalcy.

Rajesh and Latha (2001) confirmed that *Elephantopus scaber* root powder reduced the elevated levels of serum marker enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. The concentrations of total protein, total lipid, phospholipids, triglycerides and cholesterol were maintained at normal levels in carbon tetrachloride treated rats.

Reen *et al.* (2001) screened the antihepatotoxic activity of *Swertia chirata* on paracetamol toxicity in primary monolayer cultures of rat hepatocytes. Extracts of *S. chirata* reduced the LDH leakage and increased the GSH content of the cells in paracetamol toxicity.

Shenoy *et al.* (2001) concluded that intra peritoneal administration of *Ginkgo biloba* dry extract has increased the serum total protein and albumin and decreased alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels and reversed the increase in TBARS and GSH in paracetamol induced hepatotoxicity in rats.

Singh *et al.* (2001) evaluated the hepatoprotective activity of ethanolic extract of *Eclipta alba* leaves and found that hepatoprotective activity was based on the ability to improve the functional status of hepatic drug metabolizing enzymes. This is evident from shortening the hexobarbitone sleep time, zoxazolamine paralysis time and restoration of carbon tetrachloride impaired excretory capacity of hepatocytes as judged from bromsulphophthalein retention. Also significantly reduced serum alanine aminotransferase, aspartate aminotransferase and bilirubin levels.

Venukumar and Latha (2001) showed that methanolic extract of *Curculigo orchioides* reduced the elevated levels of serum enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase towards normalcy in carbon tetrachloride induced liver damage. The total protein concentration in serum and liver attained almost normal value.

Sadekar *et al.* (2002) showed that treatment with neem leaf powder initiated the reduction in raised serum enzymes level with in 48 to 76 hours in experimental male calves.

Shirwaikar *et al.* (2002) studied the hepatoprotective effect of ethanolic extract of the plant *Polygala elongata* in carbon tetrachloride induced toxicity. The total ethanolic extract exhibited significant protection against carbon tetrachloride induced increase in levels of serum enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in rats. The liver sections showed recovery of hepatic parenchyma with mild congestion and microvesicular changes.

Bhandarkar and Khan (2003) found that oral administration of aqueous suspension of alcoholic extract of *Lawsonia alba* bark to rats for ten days afforded good hepatoprotection against carbon tetrachloride induced elevation in serum marker enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) serum bilirubin, lipid peroxidation and reduction in serum total protein, serum glutathione, glutathione peroxidase, glutathione-s-transferase, glycogen, superoxide dismutase and catalase activity.

Himoliv is a polyherbal formulation containing *Picrorrhiza kurroa, Boerhaavia diffusa, Tinospora cordifolia, Andrographis paniculata* and *Phyllanthus emblica.* Bhattacharyya *et al.* (2003) revealed that in both the doses 0.5 ml/kg and 1.0 ml/kg Himoliv reduced the level of liver enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase dose dependantly in carbon tetrachloride induced hepatotoxicity. Histological studies indicated that pretreatment protected the hepatocytes from damage with mild fatty changes in hepatic parenchymal cells, which corroborated the changes observed in the hepatic enzymes. Himoliv also enhanced the protective enzymes superoxide dismutase and catalase when examined the liver homogenate and reduced the level of lipid peroxide.

Chattopadhyay (2003) found that administration of *Azadirachta indica* leaf extract significantly increased the glutathione level in liver and blood and reduced the level of liver thiobarbituric acid reactive substances in paracetamol induced hepatotoxicity in rats.

Jalapure *et al.* (2003) evaluated the hepatoprotective activity of *Piper longum* fruits in carbon tetrachloride induced toxicity. Ethanolic extract and butanol fraction of the fruit have shown marked reduction in both serum aspartate aminotransferase

and alanine aminotransferase. Liver tissue of the rats showed the presence of normal hepatic cords, absence of necrosis and less degree of infiltration.

Kale *et al.* (2003) found that treatment with *Azadirachta indica* aqueous leaf extract significantly prevented changes in serum levels of bilirubin, protein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in antitubercular drug induced hepatotoxicity in rats. Also preserved the histological structure of liver by significantly reducing the scores of degeneration necrosis and fibrosis with evidence of significant regeneration.

Aqueous extract of seeds of *Nigella sativa* were tested for hepatoprotective activity on male wistar rats against carbon tetrachloride induced toxicity. A significant reduction was observed in total bilirubin, serum enzymes like aspartate aminotransferase, alanine aminotransferase and no significant change in protein level (Mohideen *et al.,* 2003).

Nan *et al.* (2003) found that *Rhodiola sachalinensis* treatment significantly reduced the level liver hydroxy proline, malonaldehyde and serum enzymes in carbon tetrachloride induced hepatotoxicity. Immunohistological findings indicated inhibition of hepatic cell activation.

Plumbagin and petroleum ether extract of *Plumbago zeylanica* and *Plumbago rosea* significantly reduced the elevated serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein albumin and bilirubin and histopathological examination revealed preserved liver architecture, portal triad and cords of hepatocytes (Ranjith *et al.*, 2003).

Venkatesan *et al.* (2003) studied the effect of carbon tetrachloride treatment on hepatic and brain antioxidant status with aqueous extract of *Phyllanthus amarus* Linn., Nirocil (a tablet made up of aqueous extract of *Phyllanthus amarus*), phyllanthin (a bioactive liganin from *Phyllanthus amarus*) and silymarin. Plasma aspartate aminotransferase and alanine aminotransferase were estimated to monitor the extent of hepatocellular damage. Tissue lipid peroxide, ascorbic acid and total protein levels were used as markers for functional and antioxidant efficiency of liver. Phyllanthin reversed the elevated plasma aminotransferase levels but did not

affect hepatic antioxidant status. In all the paradigms tested for hepatoprotection nirocil, silymarin and aqueous extract showed significant protection.

Brahmi ghrita, an Ayurvedic panchagavya formulation containing *Bacopa monneri, Acorus calamus, Saussurea lappa, Evolvulus alsinoids* and cow ghee is evaluated for its hepatoprotective effect by Achliya *et al.* (2004). Brahmi ghrita (100mg/kg and 300mg/kg) significantly reduced the levels of serum marker enzymes aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and acid phosphatase. Histopathological examination showed normalcy of hepatic cells, central veins and portal triad.

Asha *et al.* (2004) noted that administration of *Phyllanthus mederaspatensis* extract at the dose rate of 200 mg/kg markedly prevented acetaminophen induced elevation of serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Rate of flow of bile was also enhanced by the administration of n- hexane extract of the plant and the degradation of deoxyribose to TBARS by hydroxyl radicals was markedly reduced.

Treatment with methanolic extract of *Bauhinia racemosa* stem bark at the dose of 50 mg, 100 mg, and 200 mg/kg decreased the activity of serum aspartate aminotransferase, alanine aminotransferase, bilirubin and increased the protein content in the paracetamol induced liver damage in rats. Malonaldehyde content, glutathione level, superoxide dismutase activity and catalase activity of the liver homogenate were also found to be reduced (Gupta *et al.*, 2004).

Hewawasam *et al.* (2004) investigated that pretreatment of mice with aqueous extract of *Epaltes divaricata* (0.9 g/kg) orally for seven days significantly reduced the serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase enzymes and increased the liver reduced glutathione (GSH) level after the administration of carbon tetrachloride.

Alcoholic extracts of roots of *Boerhaavia erecta* and *Boerhaavia rependa* were compared for their hepatoprotective effect in carbon tetrachloride treated rats by Krishna and Santhamma, (2004). Both of them restored the levels of serum bilirubin, serum total protein, albumin and subsequent decrease in the levels of serum globulin in experimental rats. The serum alanine aminotransferase, aspartate

aminotransferase and alkaline phosphatase activities were also restored as compared to the normal rats.

Effect of ethanolic extract of *Trianthema portulacastrum* was studied against paracetamol induced hepatotoxicity in rats (Kumar *et al.*, 2004). Treatment of rats with the extract significantly reduced the increase in the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin and significantly increased the level of total protein.

Kumar and Mishra (2004) evaluated the hepatoprotective effect of ethanolic extract of Trikathu churna, an Ayurvedic formulation containing *Piper longum* and *Zingiber officinale* in rats by inducing liver damage with carbon tetrachloride. The ethanolic extract at the rate of 150 mg/kg exhibited a significant protective effect by lowering serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin. Carbon tetrachloride induced liver peroxidation was inhibited significantly by the extract, which confirmed the protective action.

Naik *et al.* (2004) found that curcumin significantly reduced the release of LDH from liver cells and lipid peroxidation as compared to ethanol treated liver tissue. When curcumin was added along with ethanol the level of antioxidant enzymes namely superoxide dismutase, peroxidase and catalase were kept low. This indicated that curcumin by its antioxidant activity reduced the oxidative stress induced by ethanol and protected the cells *invitro.*

Ozbek *et al.* (2004) noted that treatment of animals with *Ballota glandulosissima* for seven days ameliorated the levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase elevated by carbon tetrachloride treatment and also reduced the ballooning degeneration but did not produce apoptosis of hepatocytes and bridging necrosis observed in carbon tetrachloride treatment alone.

Pandit *et al.* (2004) noted that pretreatment with ethanolic extract of *Adhatoda vasica* leaves significantly reduced the elevated levels of serum marker enzymes namely aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in carbon tetrachloride treated rats. It also reversed the elevated levels

of lipid peroxides and decreased superoxide dismutase and catalase activities and glutathione concentration in the liver of rats.

Kamilari, a polyherbal formulation was assessed for its hepatoprotective effect by Rajesh and Latha, (2004). The drug showed a significant protection against carbon tetrachloride induced alternations in the serum enzymes levels, protein, bilirubin and lipid profile.

Shon and Nan (2004) evaluated the protective effect of moutan cortex extract on acetaminophen induced hepatotoxicity in mice. Moutan cortex preexposure prevented liver injury as indicated by the decrease of serum alanine aminotransferase level and also protected against acetaminophen induced hepatic glutathione depletion, cytochrome P_{450} $2E_1$–dependant aniline and p–nitro phenol hydroxylase activities in microsome and alternate hepatic DNA damage in vivo.

Pretreatmant study (prophylactic) and posttreatment study (curative) of propolis extract prevented progression of carbon tetrachloride induced acute liver injury. Shukla *et al.* (2004) reported significant recoveries in the activities of serum transaminases in the curative study and in serum alkaline phosphatase in both the studies. Hepatic lipid peroxidation was suppressed and hepatic reduced glutathione was recovered and showed well maintained histoarchitecture.

Suja *et al.* (2004) showed that significant hepatoprotective effect was obtained against carbon tetrachloride induced liver damage, by oral administration of *Helminthostachis zeylanica* methanolic extract as evident from decreased levels of serum enzymes and an almost normal architecture of the liver in the treated groups. The extract was effective in increasing the choleretic activity of anaesthetized normal rats. It also shortened hexobarbitone induced sleeping time in mice and showed significant anti lipid peroxidant effect invitro.

Venukumar and Latha (2004) investigated the effect of methanol extract of *Coscinium fenestratum* in carbon tetrachloride induced hepatotoxicity in rats. The level of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase and glucose-6 phosphate dehydrogenase and concentrations of total protein, total lipids, phospholipids,

triglycerides and cholesterol were found recovered to almost normalcy barring slight deformity of hepatocytes and clearing of cytoplasm.

Chattopadhyay and Bandyopadhyay (2005) found that administration of *Azadirachta indica* leaf extract (500mg/kg) in paracetamol (2g/kg) induced hepatotoxicity significantly enhanced the reduced enzymatic parameters, the hepatic level of reduced glutathione dependant enzymes, superoxide dismutase and catalase.

