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Effect of *Boerhaavia diffusa* on tissue anti-oxidant defense system during ethanol-induced hepatotoxicity in rats

T. Devaki[1]*, K. S. Shivashangari[1], V. Ravikumar[1], P. Govindaraju[2]

1. Department of Biochemistry, University of Madras, Guindy Campus, Chennai - 600 025, India.
2. Department of Biochemistry, Agricultural College and Research Institute, Madurai - 625 106, India.

Abstract

Objective: The present study deals with the effect of ethanolic extract of *Boerhaavia diffusa* (BD) on tissue defense system against ethanol-induced hepatic injury in rats. Methods: Ethanol (20% alcohol - 2.5 ml/100 g body weight for 90 days) was used for the induction of hepatotoxicity. BD extract (150 mg/kg body weight/day for 30 days) was administered orally to rats intoxicated with ethanol. Results: Induction with ethanol resulted in an increase in the levels of lipid peroxides with a concomitant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione levels. Post treatment with BD root extract reversed the deleterious effects of ethanol and the enzyme activities were found to be near normal. Conclusion: These findings demonstrate the protective nature of BD root extract against ethanol-induced liver damage, proving it to be a potent antioxidant.

Key words: *Boerhaavia diffusa*, ethanol, oxidative stress, lipid peroxidation, antioxidants.

1. Introduction

The role of free radical oxidative damage in the pathophysiology of human diseases is currently a topic of considerable interest, as free radical activity has been implicated in a wide spectrum of clinical conditions [1]. Oxygen stress which enhances lipid peroxidation is well recognised to be a key step in the pathogenesis of ethanol-associated liver injury [2]. The reactive species mediated hepatotoxicity can be effectively managed upon administration of agents possessing antioxidant, free radical scavenger and anti-lipid peroxidant activities [3]. Thus antioxidants are gaining immense importance by virtue of their role in disease prevention.

Boerhaavia diffusa Linn. (Nyctaginaceae), a perennial herb commonly known as 'punarnava' has been in use as indeginous Indian medicine from time immemorial. BD has been reported to exhibit diuretic,

*Corresponding author
E-mail: devakit@yahoo.co.uk

fibrinolytic, and anti-inflammatory activities [4-6]. The roots are used for the treatment of anascara, ascites and jaundice [7].

We have recently reported the hepatoprotective action of BD against ethanol - induced liver damage [8]. Isolated compounds from the roots of BD includes punarnavine, β-sitosterol, β-D-glucoside, tetracosonoic, hexacosanoic, stearic, palmitic, arachidic, urosolic acids, hentriacontane and punarnavoside [9,10].

The present study was designed with an aim to assess the antioxidant activity of BD root extract against ethanol-induced liver damage.

2. Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade.

2.2 Plant material

BD was collected freshly in Chennai. The identity of the plant was confirmed by the Department of Botany, Captain Srinivasamurthi Drug Research Institute for Ayurveda, Chennai.

2.3 Preparation of plant extract

The roots were separated and dried in shade. The coarsely powdered dried material was 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).

2.4 Experimental animals

Male Wistar rats of body weight 100-120 g obtained from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai were used for this study. They were acclimatized to animal house conditions and were fed 10 g of low protein diet containing 5 g of wheat flour and 5 g of normal diet per day and water *ad libitum*. They were kept in a constant temperature (25 ± 2°C) and humidity (55 ± 10%) room with 12 h light period. 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 BD root extract alone (150 mg/kg body weight/day for 30 days orally). Group 3 rats were intoxicated with ethanol (2.5 ml/100 g body weight of 20% alcohol for 90 days) [11] by intragastic intubation. Group 4 rats were treated with BD root extract similar to that in group 2 after the administration of alcohol is stopped on the 90th day.

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

2.5 Biological assays

Lipid peroxidation (LPO) of tissue fractions were measured by the method of Okhaw *et al.* [12] in which malondialdehyde (MDA) released served as the index of LPO. Superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich [13] based on the oxidation of epinephrine - adrenochrome transition by the enzyme. The assay of catalase (CAT) was done as described by Beer and Sizer [14].

