Liver is the vital organ responsible for drug metabolism and appears to be a sensitive target site for substances modulating biotransformation (Ahmad et al., 2002). Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Certain medicinal agent introduced within therapeutic ranges may injure the organ sometimes. Other chemical agents those used in herbal remedies may also induce hepatotoxicity (Boerth et al., 2002). Nowadays drug induced liver injury has become a major health problem. Liver diseases such as jaundice, cirrhosis and fatty liver are very common and large public health problems in the world (Balamurugan et al., 2008). There is no rational therapy available for treating liver disorders so that management of liver diseases is still a challenge to the modern medicine. The traditional system of medicine has a major role in the treatment of liver ailments.

Azima tetracantha Lam. is a popular herb in Indian traditional medicine used as an antiarthritic, antimicrobial, hepato and nephroprotective agent. Whole plant extract of A. tetracantha contains flavonoids, amino acids, tannins, saponins and alkaloids, which may be responsible for the above activities (Jasulanth et al., 2001). In India and Sri Lanka the root, root bark and leaves are added to food as a remedy for rheumatism. The plant is considered as diuretic, used to treat dropsy, dyspepsia, chronic diarrhea and as a stimulant tonic (Bennett et al, 2004).

Tribulus terrestris is an annual plant native of Mediterranean region. T. terrestris is an important herb commonly used as folk medicine in many countries for different purposes. Fruits of T. terrestris have been shown to exhibit diuretic, antiurolithiatic, CNS stimulant, antimicrobial and antifungal activities in rats (Tuncer et al., 2009).

Iron overload is most often diagnosed when tissue...
damage occurs, especially in iron storing organ such as the liver. Hepatic fibrosis and cirrhosis are the outcomes of chronic iron overload due to inherited conditions as well as to repeated blood transfusions (Khan, 2012). Hepatotoxicity manifest as damage or dysfunction of the liver is second only to cardiovascular collapse as the cause of the death due to acute iron poisoning. An understanding of the pathogenesis of the hepatotoxicity of acute iron poisoning is central to the identification of rational and effective interventions (Firdous, 2012).

Hence the present study was undertaken to investigate hepatoprotective activity of A. tetracantha and T. terrestris against ferrous sulfate induced toxicity in experimental rats.

Materials and Methods
Animals: Healthy young albino rats weighing 130-150 g were purchased from a animal house, Manapparai, Tamilnadu and were acclimatized for 10 days under standard housing conditions (24°C ± 1°C; 45-55%RH with 12:12h light-dark cycle). The animals had free access to rat food and water ad libitum. The animals were habituated to lab conditions for two days to the experimental procedures to minimize any non specific stress. The experiment was designed and conducted in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC).

Chemicals: Ferrous sulfate (AR) was purchased from Merck (India). Silymarin (standard drug) was procured from Sigma Aldrich, Bangalore. Other solvents and chemicals were of analytical grade and purchased locally.

Plant materials: A. tetracantha leaves were collected from Pattukkottai, Tamilnadu and T. terrestris fruits were collected from Needamangalam near Thanjavur, Tamilnadu.

Dose preparation and administration: A. tetracantha leaves and T. terrestris fruits were dried at 45°C for 48 h, powdered using electric grinder and stored in a container. Powders (200 g) were extracted with hydroalcoholic mixture (ethanol and water in 1:1 proportion) at room temperature and filtrate were collected and concentrated. 1 mL of filtrate was fed to the animals orally.

Experimental design: Group I: Six rats were kept as control; Group II: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st – 24th days once daily; Group III: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st – 14th days once daily and then administered hydroalcoholic extract of fruit powder of T. terrestris on 15th – 24th days once daily; Group IV: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st – 14th days once daily and then administered hydroalcoholic extract of leaf powder of A. tetracantha on 15th–24th days once daily; Group V: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st – 14th days once daily and then administered 300 mg of silymarine on 15th–24th days once daily.

Biochemical investigation: At the end of treatment rats were sacrificed by cervical decapitation and subjected to various biochemical: sugar (Winckes and Tietz, 1971), bilirubin (Maloy et al., 1937), protein (Lowry et al., 1951), albumin, globulin (Doumas et al., 1971), alkaline phosphatase (Tiez, 1983), total cholesterol (Zlatis et al., 1951), triglycerides (Rice, 1970), lipoproteins (Fridwald et al., 1972), TBARS (Fraga et al., 1988), superoxide dismutase (Kakkar et al., 1984), catalase (Sinha, 1972) and vitamin E (Zaspal, 1983) and histopathological (Bancroft, 1977) assays.

