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RESEARCH ARTICLE

PROTECTIVE ROLE OF *FICUS BENGHALENSIS* BARK EXTRACT AGAINST ETHANOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The hepatoprotective activity of water extract of *Ficus benghalensis* (Family Moraceae) bark was studied against ethanol (3g/kg, 20% w/v *p.o*. once daily for 28 days) induced liver damage in rats. Ethanol produced significant changes in various liver parameters. It increased the biochemical parameters like AST, ALT, ALP, total bilirubin and decreased the levels of albumin and total protein along with changes in histological parameters (damage to hepatocytes). Treatment with water extract of *Ficus benghalensis* bark (at a dose of 400mg/kg, *p.o*. daily for 28 days) significantly prevented the biochemical and histological changes induced by ethanol, indicating the recovery of hepatic cells. The activity of extract was also comparable to that of silymarin, a standard hepatoprotective drug. These results demonstrate that the water extract of *Ficus benghalensis* bark is found to have significant beneficial effect on ethanol induced hepatotoxicity in Wistar rats in reference to liver function tests performed.

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INTRODUCTION

Plants have been used as a source of medicine since time immemorial. India has an ancient heritage of traditional medicine. Charaka Samhita and Sushruta Samhita give extensive description on various medicinal plants. Indian Vedas describe the widespread use of herbal products and aqueous extract of different plant parts for curing different diseases. Many plant extracts are frequently utilized in our traditional system of medicine to treat a wide variety of diseases. *Ficus benghalensis* (Family Moraceae) is a reputed plant in Ayurvedic medicine and is commonly known as "Banyan tree" in Ayurvedic literature. It is a very large tree with spreading branches attaining height of 100 ft and grows almost everywhere in India (The Wealth of India, 1985). Traditionaly it is used for wounds, fever, swollen joints, inflammation of liver and ulcers. Liver diseases contribute markedly to the global burden of mortality and disease. Alcohol remains a major cause of liver disease worldwide. Alcohol consumption has been on the increase in the recent years and has become a menace. Alcohol is toxic to liver cells, and alcoholics develop cirrhosis, a lethal condition in which the liver is so heavily damaged so that it is unable to function.

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Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can cause serious side effects.

Therefore, the current study aimed at exploring the hepatoprotective action of stem bark of *Ficus benghalensis* against alcohol induced toxicity in mice.

MATERIALS AND METHODS

Collection of Plant Material

Stem barks of *Ficus benghalensis* was purchased from Arya Vaidya Sala, Kottakkal, Trissur, Kerala. The collected stem barks were washed thoroughly under running water, cut into smaller pieces and air dried for eight days. The dried stem barks were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

Preparation of water extract of bark of *Ficus benghalensis*

100g stem bark powder of *Ficus benghalensis* was completely extracted by boiling in drinking water (1600 mL), filtered through muslin cloth and the supernatent was collected and used for the present study.

Animals

Male Wistar Albino rats of 8-12 weeks age and weighing between 170-200g were used for the hepatoprotective study. The animals were housed under standard laboratory conditions; room temperature 21.0 to 24.0^{o}C, relative humidity 57-65%, with 12 hours light and 12 hours dark cycle. Single animal was housed in a standard polysulphonate cage (Size: L 300 x B 170 x H 140 mm) and were allowed free access of standard pellet diet (Amrut lab rodent feed, manufactured by Sai Durga Feeds and Foods, Bangalore) and water *ad libitum* throughout the acclimatization and study period. The animals were acclimatized for a minimum period of five days to laboratory conditions and were observed for clinical signs daily.

The testing facilities were provided by the Department of Toxicology, CARe KERALAM Ltd, KINFRA Small Industries Park, Koratty, Trissur, Kerala, India. The animal experiments were performed after prior approval of the study protocol (Approval No**:** CKL/TOX/IAEC/20-2014) by the Institutional Animal Ethics Committee, CARe KERALAM Ltd., KINFRA Park, Koratty, Trissur, Kerala, India. The study was conducted in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision on Animals CPCSEA, New Delhi.

Experimental Protocol for hepatoprotective study

Alcohol induced hepatotoxicity

Animals, after acclimatisation (five days) in the animal house, were randomly divided into five groups of six rats each. Group I served as normal control and received distilled water orally once daily for 28 days. Group II served as negative control ethanol (3g/kg, 20% w/v *p.o*.), once daily for 28 days. Group III was administered with reference drug, silymarin (100 mg/kg *p.o.*) simultaneously with toxicant ((3g/kg, 20% w/v *p.o*.) daily for 28 days. Group IV and V received plant extract (200 mg/kg *p.o* and 400 mg/kg *p.o*. respectively) daily for 28 days simultaneously with toxicant.