Ha *et al.* (2005) reported that pretreatment of *Lycium chinense* fruit extract significantly lowered the serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase and histological examination showed that hepatocyte necrosis was either prevented or minimized. It also prevented the elevation of hepatic malonaldehyde formation and the depletion of reduced glutathione content and catalase activity in the liver of carbon tetrachloride injected rats and also displayed hydroxide scavenging activity and significantly reduced cytochrome P_2E_1 m RNA and protein compared to the liver of control group.

2.3 OTHER PHARMACOLOGICAL EFFECTS OF PLANTS UNDER STUDY

2.3.1 Azadirachta indica

Sen *et al.* (1992) evaluated the effect of *Azadirachta indica* on biochemical, immunological and visceral parameters in normal and stress rats. *Azadirachta indica* (100mg/kg) lowered blood glucose, triglyceride and aspartate aminotransferase levels in normal rats and attenuated stress induced elevation of cholesterol and urea level. *A. indica* also significantly attenuated stress induced suppression of humoral immune response and gastric ulcerogenesis.

Neem leaf alcoholic extract was investigated for its effect on ECG and blood pressure of rats by Koley and Lal (1994). Intravenous administration of neem leaf extract resulted in initial bradycardia followed by cardiac arrhythmia in rats. Neem leaf extract produced a significant and dose related fall in blood pressure, which was significant, immediate and persistent.

Talwar *et al.* (1995) found that Praneem oil Vilci (PV), purified neem oil was reported to exercise a reversible antifertility effect after a single intrauterine instillation in rodents and primates with out any adverse effects.

Bopanna *et al.* (1997) concluded that neem seed kernel powder significantly reduced the concentration of serum lipids, blood glucose and activities of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase, liver glucose-6 phosphate dehydrogenase and HMG co A reductase activity in liver and intestine of alloxan diabetic rabbits.

Kasturi *et al.* (1997) showed that oral administration of dry *Azadirachta indica* leaf powder for 24 days resulted in decrease in the weights of seminal vesicles and ventral prostate, reduction in epithelial height, nuclear diameter and the secretory material in the lumen. Biochemically, there was a decrease in the total protein and acid phosphatase activities. Seminal vesicles and ventral prostate being androgen dependant, the regressive changes histologically and biochemically suggested the antiandrogenic action of neem leaves.

Chattopadhyay (1998) reported that the water soluble portion of alcoholic extract of *Azadirachta indica* leaves at a dose of 200 mg/kg exerted significant anti inflammatory activity in cotton pellet granuloma in rats. The extract also reversed significantly the elevated levels of biochemical parameters viz. DNA, RNA, lipid peroxide, acid phosphatase and alkaline phosphatase.

Sadekar *et al.* (1998) studied the effects of feeding of powdered dry leaves of *Azadirachta indica* on humoral and cell mediated immune responses in a flock of broilers. *A. indica* (2g/kg) treatment significantly enhanced the antibody titers and also potentiated the immune responses.

Sairam *et al.* (2000) reported that NIM–76, a new vaginal contraceptive from neem oil showed antimicrobial action against bacteria like *Escherichia coli and Klebsiella pneumoniae*, antifungal activity against *Candida albicans* and antiviral ativity against polio virus replication in vero cell lines.

Khosla *et al.* (2002) investigated the effect of aqueous leaf extract of *Azadirachta indica* on isolated perfused frog and rabbit heart. Dose dependant

negative inotropic and chronotropic effects were observed in both the heart preparation. An increase in coronary blood flow in isolated rabbit heart was observed.

Godeswar *et al.* (2004) assessed the effect of *Azadirachta indica* leaf extract on cauda epididymis in wistar male rats. The electron microscopical examination revealed that the principal cells were decreased in coated micro pinocytotic vesicles, invagination of luminal surfaces, loss of apical microvilli and disruption of mitochondrial cristae and golgi apparatus.

Pai *et al.* (2004) concluded that the dental gel containing neem extract has significantly reduced the plaque index and bacterial count than that of group treated with commercially available chlorhexidine gluconate mouthwash.

Raji *et al.* (2004) evaluated the effect of ethanolic extract of *Azadirachta indica* on gastric ulceration in albino rats. The drug significantly prevented gastric ulceration induced by indomethacin and this action was accompanied by a dose dependant decrease in total gastric acidity.

4.3.2 *Aegle marmelos*

Aegle marmelos aqueous leaf extract significantly increased glucose tolerance and decreased the liver glycogen, blood urea and serum cholesterol in alloxan diabetic rats (Ponnachan *et al.*, 1993).

Sabu and Kuttan (2001) noticed that treatment with methanolic extract of *Aegle marmelos* lowers alloxan induced lipid peroxidation significantly in serum and liver and increased superoxide dismutase activity in liver and catalase activity in erythrocytes and liver to reduce the oxidative stress.

Sur *et al.* (2002) found that water extract of *Aegle marmelos* reduced the complete immobility time of spermatozoa in rats.

Kamalakkannan and Prince (2003) confirmed that depressed activities of superoxide dismutase, catalase, glutathione peroxidase and lowered glutathione content in the heart and pancreas of diabetic rats were found to increase on treatment with aqueous extract of *Aegle marmelos* fruits at dose of 250 mg/kg.

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Thirty two adult wistar rats of either sex weighing 150-200g, procured from Small Animal Breeding Station, Mannuthy were used for the study. The animals were maintained under identical managemental conditions for one week before the commencement of the study.

3.2 PREPARATION OF HERBAL AGENTS

The leaves of *Azadirachta indica* and *Aegle marmelos* were collected fresh and dried in the shade at room temperature (Figure 1 and 2). The dried leaves are then powdered well in a pulveriser. The powdered leaves are then boiled with distilled water in 1:8 ratio for 30 minutes and then filtered through a muslin cloth. The filtered solution was then evaporated to dryness and kept in the refrigerator in an airtight container. Hundred gram dried *A. indica* leaf powder gave 20g of extract and 100g dried *A. marmelos* leaf powder gave 30g of extract. A weighed quantity of dried extract was homogenized with 0.2 per cent gum acacia and was administered orally to individual rats for 12 days based on their body weight.

3.3 EXPERIMENTAL DESIGN

The study was conducted using thirty two adult albino rats weighing 150-200 g. The rats were divided into four groups of eight each. Blood was collected from all groups prior to the experiment for haematological and biochemical studies.

Group1: Served as absolute control, which received 0.2 per cent gum acacia oral suspension in distilled water for 12 days.

Group II: Rats received paracetamol (3g/kg) suspension prepared with gum acacia in distilled water for three days orally (Rao and Mishra, 1998).

Group III: *Azadirachta indica* aqueous leaf extract (500mg/kg) was given orally for 12 days. Paracetamol (3g/kg) suspension was given on day eight, nine and ten in addition to *A. indica* aqueous leaf extract.

Group IV: *Aegle marmelos* aqueous leaf extract (1g/kg) was given orally for 12 days. Paracetamol (3g/kg) suspension was given on day eight, nine and ten

Fig. 1. *Azadirachta indica* (Neem)

Fig. 2. *Aegle marmelos* (Indian Bael)

in addition to *A. marmelos* aqueous leaf extract.

Blood was collected from group II animals 48 hours after administration of paracetamol (sixth day) and from group I, III and IV animals on thirteenth day for haematological examination and serum was separated for biochemical studies and liver was taken for biochemical studies and histopathological examination.

3.4 COLLECTION OF BIOLOGICAL SAMPLES

3.4.1 Blood

Blood was collected from retro orbital plexus by puncturing with heparinised capillary tubes, into sterile vials containing Disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA Sodium) at the rate of 1mg/ml for estimation of haematological parameters.

3.4.2 Serum

Blood was collected in sterile centrifuge tubes without anticoagulant for serum for the estimation of enzymes, total protein and albumin.

3.4.3 Liver

The animals were sacrificed and dissected upon and the liver was collected. It was washed in running tap water to remove blood clots and kept in chilled 0.9 per cent sodium chloride for biochemical studies and in 10 per cent formaline for histopathological examination.

3.5 PHYTOCHEMICAL SCREENING

The aqueous extracts of *Azadirachta indica* and *Aegle marmelos* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3.5.1 Test for Detection of steroids

3.5.1.1 *Salkowski Test*

About five mg of extract was dissolved in three ml of chloroform and then shaken with three ml of concentrated sulphuric acid. Development of a red colour indicates the presence of steroids.

3.5.1.2 Leiberman burchardt Test

About five mg of the extract was dissolved in three ml of chloroform. Then five drops of acetic anhydride and one ml of concentrated sulphuric acid was added to it through the sides. A reddish ring at the junction of two layers indicates the presence of steroids.

3.5.2. Test for Detection of Alkaloids

About 0.5 g of the extract was mixed with five ml ammonia and then extracted with equal volume of chloroform. To this 0.1 N hydrochloric acid was added. The acid layer obtained was used for chemical test for the alkaloids.

3.5.2.1 Mayer's Test (potassium mercuric iodide)

To one ml of the acid layer obtained few drops of Mayer's reagent were added. If a creamy white precipitate is formed, it indicates the presence of alkaloids.

3.5.2.2 Hager's Test (saturated solution of picric acid)

To one ml of acid layer, few drops of Hager's reagent were mixed. A yellow precipitate is formed when alkaloids are present.

3.5.2.3 Dragendroff's Test (Solution of potassium bismuth iodide)

Two drops of Dragendroff's reagent was mixed with one ml of acid layer. Presence of alkaloids is indicates if a reddish brown precipitate is seen.

3.5.3 Tests for Detection of tannins

3.5.3.1 Ferric Chloride Test

Two mg of extract was mixed with three ml of one per cent ferric chloride solution .If blue green or brownish green colour is obtained, it indicates the presence of tannins.

3.5.4 Tests for Detection of Flavonoids

3.5.4.1 Ferric chloride Test

To two ml of alcoholic solution of extract (0.5g extract in 10ml methanol) few drops of neutral ferric chloride solution was mixed. Presence of flavonoids is indicated by green colour.

3.5.4.2 Lead Acetate Test

To two ml of alcoholic solution of extract, (0.5g extract in 10 ml methanol), few droops of 10 per cent lead acetate was mixed. Yellow precipitate indicates the presence of flavonoids.

3.5.5 Tests for Presence of Glycosides

3.5.5.1 Benedict's Test

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

3.5.5.2 Sodium Hydroxide Test

Dissolved a small amount of the extract (about five mg) in one ml water and added five to six drops of sodium hydroxide solution, Yellow colour indicates the presence of glycosides.

3.5.6 Tests for Presence of Phenolic Compounds

About five mg of the extract was dissolved in one ml of water and five drops of ten percent ferric chloride solution was added to it. Development of dark brown colour occurs if phenolic compounds are present

3.5.7 Tests for Detection of Diterpenes

About five mg of the extract was mixed with three ml of copper acetate solution. Presence of diterpenes is indicated by the development of green colour.

3.5.8 Tests for Presence of Triterpenes

3.5.8.1 Salkowski Test

About three mg of extract was dissolved in three ml chloroform and then shaken with concentrated sulphuric acid. Lower layer turning yellow on standing indicates presence of triterpenes.

3.5.8.2 Leibermann Burchardt Test

Few drops of acetic acid and one ml concentrated sulphuric acid was added to 30 ml chloroform solution of the extract (about three mg of extract in three ml

chloroform). Deep red ring at the junction of the two layers indicates the presence of triterpenes.