The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al.* [15] based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithio bis-(2-nitro benzoic acid) to form a complex that absorbs maximally at 412nm. Glutathione

S-transferase (GST) was assayed by the method of Habig *et al.* [16]. The changes in absorbance was recorded at 340nm and enzyme activity was calculated as nmol of 1-chloro-2,4-dinitro benzene (CDNB) conjugate formed/min/mg/ protein using a molar extinction coefficient of 9.6×10^3 min/cm.

Reduced glutathione (GSH) was estimated by the method of Moron *et al.* [17]. Virtually all the non-protein sulfhydryl content of cells is in the form of reduced glutathione. 5, 5'- dithio (2-nitrobenzoic acid) (DTNB) is a disulphide compound that is readily reduced by sulfydryl compounds forming a highly colored yellow anion. The optical density of this yellow substance was measured at 412nm. Protein was estimated by the method of Lowry *et al.* [18] using bovine serum albumin as the standard.

2.6 Statistical analysis

All the grouped data were statistically evaluated using Student's *t* - test, expressed as the mean ± S.D from 6 rats in each group. *p* value of 0.05 or less was considered to be significant.

3. Results

The levels of LPO in liver and kidney are represented in Table 1. In ethanol intoxicated group, there was a significant elevation in LPO which implicates the severity of oxidative stress. When the rats were treated with BD root extract, the levels were reduced to near normal.

Table 2 shows the activities of SOD, CAT, GPx, GST and levels of GSH in liver tissue of both normal and experimental group of rats. Activities of kidney antioxidant enzymes and GSH levels are depicted in Table 3. There was a significant decline in the activities of these enzymes in ethanol-induced rats which upon treatment with BD root extract were brought back towards their respective normal values.

4. Discussion

Hepatotoxicity of ethanol results from alcohol dehydrogenase mediated hepatic generation of reduced nicotinamide adenine dinucleotide and acetaldehyde [19]. Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the development of alcoholic liver disease and induction of cytochrome P450 2E1 (CYP2E1) by ethanol appears to be one of the mechanisms [20] through which ethanol is believed to generate such oxidative stress [21].

Lipid peroxidation is an oxygen free radical mediated process which has been implicated in alcohol-induced liver injury [22]. Prolonged consumption of ethanol enhances the activities

Table 1.
Levels of lipid peroxides (LPO) in liver and kidney of normal control and treated groups of rats (n=6 in each group)

Parameters	Group 1	Group 2	Group 3	Group 4
LPO in liver	90.91 ± 6.0	90.78 ± 6.8^{NS}	136.84 ± 10.4***	113.15 ± 8.2***
LPO in kidney	112.83 ± 6.83	111.96 ± 5.37^{NS}	164.04 ± 10.63***	126.39 ± 9.34***

Unit - nmol of malondialdehyde/100 mg.
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 2.
Activities of SOD, CAT, GPx, GST and GSH levels in liver of normal control and treated groups of rats (n=6 in each group)

Parameters	Group 1	Group 2	Group 3	Group 4
SOD	6.35 ± 0.58	6.51 ± 0.52^{NS}	4.21 ± 0.36***	6.07 ± 0.54***
CAT	40.53 ± 2.26	40.93 ± 2.94^{NS}	22.93 ± 1.75***	38.57 ± 1.83***
GPx	10.77 ± 0.61	10.81 ± 0.50^{NS}	6.21 ± 0.47**	8.83 ± 0.63***
GST	20.41± 0.20	20.43 ± 0.63^{NS}	11.17 ± 0.39***	18.29 ± 0.57***
GSH	0.51 ± 0.04	0.52 ± 0.03^{NS}	0.29 ± 0.02***	0.46 ± 0.03***

Units: SOD - units/min/mg protein; CAT - nmol of H_2O_2 decomposed/min/mg protein; GPx - nmol of GSH oxidized/min/mg protein; GST - nmol of CDNB conjugated/min/mg protein; GSH - nmol/g tissue.
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).

of the hepatic microsomal ethanol oxidizing system which results in the formation of peroxides and reduced NADP.