Results
Table I shows the levels of sugar, bilirubin, protein, albumin, globulin, A/G and alkaline phosphatase. There is a significant increased level of sugar, bilirubin and alkaline phosphatase decreased levels of protein in Group II intoxicated rats. On administration of hydroalcoholic extracts of herbal drugs and

Table II shows the levels of total cholesterol, triglycerides, HDL, LDL, VLDL, TBA RS, superoxide dismutase, catalase and vitamin E. There is a significant increased level of cholesterol, triglycerides, HDL, VLDL and TBARS decreased levels of HDL, superoxide dismutase, catalase and vitamin E in Group II intoxicated rats. On administration of hydro alcoholic extracts of herbal

| Parameters | Group I | Group II | Group III | Group IV | Group V |
|------------|---------|----------|-----------|----------|---------|
| Sugar      | 90.5 ± 4.6 | 285.5 ± 4.2 | 145.0 ± 4.1 | 128.5 ± 2.9 | 164.0 ± 2.2 |
| Bilirubin  | 0.4 ± 0.2 | 1.6 ± 0.3 | 1.2 ± 0.2 | 0.9 ± 0.2 | 1.2 ± 0.2 |
| Protein    | 6.97 ± 0.2 | 2.9 ± 0.2 | 6.6 ± 0.1 | 6.8 ± 0.3 | 6.6 ± 0.4 |
| Albumin    | 3.6 ± 0.2 | 1.9 ± 0.3 | 3.1 ± 0.2 | 3.6 ± 0.3 | 2.8 ± 0.2 |
| Globulin   | 2.87 ± 0.1 | 1 ± 0.4 | 3.7 ± 0.6 | 3.1 ± 0.2 | 3.8 ± 0.4 |
| A/G Ratio  | 1.4 ± 0.1 | 2.5 ± 2.1 | 0.9 ± 0.2 | 1.2 ± 0.2 | 0.8 ± 0.2 |
| Alkaline phosphatase | 135.5 ± 2.8 | 447.0 ± 6.4 | 2100 ± 6.3 | 1637 ± 3.6 | 2425 ± 4.1 |

Values are expressed in mean ± S.D.; *p: Significant different from Group I vs. Group II (p<0.001) **p: Significant different from Group II vs. Group III, IV and V (p<0.001)
drugs and standard drug reverse the levels near to Group I rats.

**Discussion**

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders in ethno medical practices as well as in traditional systems of medicine in India (Bhattacharya et al., 2000). Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Hence there is worldwide trend to go back to traditional medicinal plants. Many natural products of natural origin are used for treatment of liver ailments (Pulliareddy and Lokesh, 1996). Toxicity is mostly depend -dant on iron induced free radical reactions and oxidative injury. Main pathy-siological effects of iron overload on liver tissue or fibrosis, porphyria and hepatocellular carcinoma. Free radical generation and lipid per oxidation is the proposed mechanism of iron induced hepatotoxicity. Iron catalyses hydroxyl radical formation and initiate lipid per oxidation (Reilly et al., 1991).

In the present study the level of glucose and bilirubin were significantly increased in ferrous sulfate induced groups when compared to control group of rats. Emerging scientific evidence has disclosed and suspected influences between iron metabolism and glucose level. The relationship is bidirectional iron affects glucose metabolism and glucose metabolism impinges on several iron metabolic pathways (Kirsch et al., 1968). Oxidative stress and inflammatory cytokines influence this relationship, amplifying and potentiating the initiated events. In recent years, increased iron stores have been found to predict the development of increased glucose levels while iron depletion was protective against beta cells of langerhan. Urinary bilirubin is a more sensitive indicator of liver injury than serum bilirubin (Hemalatha et al., 2005). The degree of increase in serum bilirubin values has prognostic significant in chronic liver injuries but not in acute injuries.

Iron hepatotoxicity, resulted in reduction of serum total protein and albumin levels in the present study. This observation could be ascribed to changes in protein and free amino acid metabolism and their synthesis in the injured liver cells and or increased protein degradation (Ratnasooriya et al., 2005). In our study the level of lipid profile via total cholesterol, triglycerides, LDL and VLDL was significantly elevated in ferrous sulfate induced group when compared to control group of rats where as the level of HDL was found to be significantly decreased. Our data indicate that dietary intake of herbal drugs and silymarin can significantly lower the serum lipid profile levels and can significantly increase HDL levels. It can also decrease endothelial cellular surface damage, rupture and may partially repair the endothelial dysfunction resulting from hyperlipidimia (Ramanathan and Kittusamy, 2011).

