Vol 4/2 (2004) 109 - 115

JOURNAL OF NATURAL REMEDIES

Hepatoprotective activity of *Boerhaavia diffusa* on ethanol-induced liver damage in rats

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Abstract

Objective: The aim of the present study was to evaluate the effect of ethanolic extract of *Boerhaavia diffusa* root against ethanol-induced hepatic damage. Methods: Ethanol (20% alcohol - 2.5 ml/100 g body wt. for 90 days) was used for the induction of hepatotoxicity. *Boerhaavia diffusa* root extract (150 mg/kg body wt. for 30 days) was administered orally to rats intoxicated with ethanol. Results : Ethanol feeding resulted in liver injury as indicated by the significant increase in the serum activities of marker enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase. Further, there was a significant increase in the levels of cholesterol, triglycerides and free fatty acids followed by a decrease in the levels of phospholipids in serum, liver and kidney. Post treatment of *Boerhaavia diffusa* extract reversed these alterations to near normal. Conclusion : The results of this study confirm the hepatoprotective action of *Boerhaavia diffusa*.

Key words: *Boerhaavia diffusa*, ethanol, hepatoprotection, marker enzymes, lipids.

1. Introduction

Ethanol is currently recognised as the most prevalent known cause of abnormal human development. The liver is one of the important organs and is severely damaged as a result of chronic alcoholic intake [1]. Prolonged intake of alcohol leads to alcoholic hepatitis. Since alcoholism is a serious problem in the developing countries like India, treatment should be implicated against it.

Boerhaavia diffusa Linn. (Nyctaginaceae), a perennial herb commonly known as 'punarnava' has been in use as indeginous Indian medicine from time immemorial. *Boerhaavia diffusa* has been reported to exhibit diuretic, fibrinolytic, and anti-inflammatory activities [2-4]. The roots are used for the treatment of anascara, ascites and jaundice [5] and as an antidote to snake venom. The hepatoprotective activity of the *Boerhaavia diffusa* has also been reported [5,6]. Isolated compounds from the roots of *Boerhaavia diffusa* include punarnavine, β-sitosterol, β-D-glucoside, tetracosonoic, hexacosanoic, stearic, palmitic,

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arachidic acids, hentriacontane, ursolic acid and punarnavoside [7, 8].

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology which has been disturbed by a hepatotoxin. Thus the present study was taken up to determine the curative property of *Boerhaavia diffusa* against ethanol-induced hepatotoxicity.

2. Materials and Methods

2.1 Chemicals

All chemicals used were of analytical grade.

2.2 Plant material

Boerhaavia diffusa was collected freshly in the month of October 1997 in Chennai. The identity of the plant was confirmed by the Department of Botany, Captain Srinivasamurthi Drug Research Institute for Ayurveda, Chennai.

2.2 Preparation of plant extract

The roots were separated and dried in shade. The dried material was coarsely powdered and then extracted with ethanol using soxhlet apparatus. The ethanolic extract was recovered and dried using Hindhivac, Freezer drier lyophilizer, model LF6, SL.No.1008 (0.94% yield w/w in terms of dried starting material).

2.3 Experimental animals

Male Wistar rats of body weight 100-120 g obtained from Frederick Institute for Plant Protection and Toxicology (FIPPAT), Padappai, Chennai were used for this study. They were acclimatized to animal house conditions and were fed 10 gm of low protein diet containing 5 gm of wheat flour and 5 gm of normal diet per day and water *ad libitum.* The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guide lines.

The experimental animals were divided into four groups of six animals each Group 1 served as normal control. Group 2 rats were treated with ethanolic extract of *Boerhaavia diffusa* alone (150 mg/kg body wt. for 30 days orally). Group 3 rats were intoxicated with ethanol (2.5 ml/100 g body wt. of 20% alcohol for 90 days) by intragastic intubation. Group 4 rats were treated with *Boerhaavia diffusa* extract similar to that in Group 2 after the administration of alcohol is stopped on the 90^{th} day.

At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical decapitation. Blood was collected and the serum separated was used for various biochemical assays. The liver and kidney were excised and washed with ice-cold saline. A small portion of each tissue was then homogenized separately in 0.1M Tris HCl buffer and the homogenate was used for biochemical estimations.

2.4 Biological assays

The activities of serum aspartate amino-transferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [9] and the levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined by the methods of King and Armstrong [10] and King [11] respectively. Gamma glutamyl transferase (γ-GT) activity was assayed by Rosalki and Rau [12] method.

The extraction of serum and tissue lipids was done according to the procedure of Folch *et al.* [13]. The estimation of total cholesterol was carried out by the method of Zlatkis *et al.* [14] and triglycerides by the method of Foster and Dunn [15]. Free fatty acids was estimated by the method of Falholt *et al*. [16] and phospholipids by the method of Zilversmit and Davis [17].

