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Hepatoprotective activity of *Bacopa monnieri* L. against ethanol - induced hepatotoxicity in rats

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ABSTRACT - This study was designed to test the hypothesis that ethanolic extract of *Bacopa monnieri* aerial parts (EBM) protects against ethanol -induced liver injury in rats. Crude ethanolic extract of *B. monnieri* was investigated for hepatoprotective activity in albino rats at 300 mg/kg, orally and compared with standard drug Silymarin (25 mg/kg, PO). Results show that EBM was effective in blunting ethanol-induced enhanced activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), level of serum bilirubin (both total and direct), serum total cholesterol, liver weight loss and was also effective in reducing ethanol–induced lipid peroxidation both *in vitro* and *in vivo*. EBM on the other hand was found to enhance level of HDL cholesterol in ethanol-induced hepatotoxic rats. Furthermore, EBM could also blunt ethanol-induced suppressed activities of superoxide dismutase (SOD), catalase (CAT) and decreased level of reduced glutathione (GSH). Results of hepatocellular damage caused by ethanol and its recovery by EBM, suggest that it might be considered as a potential source of natural hepatoprotective agent, which could be related to the free radical scavenging properties of saponins present in high concentration in the extract.

KEY WORDS: *Bacopa monnieri* L.; Hepatoprotective activity; Antioxidants; Saponins.

INTRODUCTION

Alcohol dependency is a major health and socio-economic problem throughout the world (1, 2). It has been observed that almost all ingested alcohol is metabolized in the liver and excessive alcohol use can lead to acute and chronic liver disease (3). It has further been observed that most of the consumed alcohol is eventually broken down by the liver and the products generated and accumulated during alcohol metabolism (e.g. acetaldehyde) are more toxic than alcohol itself (3). In addition, a group of metabolic products called free radicals can damage liver cells and promote inflammation, impairing vital functions such as energy production. The body's natural defenses against free radicals (e.g. antioxidants) are inhibited by alcohol consumption, leading to increased liver damage (3). Despite great progress made in the field in the past two decades, development of suitable medications for the treatment of alcohol dependency or alcohol-induced health injury remains a challenging goal for alcohol research.

Bacopa monnieri Linn. (Scrophulariaceae) is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m (4). The plant is reported to contain tetracyclic triterpenoid saponins, bacopasides A, B, C, hersaponin, alkaloids viz. herpestine and brahmine and flavonoids (4-6). Saponins are natural products, which have been shown to possess antioxidant property (7-9). Studies have confirmed that indeed oxidative stress plays an important role in the initiation and progression of alcohol liver disease (ALD) (10-12). As *B. monnieri* contains large amounts of saponins it was thought worthwhile to investigate the hepatoprotective activity of the aerial parts of *Bacopa monnieri* Linn. in a scientific manner.

MATERIALS AND METHODS

Plant material

The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Shibpur, Howrah. After authentication, fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Salipur, Orissa, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder. The powdered plant material (400 g) was defatted with petroleum ether (60-80°C) and then extracted with 1.5 litre of ethanol (95%) in a soxhlet apparatus (13). The solvent was removed under reduced pressure, which obtained a greenish-black sticky residue (yield: 11.6% w/w with

respect to dried plant material). The dried extract was stored in a desiccator till further study.

Animals

Male Wistar rats weighing 120 ± ¤5 g were used in the experiment. They were maintained in a 12 h light/dark cycle at 25 ± ¤2° C. They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Diet and treatment

Animals were divided into four groups: Group I (control), Group II (ethanol treated), Group III (ethanol + EBM), Group IV (ethanol + silymarin). Animals of groups II, III and IV were fed with 15% (v/v) ethanol (14) at a single dose per day for 30 days by oral route. Simultaneously, but at different hours of the day, animals of groups III and group IV were fed with 300 mg/kg EBM orally and 25 mg/kg silymarin orally for 30 days at a single dose of 1 ml/100 g body wt/day respectively. Animals of group I was administered orally deionized water, 1 ml/100 g body wt/day, as vehicle. Daily records of body weight of all groups of animals were maintained during the whole experimental period.

Acute toxicity study

The test was carried out as suggested by Seth *et al*., 1972 (15). Swiss albino mice of either sex weighing between 25 – 30 g were divided into nine groups of six animals in each. The control group received normal saline (2 ml/kg, PO). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 mg/kg of the test extract, respectively. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any. The observations were made twice daily, one at 7 a.m. and another at 7 p.m.

Serum analysis

After the treatment period, the animals of all groups were anaesthetized and sacrificed. Blood was drawn from heart and serum was separated for the assay of serum glutamate oxaloacetate transaminase (SGOT) (16), serum glutamate pyruvate transaminase (SGPT) (16), alkaline phosphatase (ALP) (17), bilirubin (direct and total) (18) and cholesterol (total and HDL) (19) using analytical kits from Span Diagnostics Ltd., Surat, India.

In vitro antioxidant activity

The inhibitory effect of EBM on ethanol-induced lipid peroxidation in mice liver homogenate was determined using TBA- MDA adduct according to the modified method of Yuda *et al*., 1999 (20). A mixture containing 0.5 ml of normal liver homogenate, 0.1 ml of Tris- HCl buffer (pH 7.2), 0.1 ml of ethanol and 0.05 ml of various concentrations of EBM (0.01, 0.1 and 1.0 mg/ml) were incubated for 1hr at 37°C. After incubation 9 ml of distilled water and 2 ml of 0.6% Thiobarbituric acid (TBA) were added to 0.5 ml of incubated solution and shaken vigorously. The mixture was heated for 30 min in a boiling water bath. After cooling, 5 ml of n- butanol was added and the mixture was again shaken vigorously. The n- butanol layer was separated by centrifugation at 1000 g for 30 min and the absorbance was measured at 532 nm (Wong *et. al*., 1987) (21).