3.5.9 Test for Presence of Saponins

3.5.9.1 *Foam Test*

A small amount of extract (about five mg) was shaken with three ml of water. If the foam produced persists for 10 minutes, presence of saponins is confirmed.

3.6 PHYSIOLOGICAL PARAMETERS

3.6.1 Feed intake

The feed intake of both the treated and control groups were recorded daily and the data was analysed.

3.6.2 Body weight

The body weights of both the control and treatment groups were taken and recorded on first day of the experiment and on sixth day in group II and on thirteenth day in group I, III and IV and the data was analysed.

3.7 ESTIMATION OF BIOCHEMICAL PARAMETERS

3.7.1 Measurement of lipid peroxide level (Okhawa *et al.*, 1979).

Reagents

Potassium chloride 150mM

Sodium dodecyl sulphate 8.1 per cent (SDS)

Acetic acid 20 per cent; pH adjusted to 3.5

Aqueous solution of thiobarbituric acid 0.8 per cent (TBA)

n-butanol :pyridine mixture (15:1)

Procedure

1. One gram of tissue was mixed with 9 ml of 150 mM potassium chloride and homogenized in a tissue homogenizer.

2. Tissue homogenates (0.2ml) were taken in test tubes, added 0.2 ml of 8.1 percent SDS, 1.5 ml of 20 per cent acetic acid and 1.5 ml of TBA. Blank contained 0.2ml of potassium chloride instead of tissue homogenate.
3. Made up the volume to 4 ml with distilled water and heated on a water bath at 95^0 C for 60 minutes.
4. Added 1ml of distilled water and 5ml of n-butanol: pyridine mixture. It was shaken well and centrifuged at 15 x g for 10 minutes.
5. The absorbance of the colour of the organic layer was measured at 532nm.
6. The lipid peroxide level was calculated by using extinction coefficient of $1.56x10^5$ and the values were expressed in nmol of malonaldehyde (MDA)/g of wet tissue.

3.7.2 Measurement of superoxide dismutase level (Mimami and Yoshikawa, 1979)

Reagents

Sodium chloride 0.9 per cent

Tris cacodylic acid buffer (50mM, pH8.2)

Tris cacodylic acid 50Mm

Diethylene triamine penta acetic acid 1mM

Nitroblue tetrazolium 0.1 m

Triton X 100 0.001 per cent.

All reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1N sodium hydroxide.

Pyrogallol 0.2mM

Procedure

1. Freshly excised liver was homogenized with 10 volumes of 0.9 per cent sodium chloride followed by centrifugation at 15 x g for 10 minutes at 4^0C to harvest the supernatent.
2. The assay mixture in a total volume of 3ml consists of 1.4 ml of 50mM tris cacodylic acid buffer, 1.4ml of 0.2mM pyrogallol and 0.2ml of enzyme

preparation.

3. Blank contained distilled water instead of enzyme preparation.
4. The absorbance due to autooxidation of pyrogallol was read at 420nm using spectrophotometer.
5. One unit of SOD activity was the amount of enzyme that inhibited pyrogallol autooxidation by 50 per cent under experimental conditions.
6. The values were expressed in units/mg of protein after quantifying the protein content of supernatent by method of Lowry *et al.* (1951).

3.7.3 Esitmation of catalase (Cohen *et al.*, 1970)

Reagents

Phosphate buffer- hydrogen peroxide solution (10mM)

Phosphate buffer (0.05M pH 7.0)

0.2M sodium dihydrogen phosphate	39ml
0.2M disodium hydrogen phosphate	61ml
Distilled water	300ml

Immediately before use 0.2ml of hydrogen peroxide was added to 100ml buffer.

Procedure

1. Three ml of the phosphate buffer – hydrogen peroxide solutions were taken in test tubes.
2. Blank contained distilled water instead of hydrogen peroxide solution.
3. Samples prepared in sodium chloride (as described in case of super oxide dismutase) were added to both and the absorbance was read at 240nm at the 40^{th} second of addition of the sample.
4. The time required for the initial absorbance to decrease by 0.05 units was noted.
5. The catalase activity in units/assay mixture was calculated by using the formula $\log E_1/E_2 \times 2300/6.93 \times 1/\Delta t$.

E_1 – Initial absorbance

E_2 – Absorbance after decrease by 0.05 units

Δt – Time taken for the decrease in absorbance by 0.05 units (seconds).

3.7.4 Estimation of Serum Alanine Aminotransferase (ALT)

UV Kinetic Test (Reitman and Frankel, 1957)

Principle

Serum ALT catalyses the transfer of amino group from L- alanine to L-oxoglutarate with the 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 340nm, which is proportional to ALT activity.

Procedure

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

3.7.5 Estimation of Serum Aspartate Aminotransferase (AST)

UV Kinetic Test (Reitman and Frankel, 1957).

Principle

Aspartate aminotransferase 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 reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH in this reaction is measured as decrease in absorbance of NADH at 240nm, which is proportional to AST activity.

Procedure

Mixed well 10µl of sample and 1000µl of working reagent and measured the absorbance at 340 nm in an auto analyzer.

3.7.6 Estimation of Serum Alkaline Phosphatase (ALP)

P-NPP Method (Kind and King, 1954)

Principle

Serum alkaline phosphatase hydrolyses P-nitro phenyl phosphate in to P–nitro phenol and phosphate in the presence of oxidizing agent and Mg^{2+}. This reaction is measured, as absorbance is proportional to the ALP activity.

Procedure

Pipetted out 1000 µl of working reagent and 20 µl of sample and mixed well and measured the absorbance at 405 nm in an auto analyzer.

3.7.7 Estimation of serum Total Protein

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

Principle

The peptide bonds of protein react with copper ions in alkaline solution to form a blue–violet complex (so called biuret reaction). The complex formed 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	10µl		
Standard		10µl	
Reaction solution	1000µl	1000µl	1000µl

Mixed the solutions and incubated for ten minutes at 37^0C. Read the absorbance of standard and sample against blank at 540 nm.

Serum total protein (g/dl) = Absorbance of sample / Absorbance of standard x 6

3.7.8 Estimation of serum albumin

Bromcresol Green Dye Method (Doumas, 1971)

Principle

Albumin forms a blue green complex with bromcresol green at slightly acidic pH, which is measured photometrically.

Procedure

Sample, standard and blank were prepared as follows.

	Sample	Standard	Blank
Serum	10µl		
Standard		10µl	
Reaction solution	1000µl	1000µl	1000µl

Mixed the solutions and incubated for ten minutes at 37^0C. Read the absorbance of standard and sample against blank at 540 nm.

Serum albumin (g/dl) = Absorbance of sample / Absorbance of standard x 3

3.8 HAEMATOLOGICAL PARAMETERS

3.8.1 Total leucocyte count

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

3.8.2 Differential leucocyte count

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

3.8.3 Haemoglobin Concentration

Hemoglobin concentration was estimated by acid haematin method (Benjamin, 1985).

3.9 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

The gross and histopathological lesions in liver were studied by collecting liver at sixth day in group II and on thirteenth day in group I, III and IV.

3.9.1 Gross lesions

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

3.9.2 Histopathological examination

Liver was taken for histopathological examination to assess the hepatotoxicity. Three mm thick pieces of liver were selected randomly from both the control and experimental groups of rats and fixed in 10 per cent formalin and processed through routine paraffin embedding process, stained with haematoxylin and eosin and studied the histopathology (Sheehan and Hrapchak, 1980).

3.10 STATISTICAL ANALYSIS OF DATA

Results were analysed by using analysis of variance for comparison between groups I, II, III and IV and for parameters except body weight and body weight was analysed using analysis of covariance as described by Snedecor and Cochran (1985). Results were expressed as mean $\pm$ standard error.

4. RESULTS

4.1 PHYTOCHEMICAL SCREENING

4.1.1 Steroids

In Salkowski test red colour was obtained and Lieberman burchadt test gave a reddish ring at the junction for Aegle aqueous extract and not for Azadirachta extract. Thus it could be concluded that steroids are present in the aqueous extract of Aegle and no detectable level of steroids could be obtained in Azadirachta extract.

4.1.2 Alkaloids

A creamy white precipitate in Mayer's test and an yellow coloured precipitate in Hager's test was obtained for Azadirachta and Aegle extracts. Dragendroff's test yielded a reddish brown precipitate for the two extracts. Thus the tests revealed detectable levels of alkaloids in the aqueous extracts of Azadirachta and Aegle.

4.1.3 Tannins

Brownish green colour was obtained in ferric chloride test for Azadirachta and Aegle. The results indicated the presence of tannins in both the extracts.

4.1.4 Flavonoids

A green colour in the ferric chloride test and an yellow precipitate in lead acetate test indicated the presence of flavonoids in Azadirachta extract. Negative result was obtained for Aegle.

4.1.5 Glycosides

In Benedict's test red colour was obtained indicating the presence of glycosides in all the two samples. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides.

4.1.6 Phenolic compounds

The extract mixed with 10 percent ferric chloride produced dark brown colour indicating the presence of phenolic compounds for Azadirachta aqueous extract. No development of colour was seen in the other extract.

4.1.7 Diterpenes

Diterpenes were detected in Azadirachta and Aegle extracts as indicated by the green colour when mixed with copper sulphate solution.

4.1.8 Triterpenes

For Azadirachta and Aegle extracts, lower layer turned to yellow on standing as per Salkowski test, and by Leiberman Burchadt test, a deep ring appeared at the junction of the two layers. The results indicated the presence of triterpenes in Azadirachta and Aegle extracts.

4.1.9 Saponins

In the foam test, foam persisted for 10 minutes in the case of Azadirachta and Aegle extracts indicating the presence of saponins in Azadirachta and Aegle aqueous extracts.

The results obtained in the above study are summarized in the Table I.

4.2 PHYSIOLOGICAL PARAMETERS

4.2.1 Feed intake

The individual feed intake of rats was recorded daily and is presented in Table 2. In control group feed intake ranged from 160 to 190 g/kg. There was no marked variation in feed intake with in the group. The feed intake of paracetamol treated group was reduced to 35-50g. An increase in feed intake was observed in *Azadirachta indica* and *Aegle marmelos* treated group compared to paracetamol treated rats.

4.2.2 Body weights

The individual and mean body weights of rats (Group I, II, III and IV) were recorded on first and thirteenth day of the experiment and are presented in the Table 3. The body weight recorded on first day of group I, II, III and IV were 166.25 $\pm$ 1.52, 183.75 $\pm$ 1.39, 175 $\pm$ 1.65 and 195 $\pm$ 1.25, respectively. After treatment on

thirteenth day, body weight recorded were 170 ± 1.47, 180 ± 1.43, 172.5 ± 1.47 and 193.13 ± 1.08 g for group I, II, III and IV, respectively. A reduction in body weight shown by group II, III and IV animals and a gradual increase in body weight shown by animals of group I.

4.3 BIOCHEMICAL PARAMETERS

4.3.1 Lipid peroxide level

The results obtained are presented in Table 4 and Figure 3. The peak lipid peroxide level found was 167.94 ± 3.59 nmol of MDA/g wet tissue in animals treated with paracetamol at the rate of 3 g/kg (Group II). Group II differed significantly from Group I, which had values 58.62 ± 4.94 n mol of MDA/g of wet tissue. The group treated with plant extracts (Group III and Group IV) showed significant reduction in lipid peroxide levels compared to group II ($P<0.05$). The lipid peroxide level of animals treated with *A. indica* at the rate of 500 mg/ kg and *A. marmelos* at the rate of 1g/kg were 64.94 ± 3.31 and 68.96 ± 2.76 n mol of MDA/g of wet tissue respectively. There was no significant change in lipid peroxide level in group III animals compared to group IV.