Assessment of hepatoprotective activity

On the 29th day of the start of respective treatment blood samples were collected under light ether anaesthesia and allowed to clot for 30 minutes at room temperature. Serum separated was used for the estimation of biochemical parameters like SGPT, SGOT, ALP etc and all the animals were sacrificed by cervical dislocation. The livers were dissected immediately, fixed in 10% (w/v) buffered formalin and used for histological studies. The results of antihepatotoxic activity were presented as the Mean ± SD of six animals in each group. Results were analysed statistically using one way ANOVA followed by Dunnet's test. Values of p<0.001 were considered significant.

RESULTS

Enzymatic liver function tests

Treatment with ethanol 3g/kg resulted in significantly high levels of AST (p<0.001).Similar increase was observed in the case of ALT and ALP (p<0.001). Treatment with ethanol (3g/kg) and Silymarin (100mg/kg) caused significant normalisation of AST, ALT and ALP (p<0.001). Treatment with ethanol and *Ficus benghalensis* stem bark extract 200mg/kg did not cause any significant effect on AST and ALT. However in the case of ALP there was a significant reduction (p<0.01). Treatment with ethanol and *Ficus benghalensis* stem bark extract 400mg/kg caused significant reduction in AST and ALP (p<0.01and p<0.001 respectively). There was no effect on ALT by this treatment.

Table 1. Enzymatic Liver Function Tests

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I (Vehicle control)	118±9.80***	54±12.10***	471±64.84***
Group II (Ethanol 3g/kg)	257±37.20	259±20.30	814 ±50.70
Group III (Ethanol 3g/kg + Silymarin 100mg/kg)	189±8.73***	94±13.30***	352±45.10***
Group IV (Ethanol 3g/kg + Plant extract 200mg/kg)	228±25.80^{ns}	261±25.00^{ns}	677±49.04**
Group V (Ethanol 3g/kg + Plant extract 400mg/kg)	209±14.61**	246±15.63^{ns}	586±83.00***

Values are Mean± SD, *n*=6 animals per group. * *P*<0.05, ** *P*<0.01, *** *P*<0.001 when compared with Group II. One way analysis and Dunnet test.

Nonenzymatic liver function tests

Treatment with ethanol 3g/kg caused significant reduction in serum albumin and serum total protein (p<0.001).However serum bilirubin level was increased significantly by the ethanol (3g/kg) treatment (p<0.001). Treatment with ethanol 3g/kg and Silymarin (100mg/kg) caused significant increase in the levels of albumin and total protein (p<0.001), indicating normalisation of liver function. The same treatment caused significant reduction in bilirubin (p<0.001).

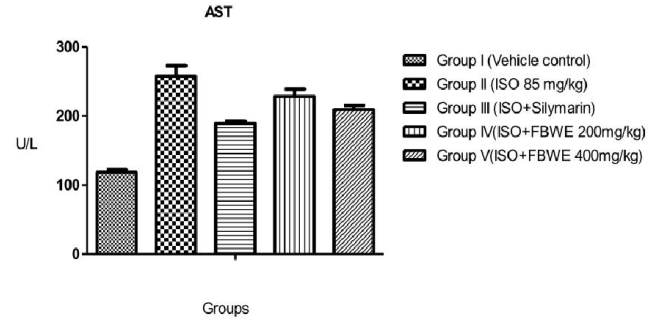
Table 2. Non Enzymatic Liver Function Tests

Groups	Albumin (g/dl)	Bilirubin Total (mg/dl)	Total protein (g/dl)
Group I (Vehicle control)	4.34±0.12***	1.32±0.15***	12.46±0.70***
Group II (Ethanol 3g/kg)	3.00±0.40	6.30±0.82	7.69±1.10
Group III (Ethanol 3g/kg + Silymarin 100mg/kg)	3.70±0.21***	2.40±0.50***	11.00±0.41***
Group IV (Ethanol 3g/kg + Plant extract 200mg/kg)	2.61±0.20*	5.70±0.40^{ns}	8.03±0.53^{ns}
Group V (Ethanol 3g/kg + Plant extract 400mg/kg)	2.90±0.14^{ns}	5.13±0.40**	9.14±0.47**

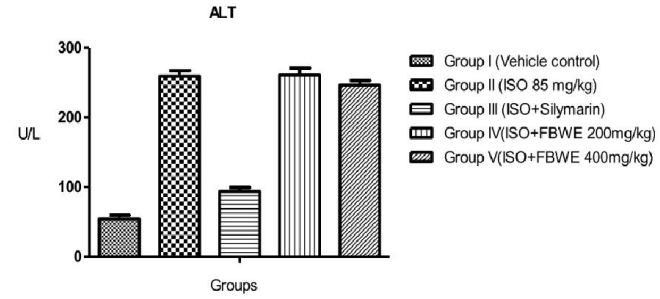
Values are Mean±SD, *n*=6 animals per group. * *P*<0.05, ** *P*<0.01, *** *P*<0.001 when compared with Group II. One way analysis and Dunnet test.