Table 1.
Levels of serum AST, ALT, ALP, LDH and γ-GT in normal control and experimental groups of rats.

Parameters	Group 1	Group 2	Group 3	Group 4
AST	82.56 ± 7.35	76.84 ± 7.31NS	270.87 ± 16.76***	87.66 ± 8.36***
ALT	28.59 ± 1.97	28.30 ± 2.56NS	57.05 ± 3.43***	31.67 ± 2.78***
ALP	62.15 ± 5.43	69.26 ± 4.21NS	88.26 ± 7.21***	66.32 ± 5.27***
LDH	2.21 ± 0.18	2.15 ± 0.13	4.57 ± 0.38***	2.78 ± 0.19***
γ-GT	5.14 ± 0.53	4.78 ± 0.75NS	31.45 ± 3.09***	11.34 ± 0.19***

Values are given as mean ± SD for groups of six animals each.
P values: ***<0.001; **<0.01; *<0.05; NS - Non significant.
Student's *t* - test (Comparisons are made between Group 1 and 2; Group 1 and 3; Group 3 and 4).
Units: AST, ALT, LDH: μ mole of pyruvate liberated/hr/lt; ALP: μ mole of p-nitrophenol liberated/hr/lt; γ-GT: IU/dl.

Table 2.
Levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum of normal control and experimental groups of rats.

Parameters	Group 1	Group 2	Group 3	Group 4
Cholesterol (mg/dl)	124.13 ± 11.35	119.32 ± 10.75^{NS}	167.90 ± 14.32***	130.74 ± 12.13***
Triglycerides (mg/dl)	51.85 ± 4.35	48.89 ± 3.78^{NS}	146.85 ± 13.87***	60.83 ± 13.87***
Free Fatty acids (mg/dl)	14.83 ± 1.28^{NS}	13.86 ± 1.25^{NS}	25.32 ± 2.36***	16.87 ± 1.48***
Phospholipids (mg/dl)	101.36 ± 9.45	99.36 ± 8.35^{NS}	127.38 ± 9.98***	101.34 ± 9.89**

Values are given as mean ± SD for groups of six animals each.
P values : ***<0.001; **<0.01; *<0.05; NS - Non significant.
Student's *t* - test (Comparisons are made between Group 1 and 2; Group 1 and 3; Group 3 and 4).

2.5 Statistical analysis

The values were expressed as mean ± SD. Statistical difference was analysed by Student's *t* - test and P values were determined.

3. Results

Ethanol fed rats alone developed significant hepatocellular damage as evident from a significant elevation in the serum activities of AST, ALT, ALP, LDH and γ-GT when compared with control (Table 1).

Table 2, Table 3 and Table 4 shows the levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum, liver and kidney respectively. There was a significant increase in the levels of cholesterol, triglycerides and free fatty acids in both serum and tissues of rats treated with ethanol when compared with control rats whereas the levels of phospholipids in both serum and tissues were decreased.

Post treatment of rats with *Boerhaavia diffusa* extract exhibited a significant reduction in the levels of enzymes and lipids leading to the reversal of hepatotoxicity significantly.

In the rats given *Boerhaavia diffusa* extract alone there was no significant change in the activities of the enzymes and lipids as compared to the control rats thereby showing the absence of any adverse toxic effects of *Boerhaavia diffusa* extract.

4. Discussion

Chronic alcohol intake is known to produce hepatocellular damage. The alcoholic liver injury appears to be generated by the effects of ethanol metabolism and the toxic effects of the immune response to alcohol or acetaldehyde altered proteins [18]. Ethanol is primarily metabolised by alcohol dehydrogenase with the formation of acetaldehyde. Several other pathways exist: cytochrome P_{450}-dependent microsomal ethanol-oxidising system, catalase and non-enzymatic ethanol oxidation [19] and the involvement of free radical species [20]. Ethanol-induced hepatic hypoxia also has been invoked as a possible cause of the potentiation of hepatotoxicity [21].

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membranes [22]. In this context, we have also observed a significant increase in the serum activities of AST, ALT, ALP, LDH and γ-GT which is in accordance with the earlier findings [23-25].

The transaminases ALT and AST are well known diagnostic indicators of liver diseases. In case of liver damage with hepatocellular lesions and parenchymal cell necrosis, non-functional enzymes such as ALT and AST are released from the damaged tissues into the blood stream [26]. Cholestasis may also contribute to the increased levels of ALT and AST [27]. ALP

Table 3.
Levels of cholesterol, triglycerides, free fatty acids and phospholipids in liver of normal control and experimental groups of rats.