4.3.2 Superoxide dismutase

The values of SOD obtained are presented in Table 5 and Figure 4. A significant increase in SOD level was noted in groups treated with plant extracts, compared to the group treated with paracetamol ($P<0.05$). Group III and group IV had SOD values of 49.48 ± 1.77 and 44.01 ± 0.77 units/mg of protein, respectively after 12 days of treatment. Group II animals had lowest value for SOD with 22.39 ± 0.71 units/mg of protein where as group I animals, had a SOD value of 48.55 ± 0.88 units/mg of protein. There was no significant difference between group III and group IV animals.

4.3.3 Catalase

The values of catalase are given in Table 6 and Figure 5. There was significant reduction in catalase values in group II animals compared to group I animals. The mean catalase values of group I and group II were 62.42 ± 3.73 and 36.56 ± 1.38 units/assay mixture respectively. There was significant increase in catalase values in group III and Group IV compared to group II ($P<0.05$). Group III

TableI. Results of phytochemical screening of *Azadirachta indica* and *Aegle marmelos* extracts

Sl. No.	Active principles	*A. indica*	*A. marmelos*
1	Steroids	Absent	Present
2	Alkaloids	Present	Present
3	Tannins	Present	Present
4	Flavonoids	Present	Absent
5	Glycosides	Present	Present
6	Phenolic compounds	Present	Absent
7	Diterpenes	Present	Present
8	Triterpenes	Present	Present
9	Saponins	Present	Present

Table 2. Effect of treatment on feed intake(g) of rats

Day	Group I	Group II	Group III	Group IV
1	160	40	160	180
2	190	35	180	190
3	180	35	175	180
4	175	40	190	190
5	190	50	200	200
6	170		170	190
7	170		165	180
8	180		180	175
9	190		70	55
10	170		70	60
11	180		85	80
12	160		125	125

Table 3. Effect of treatment on body weights (g) of rats (n=8)

Animal No.	Group I		Group II		Group III		Group IV	
	0 day	13 day	0 day	13 day	0 day	13 day	0 day	13 day
1	180	185	200	195	180	180	180	180
2	160	165	170	165	160	160	200	200
3	150	165	180	175	180	175	200	200
4	180	180	200	195	160	155	210	205
5	150	150	190	190	180	180	190	190
6	180	185	170	165	200	190	200	195
7	160	160	180	180	180	180	200	195
8	170	170	180	175	160	160	180	180
Mean	166.25	170	183.75	180	175	172.5	195	193.13
S.E.	1.52	1.47	1.39	1.43	1.65	1.47	1.25	1.08

Table 4. Effect of *Azadirachta indica* and *Aegle marmelos* on lipid peroxide level (nm of MDA /g wet tissue) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	70.3	162.2	54.6	60.87
2	72	187.2	52.12	69.62
3	34.6	172.16	64.42	80.24
4	54.6	152.4	56.31	72.76
5	53.04	175.48	74.46	78.84
6	45.12	160	67.78	62.76
7	59.76	171.6	69.62	69.62
8	79.56	162.44	80.24	56.97
Mean	**58.62** [a]	**167.94** [b]	**64.94** [a]	**68.96** [a]
S.E.	**4.94**	**3.59**	**3.31**	**2.76**

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

Table 5. Effect of *Azadirachta indica* and *Aegle marmelos* on superoxide dismutase level (units/mg protein) in paracetamol Induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	49.68	20.86	47.1	44.2
2	50.18	23.69	49.41	42.2
3	44.27	20.56	45.6	42.88
4	49.82	26.62	57.41	45.47
5	46.73	20	50.73	45.17
6	47.86	21.46	40.99	40.37
7	46.97	22.74	56	43.64
8	52.9	23.22	48.6	48.12
Mean	**48.55** [a]	**22.39** [b]	**49.48** [a]	**44.01** [a]
S.E.	**0.88**	**0.71**	**1.77**	**0.77**

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

Table 6. Effect of *Azadirachta indica* and *Aegle marmelos* on catalase level (units/assay mixture) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	80.2	33.18	61.88	74.02
2	67.69	37.08	51.12	55.23
3	50	39.67	80.57	57.52
4	59.07	31.67	61.41	70.51
5	75.36	33.23	71.88	74.17
6	62.47	42.28	66	53.69
7	54.6	41.56	70	75.31
8	50	33.77	73	63.22
Mean	**62.42** [a]	**36.56** [b]	**66.98** [a]	**65.46** [a]
S.E.	**3.73**	**1.38**	**2.97**	**3.02**

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

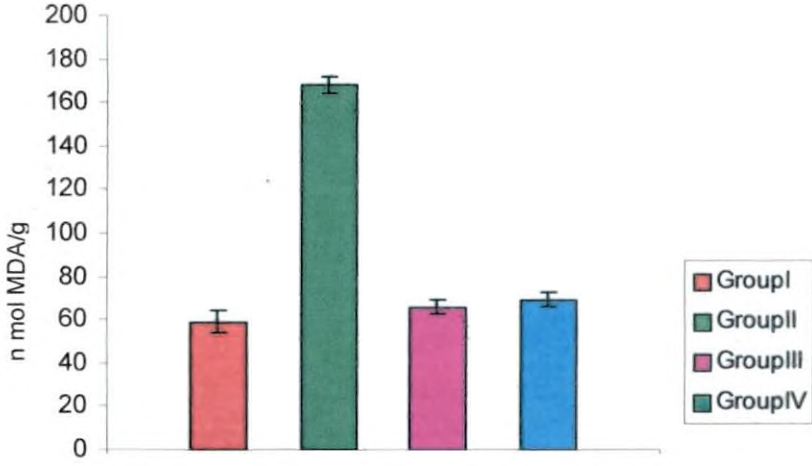

Fig. 3. Effect of treatment on lipid peroxide level (n mol MDA/g wet tissue)

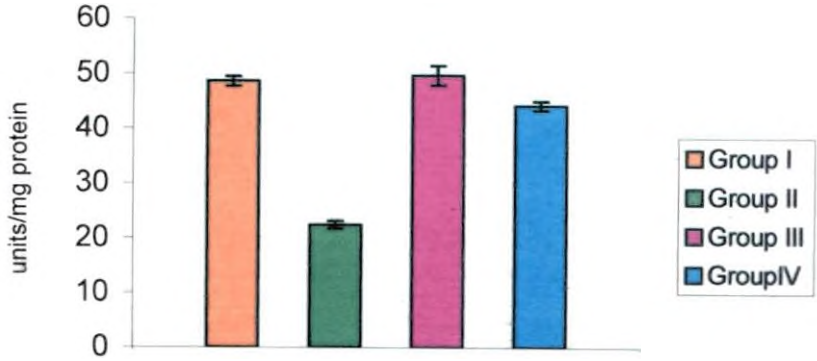

Fig. 4. Effect of treatment on SOD level (units /mg of protein)

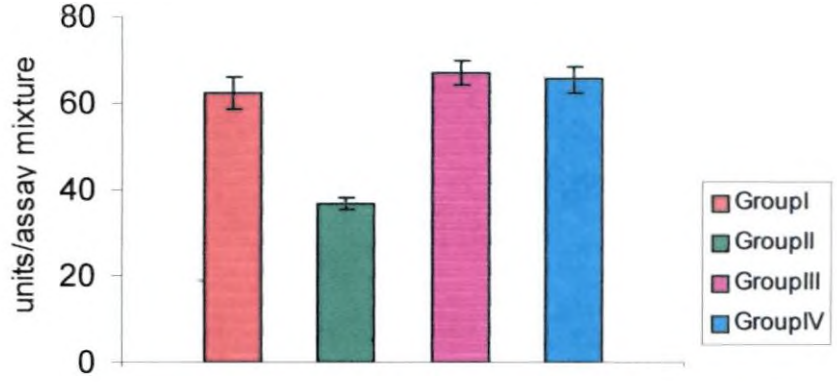

Fig. 5. Effect of treatment on catalase level (units/assay mixture)

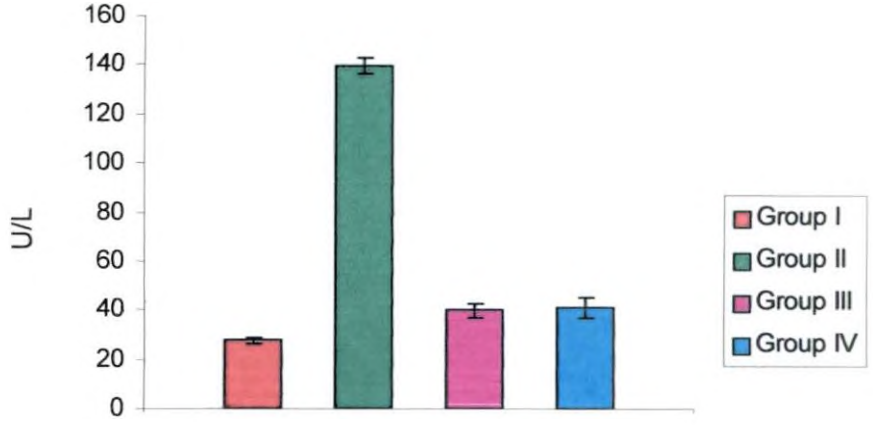

Fig.6. Effect of treatment on serum ALT level (U/L)

and Group IV had catalase values of 66.98 ± 2.97 and 65.46 ± 3.02 units/mg of assay mixture. There was no significant difference in catalase values between group III and group IV.

4.3.4 Serum Alanine Aminotransferase

The results obtained are presented in Tables 7 and 8 and Figure 6. The serum ALT level before treatment for group I, II, III and group IV were 21.75 ± 1.16, 25 ± 1.61, 24.75 ± 1.26 and 24.88 ± 1.32 U/L, respectively. The level of serum ALT was increased in group II animals after paracetamol treatment compared to group I ($P<0.05$). The mean ALT value for group I and group II were 23 ± 0.81 U/L and 137.88±3.32 U/L respectively. There was significant reduction in levels of serum ALT in group III and group IV compared to group II. The mean value for serum ALT in animals treated with *A. indica* was 33.13 ± 2.18 U/L and those treated with *A. marmelos* was 37.62 ± 1.83 U/L.

4.3.5 Serum Aspartate Aminotransferase

The results obtained are presented in Tables 9 and 10 and Figure 7. The serum AST level of group I, Group II, Group III and group IV animals before treatment were 27.63 ± 1.47, 30 ± 1.61, 29.75 ± 1.13 and 29.88 ± 1.30 U/L, respectively. Highest level of AST was obtained for paracetamol treated group of animals ie.139.75 ± 3.39 U/L. Group III and group IV animals showed significant reduction in serum AST levels compared to group II. The mean value for group III and group IV were 39.87 ± 3.14 and 41 ± 4.13 U/L, respectively. The control group had lowest value, 27.5 ± 1.24 U/L.

4.3.6 Serum Alkaline phosphatase

The results obtained are given in Tables 11 and 12 and Figure 8. Group I, Group II, Group III and Group IV animals showed mean ALP values 72.25 ± 1.89, 74.13 ± 2.79, 73.88 ± 1.55 and 73.88 ± 2.72 U/L, respectively before treatment. *A. indica* and *A. marmelos* treated animals showed significant reduction in ALP values compared to group II ($P<0.05$). Group II animals showed significant increase in serum ALP values compared to group I. The mean ALP values after treatment for group I, group II, group III and group IV were 72.63 ± 2.38, 153.63 ± 4.46, 83.88 ± 4.38 and 85.13 ± 4.49 U/L, respectively.