Iron poison is associated primarily with necrosis of the periporal areas, the site of hepatic regeneration. The periportal area receives the blood which is rich in oxygen and iron, both of which substrates for free radical generation. Iron catalyses the hydroxyl radical formation, the hydroxyl ion attacks all biological molecules, including cell membrane lipids, to initiate lipid per oxidation. The highly toxic peroxidative metabolite induces widespread cellular injury. Hepatic injury resulted in the leakage of cellular enzyme (alkaline phosphatase) into the blood stream, resulting in the augmented levels of serum enzyme (Kaplan, 1986). Serum levels of this enzyme are excellent indicator of hepatic parenchymal damage and dysfunction.

Oxidative stress is a common pathogenic mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. Cell damage occurs when there is an excess of reactive species derived from oxygen and nitrogen or a defect of antioxidant molecule (Akhtar, 2013). In the present study significant decrease in the activity of liver superoxide dismutase, catalase and vitamin E in iron induced rats were observed which may be due to increased reactive oxygen species generation.

| Parameters      | Group I       | Group II      | Group III     | Group IV      | Group V       |
|-----------------|---------------|---------------|---------------|---------------|---------------|
| Total cholesterol | 115.0 ± 3.5   | 356.2 ± 1.7a  | 190.0 ± 4.5a  | 172.5 ± 2.1b  | 212.5 ± 6.7a  |
| Triglycerides   | 90.0 ± 2.9    | 200.0 ± 2.3a  | 99.7 ± 2.3a   | 120.5 ± 3.7a  | 110.8 ± 4.0a  |
| HDL             | 47.3 ± 4.6    | 28.5 ± 2.1a   | 42.8 ± 2.9a   | 45.3 ± 3.3a   | 37.5 ± 3.8a   |
| LDL             | 49.8 ± 8.1    | 357.7 ± 1.3a  | 127.3 ± 7.0a  | 103.7 ± 1.7a  | 150.8 ± 8.7a  |
| VLDL            | 18.0 ± 0.5    | 40.0 ± 0.4a   | 19.9 ± 0.9a   | 24.1 ± 0.7a   | 22.1 ± 0.1a   |
| TBARS           | 0.4 ± 0.1     | 0.8 ± 0.1a    | 0.5 ± 0.1a    | 0.46 ± 0.2a   | 0.53 ± 0.1a   |
| Superoxide dismutase | 36.0 ± 2.1   | 18.7 ± 5.9a   | 32.5 ± 3.9a   | 35.2 ± 3.7a   | 30.8 ± 5.1a   |
| Catalase        | 56.9 ± 0.6    | 45.4 ± 0.3a   | 65.2 ± 0.3a   | 71.7 ± 0.2a   | 61.2 ± 0.1a   |
| Vitamin E       | 152.7 ± 3.5   | 82.7 ± 2.1a   | 138.7 ± 12.0a | 148.5 ± 6.7a  | 127.2 ± 6.2a  |

Values are expressed in mean ± S.D.; *p*: Significant different from Group I vs. Group II (p<0.001); **p**: Significant different from Group II vs. Group III, IV and V (p<0.001).
The rat liver was dissected out and fixed in 10% formalin and further processed for histopathological investigations. Histopathology of the liver of control group showed normal hepatic cells were as administration of ferrous sulfate in intoxicated group showed lesion with congestion and sign of necrosis. Treatment with silimar showed normal histological appearance with regenerative changes and no sign of necrosis. A. tetracantha treated group showed slight evidence of necrosis and the overall architecture was near normal. T. terrestris treated group showed no infiltration and no necrosis. Thus the result of histopathology of the liver further confirmed the hepatoprotective activity of hydroalcoholic extract of A. tetracantha and T. terrestris.

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References
Ahmad A, Najmi AK, Ahmad S, Pal SN, Balani DK. Evaluation of hepatoprotective potential of Jigrine post-treatment against thioacetamide induced hepatic damage. J Ethnopharmacol. 2002; 9: 35-41.

Akhtar MS, Asjad HMM, Bashir S, Malik A, Khalid R, Gulzar F, Irshad N. Evaluation of antioxidant and hepatoprotective effects of Khamira Gazabani Amtri Jadwar God Sadab Wala (KGA). Bangladesh J Pharmacol. 2013; 8: 44-48.

Balamurugan C, Muthusamy P, Dhonde SM. Observation of the hepatoprotective effect and antioxidant activities of Triaitnema decandra Linn. (Ballai sharunnai) roots on carbon tetrachloride treated rats. Bangladesh J Pharmacol. 2008; 32: 83-89.