Parameters	Group 1	Group 2	Group 3	Group 4
Cholesterol (mg/gm)	6.37 ± 0.73	5.96 ± 0.63^{NS}	11.36 ± 1.93***	8.12 ± 0.97**
Triglycerides (mg/gm)	17.83 ± 1.58	17.43 ± 1.67^{NS}	30.62 ± 2.85***	20.48 ± 1.92***
Free Fatty acids (mg/gm)	7.12 ± 0.68	7.02 ± 0.63	16.18 ± 1.43***	11.72 ± 1.08***
Phospholipids (mg/gm)	33.20 ± 2.62	31.32 ± 2.82^{NS}	26.43 ± 1.38***	30.36 ± 2.53**

Values are given as mean ± SD for groups of six animals each.
P values : ***<0.001; **<0.01; *<0.05; NS - Non significant.
Student's *t* - test (Comparisons are made between Group 1 and 2; Group 1 and 3; Group 3 and 4).

Table 4.
Levels of cholesterol, triglycerides, free fatty acids and phospholipids in kidney of normal control and experimental groups of rats

Parameters	Group 1	Group 2	Group 3	Group 4
Cholesterol (mg/gm)	10.81 ± 0.91	10.45 ± 1.71^{NS}	14.93 ± 1.23***	11.53 ± 1.15***
Triglycerides (mg/gm)	7.32 ± 0.83	6.53 ± 0.73^{NS}	11.65 ± 1.93***	8.53 ± 0.97**
Free Fatty acids (mg/gm)	4.36 ± 0.39	4.11 ± 0.40^{NS}	6.35 ± 0.61***	5.01 ± 0.61**
Phospholipids (mg/gm)	14.77 ± 1.03	14.57 ± 1.53^{NS}	18.31 ± 1.06***	16.23 ± 1.53**

Values are given as mean ± SD for groups of six animals each.
Values : ***<0.001; **<0.01; *<0.05; NS - Non significant.
Student's *t* - test (Comparisons are made between Group 1 and 2; Group 1 and 3; Group 3 and 4).

activity is related to functioning of the hepatic cell. Increase in serum levels of ALP is due to increased synthesis in presence of increasing biliary pressure [28].

The LDH activity was found to be directly proportional to the concurrent reduction of NAD^+ which is used for the oxidation of alcohol to acetaldehyde [29]. NADH + H^+ generated may be utilised by pyruvate which results in the formation of excess lactate and thereby increases the LDH activity.

The peroxidation of membrane lipids results in loss of membrane structure and integrity resulting in elevated levels of γ-glutamyl transpeptidase, a membrane bound enzyme in the serum [25]. In alcoholic fatty liver, an increase in γ-GT may present the only clinico-chemical syndrome. The assay of γ-GT is thus a helpful adjunct in detecting toxic liver damage [30] and used as an index of ethanol-induced hepatic damage.

Post treatment with *Boerhaavia diffusa* extract afforded a significant protection against ethanol-induced liver damage by ameliorating the increase in serum marker enzyme levels evidencing its hepatoprotective activity. The possible mechanism may be that *Boerhaavia diffusa* being able to induce accelerated regeneration of liver cells by reducing the leakage of marker enzymes into the blood and thereby lowering their values in the serum.

Marked alterations in lipid metabolism have been reported in chronic ethanolic feeding [31, 32]. Scheig and Isselbacher [33] also demonstrated that direct addition of ethanol to rat liver slices enhances *in vitro* lipogenesis.

An enhanced cholesterol synthesis is theoretically possible, since chronic ethanol consumption is associated with proliferation of the smooth endoplasmic reticulum [34, 35] which is the site of major steps in cholesterol synthesis [36 - 38]. The proliferation of the smooth endoplasmic reticulum induced by ethanol may be linked to the fact that the hepatic microsomes which comprise the smooth endoplasmic reticulum contain a microsomal ethanol-oxidising system that increases in activity upon ethanol feeding [39].

The increase of lipids by alcohol was due mainly to the increase of triglyceride because other lipids were significantly changed except for cholesteryl ester which made only a small contribution to the weight of total lipid. However as fatty infiltration is developed in the liver, cholesteryl ester increases remarkably accompanied with an increase of hepatic triglyceride [40, 41]. Antonenkov *et al.* [42] have reported that chronic ethanol results in moderate hypercholesterolemia and triglyceridemia.

The increased free fatty acid accumulation may be directly due to lipid breakdown and indirectly due to the oxidation of ethanol by the liver to acetate and its conversion to fatty acids which is a means of removal of excess hydrogen generated by ethanol [23].

In free radical mediated tissue injury, lipid peroxidation leads to degradation of phospholipids and alteration in the membrane fluidity, which is essential for liver cell function [43]. This may account for the decreased levels of phospholipids in ethanol intoxicated rats. Jaya *et al.* [44] have also reported a decrease in the phospholipid content of liver of alcohol fed rats.

These alterations were very much reduced in rats treated with *Boerhaavia diffusa* extract after alcohol intoxication, thus establishing its broad spectrum of hepatoprotective potential.

The results indicate that post treatment with *Boerhaavia diffusa* extract protects the liver against ethanol-induced hepatotoxicity. Thus from this study it is clear that *Boerhaavia diffusa* possesses hepatoprotective property.

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