4.3.7 Serum Total protein

The values of serum total protein obtained before and after treatment are presented in Table 13 and 14 and Figure 9. The mean values of serum total protein before treatment were 5.96 ± 0.12, 5.83 ± 0.07, 6.03 ± 0.13, 5.85 ± 0.10 g/dl for group I, II, III and IV respectively. Compared to control group paracetamol treated group showed significant reduction in levels of total protein. Control group had a total protein value of 5.98 ± 0.1 g/dl and paracetamol treated group had a mean value of 4.85 ± 0.06 g/dl *A. marmelos* and *A. indica* treated group showed significant elevation in levels of total protein compared to paracetamol treated group ($P<0.05$). The group of animals treated with *A. indica* aqueous leaf extract had serum total protein value of 5.98 ± 0.14 g/dl and animals treated with *A. marmelos* had total protein value 5.63 ± 0.14 g /dl.

4.3.8 Serum albumin

The values of serum albumin before and after treatment are shown in Table15 and 16 and Figure10. The serum albumin values before treatment for group I, group II, group III and group IV animals were 2.86 ± 0.07, 2.85 ± 0.06, 2.89 ± 0.04 and 3 ± 0.09 g/dl, respectively. Animals treated with paracetamol (group II) showed a significant reduction on serum albumin values compared to group I animals. The mean albumin value of group I and group II were 2.90 ± 0.05 and 1.88 ± 0.08 g/dl, respectively. The values of serum albumin in group III and group IV increased significantly compared to group II. The values obtained for group III and group IV were 2.8 ± 0.43 and 2.88 ± 0.07 g/dl respectively.

4.4 HAEMATOLOGICAL PARAMETERS

4.4.1 Haemoglobin concentration

The means values are presented in Table 17 and 18. The haemoglobin concentration of group I, group II, group III and group IV were 12.85 ± 0.26, 12.84 ± 0.1, 12.69 ± 0.31 and 12.68 ± 0.14 g/dl, respectively, before treatment and 12.78 ± 0.32, 12.44 ± 0.15,12.7 ± 0.32 and 12.6 ± 0.14 g/dl after treatment and no significant difference could be seen between groups. The values were with in normal range on both the occasions in all groups, which is 10-18 g/dl.

4.4.2 Total Leucocyte Count

The results are given in Table 17 and 18. The values of total leucocyte count were in normal range in all groups before and after treatment. The values obtained for group I, group II, group III and group IV were10.83 ± 0.37, 10.09 ± 0.20, 10.43 ± 0.28 and 10.21 ± 0.32 X10 ^{3} /µl of blood after treatment and 10.86 ± 0.35, 10.19 ± 0.27, 10.42 ± 0.29 and 10.11 ± 0.33 X10 ^{3}/µl of blood before treatment. The values did not differ significantly between groups.

4.4.3 Differential Leucocyte Count

4.4.3.1 Lymphocytes

The results obtained are presented in Table 17 and 18. The lymphocyte count in grup I, groupII, group III and group IV were similar with values of 81.13 ±1.14, 82.38 ± 16.46, 79.25 ± 1.14 and 79.95 ± 1.23 per cent, respectively, before treatment and 81 ± 1.06, 81.25 ± 1.01, 79.25 ± 0.93 and 79.75 ± 1.23 per cent after treatment

4.4.3.2 Neutrophils

The data presented in Table 17 and 18. The neutrophil count of the rats before treatment were 18.13 ± 1.05, 19.59 ± 3.02, 20.88 ± 0.89 and 20 ± 1.25 percent and after treatment were 18.5 ± 1.17, 18.75 ± 1.01, 20.38 ± 0.88 and 20 ± 1.25 per cent .

4.4.3.3 Eosinophils

The data is presented in Table 17 and 18. The eosinophil counts of different groups did not differ significantly after treatment. The values were 0.50 ± 0.35, 0.43 ± 0.03, 0.5 ± 0.35 and 0.13 ± 0.12 per cent for group I, group II, Group III and group IV, respectively, before treatment and 0.38 ± 0.35, 0.13 ± 0.12, 0.25 ± 0.15 and 0.13 ± 0.12 per cent after treatment.

4.4.3.4 Monocytes

The data is present in Table 17 and 18. The monocyte counts of different groups did not differ significantly after treatment. The mean values were 0.13 ± 0.12, 0, 0.13 ± 0.12 and 0.13 + 0.12 per cent, respectively, before and after treatment.

4.4.3.5 Basophils

No basophils were observed before and after treatment.

4.5 GROSS AND HISTOPATHOLOGICAL EXAMINATION

Gross examination of liver from control group showed normal appearance and colour (Figure 11). Paracetamol treated liver showed greyish white areas of necrosis (Figure 12). Livers of *A. indica* and *A. marmelos* pretreated rats were almost normal in appearance.

Histopathological examination of control group revealed normal hepatic architecture (Figure13). Liver of paracetamol treated group revealed centrilobular coagulative necrosis (figure 14). The nuclei of necrotic hepatocytes were in varying stages of pyknosis. The cytoplasm was homogenous and stained more pink. The normal sharp contours of the cells could not be appreciated. Disruption of hepatic cords was also evident in focal areas. The liver of *A. indica* and *A. marmelos* treated rats showed normal hepatic cords, absence of necrosis, mild congestion and less degree of infiltration (Figure 15 and 16).

Table 7. Serum ALT level (U/L) before treatment in rats

Animal No.	Group I	Group II	Group III	Group IV
1	25	20	20	20
2	18	18	28	28
3	20	25	25	22
4	18	30	20	28
5	20	32	30	22
6	23	28	22	21
7	22	25	28	30
8	28	22	25	28
Mean	21.75	25	24.75	24.88
S.E.	1.16	1.61	1.26	1.32

Table 8. Effect of *Azadirachta indica* and *Aegle marmelos* on serum ALT level (U/L) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	24	134	27	38
2	20	140	41	35
3	20	145	29	38
4	22	146	29	48
5	24	120	41	30
6	25	150	27	33
7	22	140	30	42
8	27	128	41	37
Mean	23 [a]	137.88 [b]	33.13 [c]	37.63 [c]
S.E.	0.81	3.32	2.18	1.83

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

Table 9. Serum AST level (U/L) before treatment in rats

Animal No.	Group I	Group II	Group III	Group IV
1	23	34	28	28
2	20	24	28	25
3	25	38	30	29
4	30	34	32	27
5	32	28	24	29
6	30	25	30	32
7	29	29	35	31
8	32	28	32	38
Mean	27.63	30.00	29.75	29.88
S.E.	1.47	1.61	1.13	1.30

Table 10. Effect of *Azadirachta indica* and *Aegle. marmelos* on serum AST level (U/L) in paracetamol induced hepatotoxicity in rats(n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	25	128	30	66
2	21	125	48	35
3	24	140	33	49
4	28	155	35	34
5	30	135	30	34
6	31	146	46	48
7	30	149	56	34
8	31	140	41	28
Mean	27.5^{a}	139.75^{b}	39.87^{c}	41^{c}
S.E.	1.24	3.39	3.14	4.13

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

Table 11. Serum ALP level (U/L) before treatment in rats

Animal No.	Group I	Group II	Group III	Group IV
1	78	75	70	73
2	70	80	75	65
3	68	82	80	62
4	65	63	78	80
5	75	69	75	69
6	76	83	73	75
7	80	79	75	82
8	66	62	65	85
Mean	72.25	74.13	73.88	73.88
S.E.	1.89	2.79	1.55	2.72

Table12. Effect of *Azadirachta indica* and *Aegle marmelos* on serum ALP level (U/L) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	80	160	75	79
2	68	145	95	70
3	70	150	100	80
4	65	142	93	105
5	78	140	90	78
6	73	178	73	99
7	82	162	80	95
8	65	152	65	75
Mean	72.63 [a]	153.63 [b]	83.88 [a]	85.13 [a]
S.E.	2.38	4.46	4.38	4.49

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

Table13. Serum total protein level (g/dl) before treatment in rats

Animal No.	Group I	Group II	Group III	Group IV
1	6.5	5.8	6.8	6.2
2	6	5.9	6	6.1
3	5.3	5.5	6	5.9
4	5.8	6	6.1	6
5	6.2	6.1	5.8	5.5
6	6.1	6	6	5.8
7	5.8	5.8	5.4	5.3
8	6	5.5	6.1	6
Mean	5.96	5.83	6.03	5.85
S.E.	0.12	0.07	0.13	0.10

Table14. Effect of *Azadirachta indica* and *Aegle marmelos* on serum total protein level (g/dl) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	6.5	4.9	6.8	6
2	6	4.6	6	6
3	5.5	5.1	5.9	5.7
4	5.8	4.9	6	6
5	6.2	5	6	5.1
6	6	4.6	5.8	5.4
7	5.8	4.7	5.3	5
8	6	5	6	5.8
Mean	5.98 [a]	4.85 [b]	5.98 [a]	5.63 [a]
S.E.	0.10	0.06	0.14	0.14

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

Table15. Serum albumin level (g/dl) before treatment in rats

Animal No.	Group I	Group II	Group III	Group IV
1	2.8	2.8	2.9	3
2	3	3	2.7	3.5
3	3.1	3	3	3.1
4	2.6	2.5	2.7	3
5	2.5	2.8	3	3
6	2.9	3	2.8	2.9
7	3	2.9	3	2.5
8	3	2.8	3	3
Mean	2.86	2.85	2.89	3.00
S.E.	0.07	0.06	0.04	0.09

Table16. Effect of *Azadirachta indica* and *Aegle marmelos* on serum albumin level (g/dl) in paracetamol induced hepatotoxicity in rats (n=8)

Animal No.	Group I	Group II	Group III	Group IV
1	2.8	2.3	2.9	3
2	3.1	1.6	2.7	3.2
3	3	1.8	2.8	3
4	2.9	2.1	2.6	2.8
5	2.6	1.7	3	2.8
6	2.8	2	2.7	2.9
7	3	1.8	2.8	2.8
8	3	1.7	2.9	2.5
Mean	2.90^{a}	1.88^{b}	2.80^{a}	2.88^{a}
S.E.	0.05	0.08	0.04	0.07

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

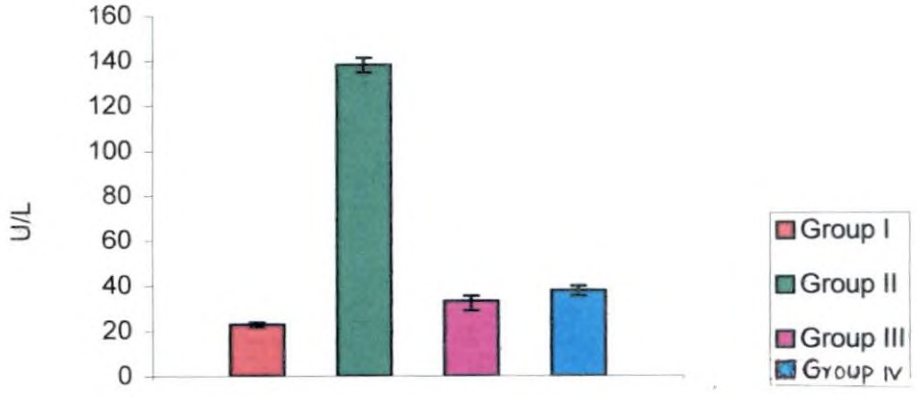

Fig. 7. Effect of treatment on serum AST level (U/L)