Bancroft J. Theory and practice of histological technique. 1977, p 411.

Bennett RN, Mellon FA, Roa Ra EA, Perkins L, Kroon PA. Profiling glucosinolates, flavonoids, alkaloids and other secondary metabolites in tissues of Azima tetracantha Lam. J Agr Food Chem. 2004; 52: 5856-62.

Bhattacharya A, Ramanathan M, Ghosals, Bhattacharya SK. Effect of withania somnifera glycowithanolides on iron-induced hepatotoxicity in rats. Phytother Res. 2000; 14: 568-70.

Boerth J, Strong KM. The clinical utility of milk thistle (Silybum marianum) in cirrhosis of the liver. J Herbal Pharmacotherapy 2002; 2: 11-17.

Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta. 1971; 41: 21-30.

Firdous SM, Sarvanti K, Debnath R, Neeraja K. Protective effect of ethanolic extract and its ethyl acetate and n-butanol fractions of Sechium edule fruits against carbon tetrachloride induced hepatic injury in rats. Int J Pharm Pharm Sci. 2012; 4: 354-59.

Fraga CG, Lebovitz BE, Toppel AL. Lipid peroxidation measured as TBARS in tissue. Free Radiol Med. 1988; 4: 155-61.

Fridwald WT, Levy RT, Fredricleona DS. Estimation of lipoproteins in plasma without use of preparative centrifuge. Clin Chem. 1972; 23: 499.

Hemalatha K, Geetha M, Senthavarnor R. The hepatoprotective activity of lawsonia alba on carbon tetrachloride treated rats. Geobios. 2005; 2: 79-82.

Jasulanth A, Begum VH, Akilandeswari S, Bangu M, Manimaran S, Ruckmani K. Effects of Azima tetracantha on dermal wound healing in rats. Hamdard Medicus. 2001; 44: 13-16.

Kakkar R, Das B, Vishwanathan PN. Modified spectrophotometric method of super oxide dismutase. Indian J Biochim Bio. 1984; 21: 130-32.

Kaplan MM. Serum alkaline phosphatase-another piece is added to the puzzle. Hepatology. 1986; 6: 526-28.

Khan I, Singh V, Chaudhary AK. Hepatoprotective activity of Pinus roxburghii Sarg. wood oil against carbon tetrachloride and ethanol induced hepatotoxicity. Bangladesh J Pharmacol 2012; 7: 94-99.

Kirsch KR, Frith L, Black E, Hoffenberg R. Regulation of albumin synthesis and catabolism by alteration of dietary protein. Nature 1968; 217: 578-79.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem. 1951; 193: 265-75.

Malloy E, Evelyn K. The determination of bilirubin with the photoelectric calorimeter. J Biol Chem. 1937; 119: 481-85.

Pullareddy A, Lokesh BR. Effect of curcumin and aegon on iron-induced hepatic toxicities in rats. Toxicology 1996; 107: 39-45.

Ramanathan A, Kuttunsamy A. Antihypertensive effect of isolated chitin from Rhizopus oryzae against paracetamol-induced hepatotoxicity. Bangladesh J Pharmacol. 2011; 6: 64-67.

Ratnasooriya WD, Jayakody JRA, Premakumara GAS, Ediviweera ERH. Antioxidant activity of water extract of scoparia dulcis. Fitoterapia. 2005; 76: 220-22.

Reilly PM, Schiller HJ, Bulkey GB. Pharmacological approach to tissue injury mediated by free radical and other reactive oxygen metabolites. Am J Surg. 1981; 61: 488-503.

Rice EW. Standard methods in clinical chemistry. Vol VI. New York, Academic Press, 1970; pp. 215-22.

Singh KA. Colorimetric method of catalase. 1972; 47: 389-94.

Tiez NW. Study group on alkaline phosphatase: A reference method of measurement of alkaline phosphatase activity in human serum. Clin Chem. 1983; 29: 751.

Tuncer MA, Yaaymaci B, Sahi L, Cayli S, Acar G, Altug T, Demir R. Influence of Tribulus terrestris Linn. on lipid profile and endothelial structure in developing atherosclerotic lesions in the aorta of rabbits on a high-cholesterol diet. Acta Histochem. 2009; 142-143.

Winckes J, Tietz NW. Clinical guide to laboratory tests. 1971, p. 246.

Zapal BJ. Determination of tocopherol in tissue and plasma. Anal Biochem. 1983; 130: 146-50.

Zlatis, Zak, Boyle JA. New method for the direct determination of serum cholesterol. J Lab Clin Med. 1953; 41: 486.