The associated increase in the activity of NADPH oxidase, increased production of hydrogen peroxide from NADPH and oxygen may favour lipid peroxidation in the endoplasmic reticulum, a key metabolic site [23]. The levels of lipid peroxides have been found to increase after chronic ingestion of ethanol.

These findings corroborate the evidence that free radicals and lipid peroxides are involved in various forms of pathology such as fatty infiltration of liver [24] which is a prominent characteristic of alcoholism. Post treatment with BD root extract reduced the levels of lipid peroxides and maintained to near normal.

Free radical scavenging enzymes such as SOD, CAT, GPx, GR and GST are the first line of defense against oxidative injury decomposing O_2 and H_2O_2 before interacting to form the more reactive hydroxyl radical (OH^{-1}). The equilibrium between these enzymes is an important process for the effective removal of oxidative stress in intracellular organelles [25].

A number of previous reports suggested that ethanol or its metabolites could alter the balance in the liver towards auto oxidation, by either acting as pro-oxidants or by reducing the antioxidant level or by a combination of both [26,27]. Lowered activities of antioxidants in alcohol intoxicated rats were observed as reported by other researchers [28, 29].

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of O_2^-[30]. CAT is found in peroxisomes in most tissues and probably serves to remove peroxide which is generated by peroxisomal oxidase enzymes [31]. The production of superoxide radicals in the process to counteract the ethanol treated inflammation modulates SOD and CAT resulting in the accumulation of superoxide anion, thus damaging the hepatic cells. The decreased activities of these enzymes in alcohol intoxicated rats suggest increased peroxidation and necrotic changes [32]. Administration of BD root extract to the alcohol administered rats stimulates the activities of SOD and CAT bringing back to near normal.

Table 3.
Activities of SOD, CAT, GPx, GST and GSH levels in kidney of normal control and treated groups of rats (n=6 in each group)

Parameters	Group 1	Group 2	Group 3	Group 4
SOD	6.23 ± 0.50	6.39 ± 0.57^{NS}	4.47 ± 0.39***	6.02 ± 0.48***
CAT	37.36 ± 1.51	37.47 ± 2.21	17.36 ± 1.53***	25.72 ± 1.94***
GPx	38.78 ± 1.37	39.03 ± 0.97^{NS}	30.03 ± 2.95***	35.75 ± 2.91**
GST	9.76 ± 0.18	9.78 ± 0.29	5.93 ± 0.32***	8.85 ± 0.31***
GSH	0.29 ± 0.02	0.31 ± 0.02	0.20 ± 0.02***	0.25 ± 0.01***

Units : SOD - units/min/mg protein; CAT - nmol of H_2O_2 decomposed/min/mg protein; GPx - nmol of GSH oxidized/min/mg protein; GST - nmol of CDNB conjugated/min/mg protein; GSH - nmol/g tissue.
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).

GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. GST plays an important role in liver by eliminating toxic compounds by conjugating them with glutathione [30]. The activities of GPx and GST were lowered on alcohol treatment. Reduction in the activities of these enzymes by ethanol intoxication indicates that glutathione was consumed during the reaction with oxygen and peroxide radicals. When ethanol intoxicated rats were treated with BD root extract, these enzymes were restored to an almost normal activity.

GSH is an important biological molecule involved in many processes particularly in cellular protection against oxidative stress. It could be represented as a marker for the evaluation of oxidative stress [33]. GSH levels were significantly declined in ethanol fed rats. Decreased intracellular GSH content associated with acute ethanol liver injury has also been reported [20]. The possible mechanism for decreased levels of GSH in ethanol treated rats may be due to increased concentration of NADPH. So the activity of NADPH dependent GSH would be increased and the concentration of GSH is reduced in ethanol fed rats. When the rats were treated with BD root extract there was an enhancement in the levels of GSH probably by removing the NADPH available.

The present study shows that BD root extract could offer protection to the experimental animals from the deleterious effects of ethanol-induced hepatotoxicity. Thus the protective action of BD root extract may be attributed to its ability to act as antioxidant and singlet oxygen quencher, thereby inhibiting the destructive effects of reactive oxygen species.

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