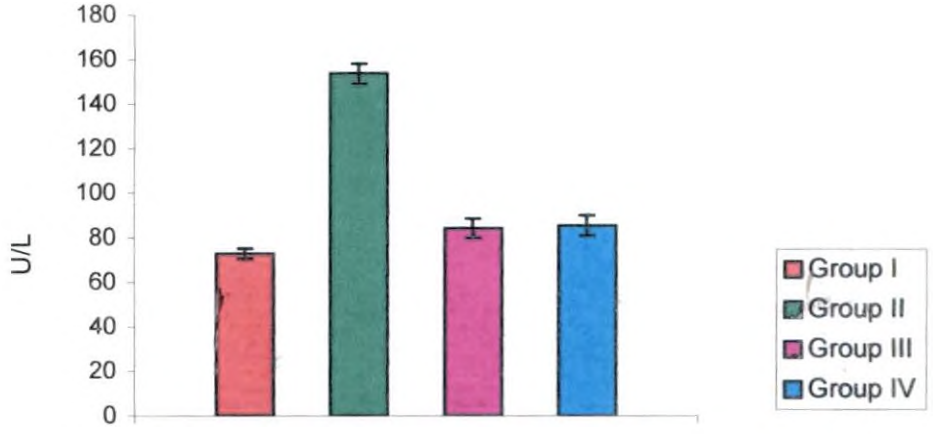

Fig. 8. Effect of treatment on serum ALP level (U/L)

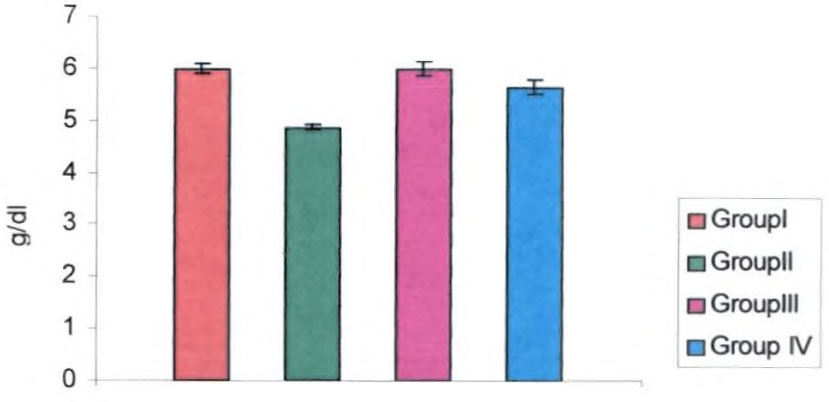

Fig.9. Effect of treatment on serum total protein level (g/dl)

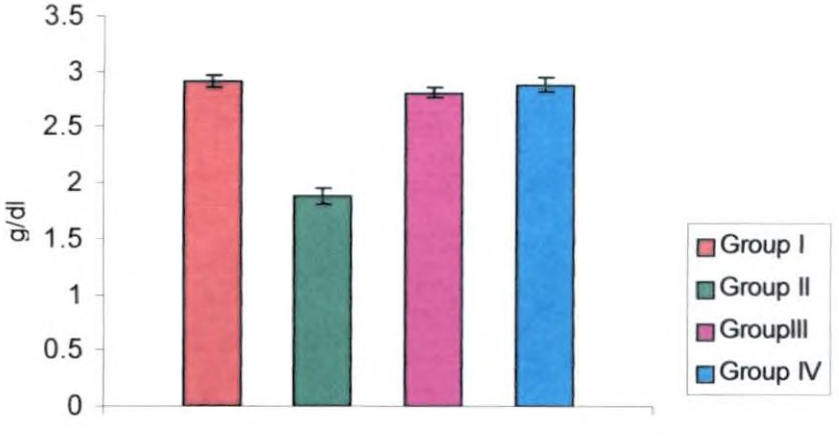

Fig.10. Effect of treatment on serum albumin level (g/dl)

Table 17. Haematological values before treatment in rats.

		First day						
				Differential Leucocyte count (%)				
		Hemoglobin(g/dl)	Leucocyte count(10^3/µl)	Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
Group I	Mean	12.85	10.86	81.13	18.13	0.50	0.13	0.00
	S.E.	0.26	0.35	1.14	1.05	0.35	0.12	0.00
Group II	Mean	12.84	10.19	82.38	19.59	0.43	0.00	0.00
	S.E.	0.1	0.27	16.46	3.02	0.03	0.00	0.00
GroupIII	Mean	12.69	10.42	79.25	20.88	0.50	0.13	0.00
	S.E.	0.31	0.29	1.14	0.89	0.35	0.12	0.00
Group IV	Mean	12.68	10.11	79.75	20.00	0.13	0.13	0.00
	S.E.	0.14	0.33	1.23	1.25	0.12	0.12	0.00

Table 18. Effect of *Azadirachta indica* and *Aegle marmelos* on haematological parameters in paracetamol induced hepatotoxicity in rats (n=8)

		Thirteenth day						
		Hemoglobin(g/dl)	Leucocyte count(10^3/µl)	Differential Leucocyte count (%)				
				Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
Group I	Mean	12.78	10.83	81	18.5	0.38	0.13	0.00
	S.E.	0.32	0.37	1.06	1.17	0.35	0.12	0.00
Group II	Mean	12.44	10.09	81.25	18.75	0.13	0.00	0.00
	S.E.	0.15	0.20	1.01	1.01	0.12	0.00	0.00
Group III	Mean	12.70	10.43	79.25	20.38	0.25	0.13	0.00
	S.E.	0.32	0.28	0.93	0.88	0.15	0.12	0.00
Group IV	Mean	12.60	10.21	79.75	20	0.13	0.13	0.00
	S.E.	0.14	0.32	1.23	1.25	0.12	0.12	0.00

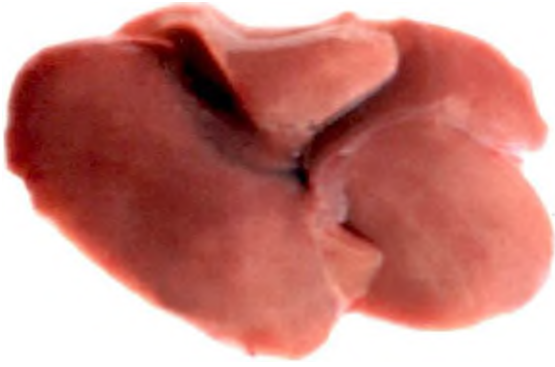

Fig. 11. Normal liver

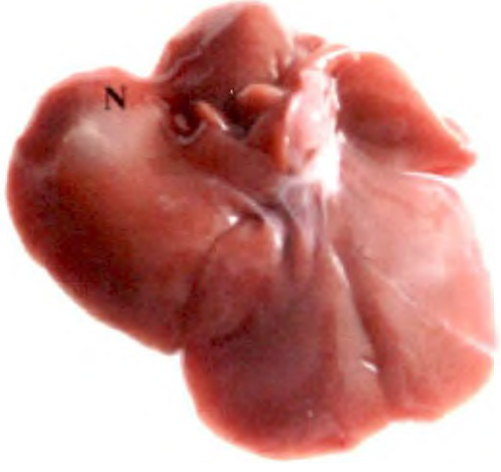

Fig. 12. Liver of paracetamol treated rats showing areas of necrosis (N)

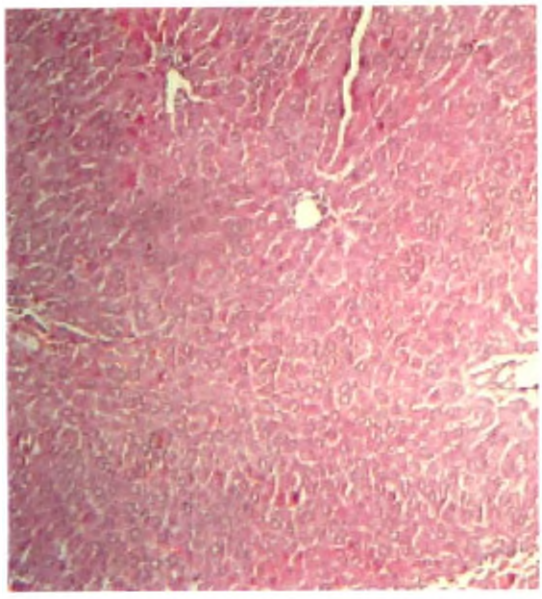

Fig. 13.

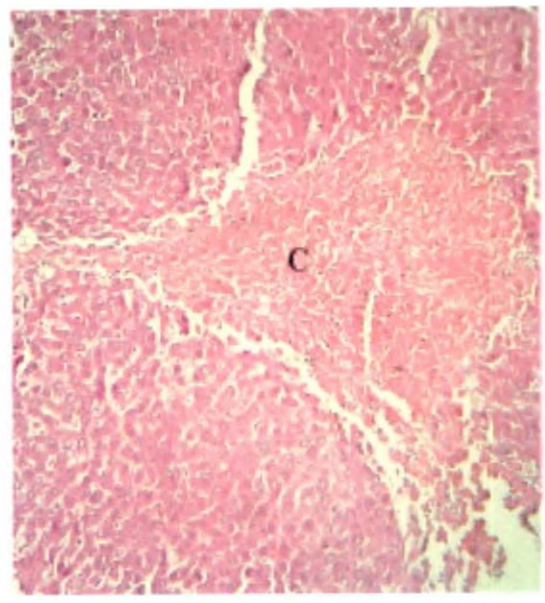

Fig. 14.

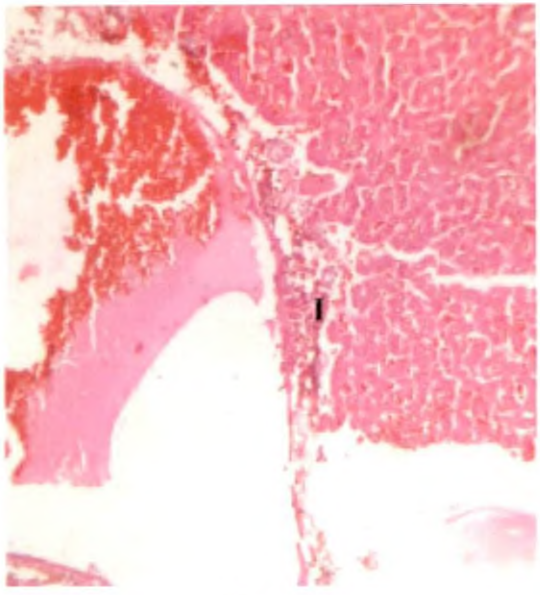

Fig. 15.

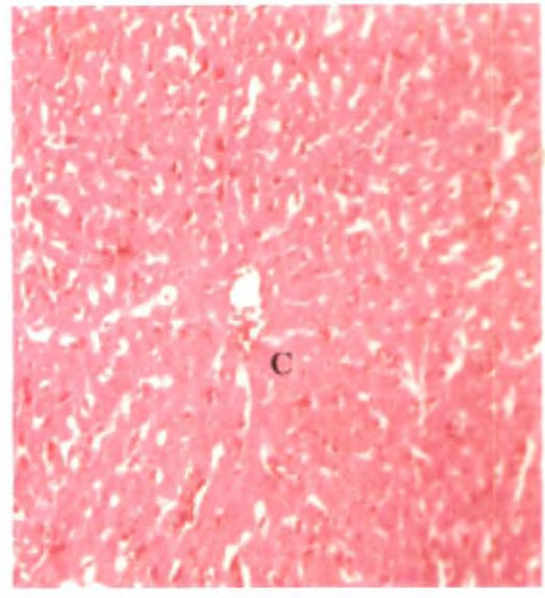

Fig. 16.