Treatment with ethanol and *Ficus benghalensis* stem bark extract 200mg/kg did not cause any appreciable effect on albumin, bilirubin and total protein levels. Nevertheless the treatment with ethanol 3g/kg and *Ficus benghalensis* stem bark extract 400mg/kg reduced the increased level of bilirubin caused by ethanol treatment ($p<0.001$) and total protein levels increased significantly ($p<0.01$) suggesting improved liver function by *Ficus benghalensis*.

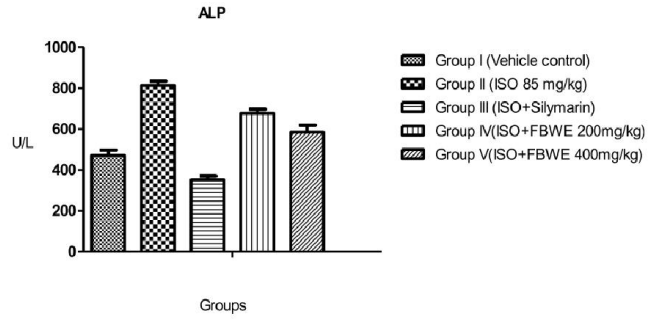
Graph 1 AST



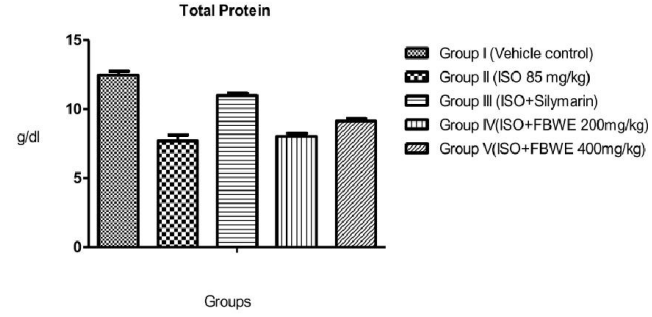
Graph 2: ALT



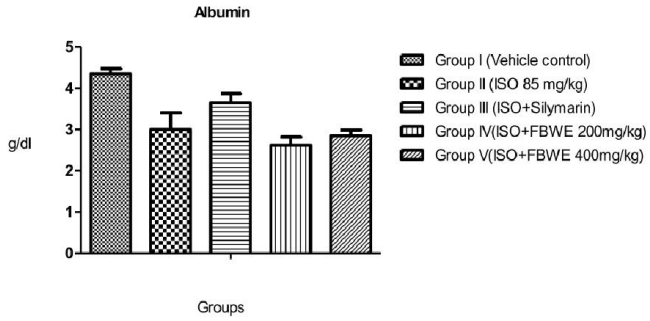
Graph 3: ALP



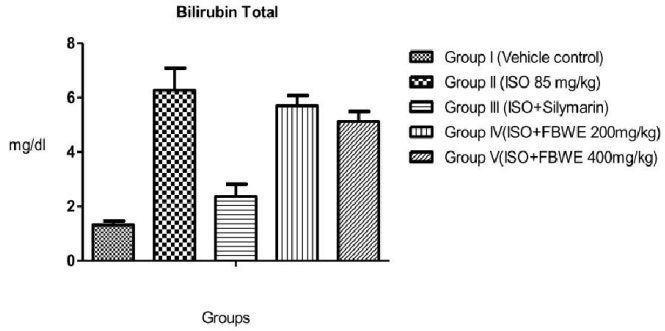
Graph 4: Total Protein

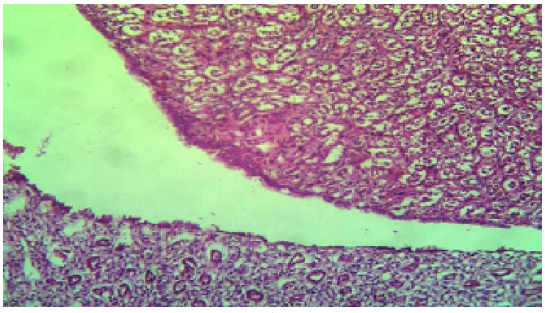


Graph 5 Albumin



Graph 6: Bilirubin Total





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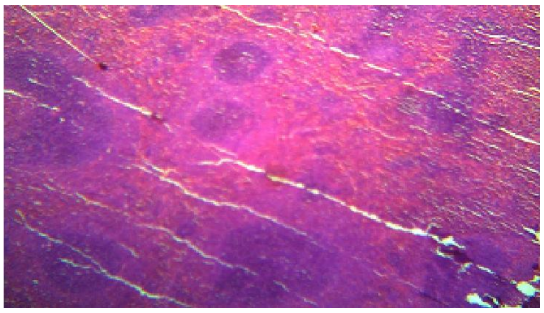