Fig. 13. Liver- cross section showing normal arrangement of hepatocytes with nuclei (H&Ex125)

Fig. 14. Liver- cross section of paracetamol treated rats showing centrilobular coagulative necrosis (C) (H&Ex125)

Fig. 15. Liver- cross section showing mild degree of infiltration (I) after treatment (H&Ex125)

Fig. 16. Liver- cross section showing mild congestion (C) after treatment (H&Ex125)

Discussion

5. DISCUSSION

The present study was undertaken to assess the hepatoprotective activity of *Azadirachta indica* and *Aegle marmelos* in paracetamol induced hepatotoxicity in rats. Paracetamol is a widely used antipyretic and analgesic, produces acute liver damage, if overdose is consumed. The liver damage was evidenced by the elevation of liver enzymes and ultimate hepatic necrosis brought about by increased lipid peroxidation or depletion of glutathione (Shenoy *et al.*, 2002). Plant derived natural products such as flavonoids, terpinoids and steroids, have received considerable attention in recent years due to their diverse pharmacological properties such as antioxidant and hepatoprotective activity (Gupta *et al.*, 2004). A combination of different herbal extracts or fractions are likely to provide desired activities to cure severe liver diseases.

In the present study, paracetamol was given to rats of group II for three days. Animals of group III were given *A. indica* aqueous leaf extract at the dose rate of 500 mg/kg for 12 days and paracetamol suspension on day eight, nine and ten. Animals of group IV were given *A. marmelos* at the rate of 1g/kg for 12 days and paracetamol suspension on days eight, nine and ten. Blood was collected from group II animals 48 hours after administration of paracetamol (sixth day) and group I, III and IV animals on thirteenth day for haematological examination and serum was separated for biochemical studies and liver was taken for biochemical studies and histopathological examination. Various haematological, physiological, biochemical parameters and histopathological examination were carried out which included feed intake, body weight liver lipid peroxide, superoxide dismutase and catalase, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein and albumin, total and differential leucocyte counts and haemoglobin estimation.

From the results it is obvious that the herbal agents tried namely aqueous extract of *A. indica* at the rate of 500 mg/kg as well as *A. marmelos* at the rate of 1g/kg reduced the lipid peroxidation and elevated serum levels of alanine amino transferase, aspartate aminotransferase and alkaline phosphatase and increased the

reduced levels of superoxide dismutase, catalase, total protein and albumin in paracetamol induced hepatotoxicity in rats.

5.1 PHYTOCHEMICAL SCREENING

Phytochemical screening of aqueous extract of *A. indica* revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins but no detectable level of steroids. Bhanwra *et al.* (2000) also found that flavonoids present in neem leaf aqueous extract are well known antioxidants. Vallachira (1998) reported that the dried bark and leaves of *Melia azadirachta* contained a bitter amorphous resin, an alkaloid margosine and margosic acid. The present study also revealed the presence of alkaloids. Kale *et al.* (2003) found that six flavonol – o- glycosides, which are known to be responsible for blood sugar lowering activity, are also responsible for hepatoprotective activity.

Screening for active principles of *A. marmelos* aqueous leaf extract gave positive test for steroids, alkaloids, glycosides, phenolic compounds, tannins, diterpenes, triterpenes and saponins but no detectable level of phenolic compounds and flavonoids. Kamalakkannan and Prince (2003) found that alkaloids, coumarins and tannins present in the fruits of *A. marmelos* have antioxidant effect. The following constituents are reported to present in aegle fruit like aegelin, alloimperatorin, imperatorin, marmelide, marmelosine, marmesin, psoralen, rhamnose, scoparone, scopoletin, skimmin, tannic acid, umbelliferone and xanthotoxol.

5.2 PHYSIOLOGICAL PARAMETERS

5.2.1 Feed intake

The feed intake of paracetamol treated rats were reduced compared to group I animals. In *A. indica* and *A. marmelos* treated rats the feed intake was normal upto 7 days and feed intake reduced after paracetamol treatment. General symptoms of paracetamol over dosage in the first 24 hours are palor, nausea, vomiting, anorexia and abdominal pain. Liver damage may become apparent 12 to 24 hours after ingestion of paracetamol (Roberts and Morrow, 2001).

5.2.2 Body weight

The control group showed a gradual increase in body weight. The paracetamol treated group showed a reduction in body weight of 3.75 g. The body weight of Group III and group IV were decreased during the experiment, being less pronounced than the paracetamol treated rats.

5.3 BIOCHEMICAL PARAMETERS

5.3.1 Lipid peroxide

Lipid peroxidation is oxidative deterioration of polyunsaturated lipids and it involves reactive oxygen species and transition metal ions. It is a molecular mechanism of cell injury leading to generation of peroxides and lipid peroxides, which can be decomposed to yield a wide range of cytotoxic products, most of which are aldehydes, exemplified by malonaldehyde (MDA), 4- hydroxy nonenal etc. (Shukla *et al.*, 2004).

The lipid peroxide levels were higher in paracetamol treated animals compared to the control and plant extract treated groups (Table 4). *A. indica* at the rate of 500 mg/kg and *A. marmelos* at the rate of 1g/kg reduced the elevated lipid peroxide levels.

Paracetamol produced acute liver damage by the covalent binding of N- acetyl p- benzo quinone imine, an oxidation product of paracetamol, to sulfhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver (Kapur *et al.*, 1994).

A. indica leaf extract decreased the lipid peroxidation in the liver in murine carcenogenesis model systems by increasing glutathione activity above basal level in the liver and in the extrahepatic organs (Dasgupta *et al.*, 2004). The process of free radical production may be deleterious due to the increased oxidative stress. Reactive oxygen species has been shown to modify enzymes and induce signaling including activation of protein kinase, induce protein phosphorylation and also act as second messenger for the expression of genes involved in immune response. Treatment

with *A. marmelos* was able to decrease the serum lipid peroxide indicating that these treatments lowered oxidative stress in diabetic rats (Sabu and Kuttan, 2001).

5.3.2. Superoxide dismutase

Superoxide dismutase level was least in animals treated with paracetamol. There was a significant increase in the level of superoxide dismutase in plant extract treated group (Table 5 and Fig 4). There was no significant difference in the activity of superoxide dismutase between *A. indica* and *A. marmelos* treated rats.

Paracetamol can cause cellular damage through metabolic activation of chemical to highly reactive compounds like free radicals. Cells have a number of mechanisms to protect themselves from the toxic effects of reactive oxygen species. Superoxide dismutase removes superoxide by converting it into hydrogen peroxide that can be rapidly converted into water by catalase and glutathione peroxidase (Gupta *et al.*, 2004).

Treatment with *Holostema adakodien* reduced the levels of lipid peroxide and increased superoxide dismutase, catalase and reduced glutathione suggesting that the drug could have prevented the free radical mediated membrane peroxidation in carbon tetrachloride induced hepatotoxicity (Jelly *et al.*, 2004). Similar results were also obtained in the present study. Liver superoxide dismutase activity was increased significantly in rats treated with aqueous extract of *A. marmelos* when compared to alloxan induced diabetic rats (Sabu and Kuttan, 2001).

5.3.3 Catalase

As stated earlier in case of superoxide dismutase, the level of catalase was also lowest in the paracetamol (3g/kg) treated rats. Similar observations are made by Ahmed and Khater (2001) when rats were treated with paracetamol at the dose rate of 640 mg/kg. There was increase in the activity of catalase in the *A. indica* and *A. marmelos* treated groups.

Catalase activity was increased in erythrocytes and liver with *A. marmelos* treatment in alloxan diabetic rabbits indicating that the treatment may help to lower hydrogen peroxide concentration by its decomposition with catalase and

subsequently reduction of oxidative stress (Jose and Kuttan, 2000). Present study also confirmed the elevation of activity of catalase in *A. marmelos* treated rats.

Treatment with aqueous extract of *A. indica* and *Abroma augusta* increased the level of antioxidant enzymes like superoxide dismutase, catalase, glutathione transferase and glutathione peroxidase in alloxan diabetic rats (Halim, 2003).

5.3.4 Serum Alanine Aminotransferase

Serum alanine aminotransferase level was highest in animals treated with paracetamol (groupII). There was significant reduction in levels of alanine amino transferase in plant extract treated groups (Tables 7 and 8 and Figure 6).

Liver damage is assessed in paracetamol hepatotoxicity by the determination of enzymes levels such as alanine aminotransferase and aspartate aminotransferase. Liver necrosis or membrane damage releases the enzymes in to the circulation, therefore it can be measured in the serum. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatic cellular damage. Alanine aminotransferase is more specific to liver and thus a better parameter for detecting liver injury (Bhanwra *et al.*, 2000; Gupta *et al.*, 2004; Kumar *et al.*, 2004).

The highly significant reduction in levels of serum alanine aminotransferase and aspartate aminotransferase were observed in rats treated with neem leaf extract and paracetamol compared to the rats treated with paracetamol alone, which indicated that neem leaf affects important biochemical reactions which may be beneficial in reducing hepatic damage (Bhanwra *et al.*, 2000).

5.3.5 Serum Aspartate Aminotransferase

Aspartate aminotransferase activity was highest in the paracetamol treated group compared to the control group. There was significant reduction in levels of aspartate aminotransferase in groups treated with *A. indica* and *A. marmelos* aqueous leaf extract compared to group II.

Hepatocellular necrosis by paracetamol leads to elevation of the serum marker enzymes alanine aminotransferase, aspartate aminotransferase and alkaline

phosphatase which are released from liver into blood. Elevation of these enzymes in serum indicates membrane damage (Shenoy *et al.*, 2002).

Treatment with neem leaf powder initiated the reduction in the raised serum enzymes levels, viz. alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase with in 48 to 72 hour and restored them back to near normal with in a week period (Sadekar *et al.*, 2002).

The ethanolic extract of *Trianthema portulacastrum* preserved the structural integrity of the hepatocellular membrane in a dose dependant manner in paracetamol and thioacetamide induced hepatotoxicity as evident from the reduction in levels of elevated serum enzymes ALT, AST and ALP (Kumar *et al.*, 2004). Similar results were obtained in the present study with *A. indica* and *A. marmelos* in paracetamol induced hepatotoxicity.

5.3.6 Serum alkaline phosphatase

An elevated level of serum enzyme alkaline phosphatase is often associated with liver damage. In the present study groups treated with *A. indica* and *A. marmelos* aqueous leaf extract showed reduction in the elevated levels of enzyme alkaline phosphatase compared to the animals treated with paracetamol. The animals of group II which received paracetamol at the dose rate of 3g/kg had a higher serum alkaline phosphatase level compared to that of the control group. Aspartate aminotransferase activity was highest in the paracetamol treated group compared to the control group. There was a significant reduction in levels of aspartate aminotransferase in groups treated with *A. indica* and *A. marmelos* aqueous leaf extract compared to group II.

Rajesh and Latha (2001) observed that when carbon tetrachloride treated rats were administered with *Elephantopus scaber* root powder, there was significant reduction in the elelvated activities of serum enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase.

Chattopadhyay *et al.* (1992) observed that the values of serum enzymes alanine aminotransferase and alkaline phosphatase were much lower in animals

receiving *A. indica* leaf extract and paracetamol alone indicating that the degree of hepatic cell damage was of lesser magnitude in the extract treated group.

5.3.7 Serum total protein

The site of specific oxidative damage of the susceptible aminoacids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis of liver disorders. Hypoalbuminaemia is more frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content has been deemed as a useful index of the severity of cellular dysfunction in liver diseases. The lowered level of total protein in the serum of carbon tetrachloride treated rats revealed the severity of hepatotoxicity (Venukumar and Latha, 2001).