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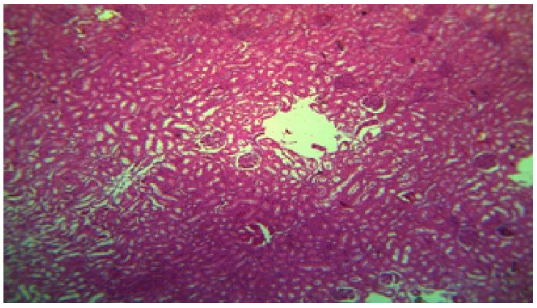

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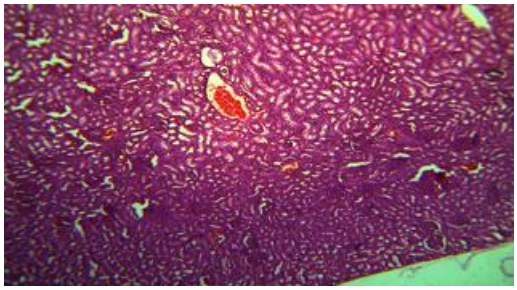
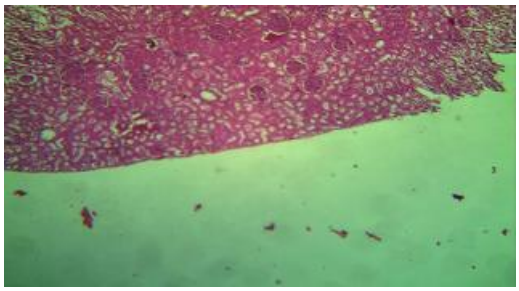


d)



e)

Fig. 1. Histopathology slides of rat liver sections of different treatment groups (a) Control; (b) Ethanol 3g/kg; (c) Ethanol 3g/kg + Silymarin 100mg/kg); (d) Ethanol 3g/kg + Plant extract 200mg/kg); (e) (Ethanol 3g/kg + Plant extract 400mg/kg)

DISCUSSION

The water extract of *Ficus benghalensis* stem bark, administered orally, exhibited significant protection against alcohol induced liver injury as manifested by the reduction in toxin mediated rise in AST, ALP and total bilirubin in rats. The liver can be injured by many chemicals and drugs. In this study ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Ethanol alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations (Parmar *et al*., 2009). Generally measurement of AST, ALT and ALP are commonly used marker enzymes of hepatotoxicity (Sivaraj *et al.,* 2011; Modi *et al*., 2011; Panda *et al*., 2012).

In the present study the significant decrease in the marker enzymes and bilirubin in plant extract administered animals might be due to decreased leakage of the enzymes in the liver cells. This suggest that *Ficus benghalensis* stem bark extract could repair the hepatic injury and/or restore the cellular permeability, thus reducing the toxic effect of ethanol induced liver toxicity and preventing enzymes leakage into the circulation. These results were comparable to earlier reports by other investigators (Sivaraj *et al.,* 2011; Parmar *et al.,* 2009; Suryavanshi, 2011). A comparative histopathological study of the livers from different groups further corroborated the hepatoprotective potential (Panda *et al.,*2012).

The preliminary phytochemical screening indicated the presence of alkaloids, flavanoids, phenolic compounds, tannins, saponins etc in the water extract of *Ficus benghalensis*. Since phytoconstituents like flavonoids are well known for their antioxidant and hepatoprotective activities (Parmar *et al.,* 2009), it may be speculated that these constituents of *Ficus benghalensis* are be responsible for the observed protective effects. Further, investigations are underway to determine the exact phytoconstituents of this medicinally important plant which are responsible for its hepatoprotective activities.

CONCLUSION

The water extract of *Ficus benghalensis* bark has shown the hepatoprotective activity against alcohol induced liver damage in rats. From this preliminary study, it can be concluded that the water extract of *Ficus benghalensis* bark, is proved to be one of the herbal remedies for liver ailment. Further studies are in progress in our laboratory for isolation and characterisation of the phytoconstituents which are present in this medicinally important tree.

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