Total protein level was lowest in the case of animals treated with paracetamol at the rate of 3 g/kg compared to other groups. The lowered total protein level was significantly elevated by treatment with aqueous extract of *A. indica* and *A. marmelos.*

Decrease in serum total protein and albumin in carbon tetrachloride induced hepatotoxicity in rats may be associated with decrease in the number of hepatocytes which in turn may result in the decreased hepatic capacity to synthesize protein and glycogen. Feeding of *Lawsonia alba* extract restored the decreased level of serum total protein confirming the hepatoprotective activity (Bhandarkar and Khan, 2003). Similar results were obtained in the present study with *A. indica* and *A. marmelos*.

3.8 Serum albumin

As stated earlier in case of total protein, serum albumin level was lowest in paracetamol treated rats (Group II and Table 15 and 16). There was a significant elevation in reduced albumin level in groups treated with *A. indica and A. marmelos* compared to group II.

Shenoy *et al.* (2002) revealed that paracetamol at the rate of 2g/kg produced liver damage in rats as indicated by a significant reduction of serum total protein and albumin. Shenoy *et al.* (2001) found that *Ginkgo biloba* has increased the level of total protein and albumin in the serum, which indicates hepatoprotective activity.

Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates regeneration process and the production of liver cells.

5.4 HAEMATOLOGICAL PARAMETERS

Haematological parameters are presented in Table 17 and 18. The study of haematological parameters of all the groups revealed no significant changes and all values fall within normal ranges of blood values for the experimental animal under study.

5.5 GROSS AND HISTOPATHOLOGICAL EXAMINATION

Gross examination of liver from control group showed normal appearance and colour. Liver of animals treated with paracetamol showed grayish white areas of necrosis. Liver of *A. indica* and *A. marmelos* pretreated rats were almost normal in appearance.

Bhanwra *et al.* (2000) observed that liver of most of the paracetamol treated rats were covered with white slough and multiple white patches indicating necrosis. Livers from neem leaf extract treated group were almost normal in appearance regarding colour and organ weight.

Histopathological examination of control group revealed normal hepatic architecture. Liver of paracetamol treated group revealed centrilobular coagulative necrosis. The nuclei of necrotic hepatocytes were in varying stages of pyknosis. The cytoplasm was homogenous and stained more pink. The normal sharp contours of the cells could not be appreciated. Disruption of hepatic cords was also evident in focal areas. The liver of *A. indica* and *A. marmelos* treated rats showed amelioration of liver injury as evident from presence of normal hepatic cords, mild congestion and less degree of infiltration.

The covalent binding of oxidation products of paracetamol, to sulfhydryl groups of protein resulting in cell necrosis. Histopathological examination revealed centrilobular hepatocytes characterised by nuclear pyknosis and eosinophilic cytoplasm (Kapur *et al.,* 1994). Bhanwra *et al.* (2000) revealed that paracetamol

treated liver sections showed confluent centrilobular necrosis, cloudy swelling, pyknotic nuclei, loss of ribosomes, cytoplasmic matrix swelling and eosinophilic cytoplasm. There were small lipid globules in surviving hepatocytes, congestion of sinusoids and gross hydropic vacuolation was prominent. Histology of liver sections from neem leaf extract and paracetamol treated rats revealed fine areas of congestion with mild fatty change.

Kale *et al.* (2003) found that concurrent administration of *A. indica* aqueous leaf extract to antitubercular drug treated rats preserved the histological structure of liver by significantly reducing scores of necrosis, degeneration and fibrosis with evidence of significant regeneration.

The results of the present study confirmed the strong hepatoprotective activity of aqueous extract of *A. indica* and *A. marmelos* leaves in paracetamol induced hepatotoxicity in albino rats. *A. indica* aqueous leaf extract itself could act as a 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 (Chattopadhyay, 2003). The results of present study also showed that *A. marmelos* aqueous leaf extract induces antioxidant defence system and reduces lipid peroxidation and serum enzymes levels and elevated superoxide dismutase, catalase, total protein and albumin.

The present investigation confirms hepatoprotective effect of *A. indica* and *A. marmelos* in paracetamol induced hepatotoxicity in albino rats.

6. SUMMARY

The present study was undertaken to assess the hepatoprotective effect of aqueous extract of *Azadirachta indica* and *Aegle marmelos* leaves on paracetamol induced hepatotoxicity in rats.

The study was conducted using 32 adult albino rats, divided into four groups of eight each. Group1 animals served as absolute control, which received 0.2 per cent gum acacia oral suspension in distilled water for 12 days. Group II animals received paracetamol (3g/kg) suspension prepared with gum acacia in distilled water for three days orally. Group III animals received *A. indica* aqueous leaf extract (500mg/kg) orally for 12 days and paracetamol (3g/kg) suspension on day eight, nine and ten. Group IV animals received *A. marmelos* aqueous leaf extract (1g/kg) orally for 12 days and paracetamol (3g/kg) suspension on day eight, nine and ten. Blood was collected from all groups prior to the experiment and from group II animals 48 hours after administration of paracetamol (sixth day) and from group 1, III and IV animals on thirteenth day for haematological examination and serum was separated for biochemical studies and liver was taken for biochemical studies and histopathological examination.

Various haematological, physiological, biochemical parameters and histopathological examination were carried out which include feed intake, body weight, liver lipid peroxide, superoxide dismutase and catalase, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein and albumin, total and differential leucocyte count and haemoglobin estimation.

Phytochemical screening of aqueous extracts of *A. indica* revealed the presence of alkaloids, tannins, flavonoids, phenolic compounds, glycosides, diterpenes, triterpenes and saponins, but no detectable level of steroids. Screening for active principles of *A. marmelos* aqueous leaf extract gave positive test for steroids, alkaloids, tannins, glycosides, diterpenes, triterpenes and saponins but no detectable level of phenolic compounds and flavonoids.

The feed intake of paracetamol treated rats were reduced compared to group I animals. In *A. indica* and *A. marmelos* treated rats the feed intake was normal upto 7 days and feed intake reduced after paracetamol treatment. The control group (group I) showed a gradual increase in body weight. The paracetamol treated group showed reduction in body weight during five day experimental period. The body weight of Group III and group IV were decreased during the experiment, being less pronounced than the paracetamol treated rats.

The lipid peroxide level was higher in paracetamol treated animals compared to the control and plant extract treated groups. *A. indica* at the rate of 500 mg/kg and *A. marmelos* at the rate of 1g/kg reduced the elevated lipid peroxide levels. Superoxide dismutase level was least in animals treated with paracetamol. There was significant increase in the level of superoxide dismutase in plant extract treated groups. The level of catalase was also lowest in the paracetamol (3g/kg) treated rats. There was increase in the activity of catalase in the *A. indica* and *A. marmelos* treated groups.

Serum alanine aminotransferase level was highest in animals treated with paracetamol. There was significant reduction in levels of alanine amino transferase in plant extract treated groups. Aspartate aminotransferase activity was highest in the paracetamol treated group compared to the control group. There was significant reduction in levels of aspartate aminotransferase in groups treated with *A. indica* and *A. marmelos* aqueous leaf extract compared to group II. The elevated alkaline phosphatase activity in paracetamol treated group was also reduced significantly in groups treated with *A. indica* and *A. marmelos* aqueous leaf extract.

Total protein level was lowest in the case of animals treated with paracetamol at the rate of 3 g/kg compared to the other groups. The lowered total protein level was significantly elevated by treating with aqueous extract of *A. indica* and *A. marmelos*. The lowered serum albumin level in paracetamol treated rats were significantly elevated in groups treated with *A. indica and A. marmelos* aqueous leaf extract.

The study of haematological parameters of all the groups revealed no significant changes and all values fall within normal ranges of blood values for the experimental animal under study.

Gross examination of liver from control group showed normal appearance and colour. Paracetamol treated liver showed greyish white areas of necrosis. Liver of *A. indica* and *A. marmelos* pretreated rats were almost normal in appearance. Histopathological examination of control group revealed normal hepatic architecture. Liver of paracetamol treated group revealed centrilobular coagulative necrosis. The liver of *A. indica* and *A. marmelos* treated rats showed amelioration of liver injury as evident from presence of normal hepatic cords, absence of necrosis, mild congestion and less degree of infiltration.

The results of the present study confirmed the strong hepatoprotective activity of aqueous extract of *A. indica* and *A. marmelos* leaves in paracetamol induced hepatotoxicity in rats.

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HEPATO PROTECTIVE EFFECT OF *Aegle marmelos* (INDIAN BAEL) AND *Azadirachta indica* (NEEM) AQUEOUS LEAF EXTRACT ON PARACETAMOL INDUCED TOXICITY IN RATS

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ABSTRACT

The present study was undertaken to assess the hepatoprotective effect of aqueous extract of leaves of *Azadirachta indica* at the rate of 500 mg / kg and *Aegle marmelos* at the rate of 1 g/kg on paracetamol induced hepatotoxicity in rats.

The study was conducted using thirty two adult albino rats weighting 150-200g. The rats were divided into four groups of eight each. Animals of Group1 served as absolute control, which received 0.2 per cent gum acacia oral suspension in distilled water for 12 days. Group II animals received paracetamol (3g/kg) suspension prepared with gum acacia in distilled water for three days orally. Group III animals received *A. indica* aqueous leaf extract (500mg/kg) orally for 12 days and paracetamol (3g/kg) suspension on day eight, nine and ten. Group IV animals received *A. marmelos* aqueous leaf extract (1g/kg) orally for 12 days and paracetamol (3g/kg) suspension on day eight, nine and ten. Blood was collected from all groups before and after treatment for haematological and biochemical examination and liver was taken for biochemical and histopathological examination.

The feed intake of paracetamol treated rats was reduced compared to animals group I. In *A. indica* and *A. marmelos* treated rats the feed intake was normal upto seven days and feed intake reduced after paracetamol treatment. The animals of group I showed a gradual increase in body weight. The paracetamol treated group showed reduction weight. The body weight of Group III and group IV were decreased during the experiment, being less pronounced than the paracetamol treated rats.

The lipid peroxide levels were higher in paracetamol treated animals compared to the control and plant extract treated groups. *A. indica* at the rate of 500 mg/kg and *A. marmelos* at the rate of 1g/kg reduced the elevated lipid peroxide levels. The level of superoxide dismutase and catalase were lowest in the paracetamol (3g/kg) treated rats. There was increase in the activity of superoxide dismutase and catalase in the treatment groups.

Serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activity were higher in the paracetamol treated group compared to the control group. There was significant reduction in levels of these enzymes in group

III and group IV compared to group II. Total protein and albumin levels were lowest in the case of animals treated with paracetamol compared to the other groups. The lowered total protein and albumin levels in group II animals were significantly elevated in group III and IV animals treated with aqueous extract of *A. indica* and *A. marmelos*.

The study of haematological parameters of all the groups revealed no significant changes.

Gross examination of liver from control group showed normal appearance and liver of paracetamol treated animals showed greyish white areas of necrosis. Livers of *A. indica* and *A. marmelos* pretreated rats were almost normal in appearance. Histopathological examination of control group revealed normal hepatic architecture. Liver of paracetamol treated group revealed centrilobular coagulative necrosis. The liver of *A. indica* and *A. marmelos* treated rats showed normal hepatic cords, absence of necrosis, mild congestion and less degree of infiltration.

The results of the present study confirmed the strong hepatoprotective activity of *A. indica* and *A. marmelos* aqueous leaf extract in paracetamol induced hepatotoxicity in rats.