In vivo antioxidant activity

After the treatment period following study, the animals were deprived of food overnight and sacrificed by cervical dislocation. The livers were dissected out, washed in ice-cold saline, patted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation (LPO) by the method of Fraga et al., 1988 (22). A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of reduced glutathione (GSH) by the method of Ellman et al., 1959 (23). The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) by the method described by Kakkar *et al*., 1984 (24) and catalase (CAT) activity was measured by the method of Maehly *et al*., 1954 (25). Protein estimation was done as per the method of Lowry *et. al*., 1951 (26).

Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t-test ($P < 0.05$) to compare group means.

RESULTS

Serum analysis

In acute toxicity study, it was found that the extract induced sedation and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation. Rats subjected to ethanol only, developed significant (P<0.05) hepatocellular damage as evident from significant increase in serum activities of GOT, GPT, ALP and bilirubin concentration as compared to

Table 1: Effect of EBM (300 mg/kg p.o.) on SGOT, SGPT, ALP, bilirubin (total and direct), cholesterol (total and HDL) and liver weight in ethanol-induced hepatotoxic rats

Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	ALP (KA units)	Bilirubin (mg/dl)		Cholesterol (mg/dl)		Liver weight (g)
					Total	Direct	Total	HDL	
I	Control	46.33 ± 0.95	55.33 ± 0.67	78.00 ± 1.79	0.57 ± 0.02	0.08 ± 0.01	116.42 ± 1.90	9.98 ± 0.61	10.90 ± 0.45
II	Ethanol treated	135.17 ± 3.11*	117.33 ± 3.98*	159.33 ± 4.53*	6.05 ± 0.33*	0.73 ± 0.04*	184.78 ± 3.63*	1.51 ± 0.06*	8.53 ± 0.25*
III	Ethanol + Extract	62.33 ± 3.62**	64.50 ± 2.88**	112.83 ± 4.49**	0.95 ± 0.05**	0.21 ± 0.02**	136.77 ± 3.59**	11.17 ± 1.48**	10.75 ± 0.30**
IV	Ethanol + Silymarin	53.50 ± 2.95**	62.00 ± 2.92**	91.33 ± 5.06**	0.96 ± 0.06**	0.34 ± 0.04**	143.7 ± 4.28**	20.18 ± 1.41**	10.50 ± 0.38**

*All values are Mean ±SEM, n=6 rats in each group *P < 0.05 as compared with Group I **P < 0.05 as compared with Group II.*

Table 2: Effect of EBM on ethanol-induced in vitro lipid peroxidation

Sample	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
Ethanolic extract	50	22.66 ± 1.20	216.59
	100	32.45 ± 2.52	
	200	47.86 ± 2.37	
	300	57.59 ± 1.85	
	400	62.22 ± 2.41	
α-tocopherol			190.22

n=3, Values are Mean ± S.EM.

Table 3: Effect of EBM on LPO, antioxidant enzymes and GSH in liver of ethanol-induced hepatotoxic rats in vivo

Group	Treatment	LPO[a]	SOD[b]	CAT[c]	GSH[d]
I	Control	1.69 ± 0.27	12.59 ± 0.42	64.40 ± 3.25	61.59 ± 2.11
II	Ethanol treated	7.32 ± 0.23*	3.66 ± 0.17*	39.80 ± 2.13*	34.84 ± 1.44*
III	Ethanol + Extract	3.79 ± 0.33**	8.74 ± 0.26**	54.52 ± 3.71**	58.00 ± 1.56**
IV	Ethanol + Silymarin	3.23 ± 0.15**	9.49 ± 0.40**	56.64 ± 2.80**	54.68 ± 1.88**

*All values are Mean ± SEM, n=6 rats in each group *P < 0.05 as compared with Group I **P < 0.05 as compared with Group II. a= nmole of MDA/mg of protein. b= Units/mg of protein c= μmole of H_2O_2 consumed/min/mg of protein. d= μg/mg of protein.*

normal control group, which has been used as reliable markers of hepatotoxicity (Table 1). Oral administration of EBM (300 mg/kg) exhibited significant reduction (P<0.05) in ethanol-induced increase in levels of GOT, GPT, ALP and bilirubin concentration. Treatment with silymarin also reversed the hepatotoxicity significantly (P<0.05). Table 1 also revealed that total cholesterol level of serum of rats treated only with ethanol increased significantly (p<0.05) while HDL level decreased significantly (p<0.05) with respect to control group. But, EBM was successful in blunting this ethanol-induced increase in serum cholesterol level and decrease in HDL level, which is comparable with the reference drug silymarin.

Liver weight

Liver weight of rats treated with ethanol only decreased significantly (p<0.05), which is prevented by EBM and silymarin (Table 1).

In vitro lipid peroxidation

Ethanol-induced *in vitro* lipid peroxidation study revealed that EBM has significant anti lipid peroxidation potential with IC_{50} value being 216.59 μg/ml, which is comparable with the reference drug α-tocopherol (Table 2).

In vivo antioxidant activity

In vivo lipid peroxidation study revealed that ethanol treated group showed significant increase (p<0.05) in malondialdehyde (MDA) level when compared with normal control group. EBM and silymarin were able to significantly prevent (p<0.05) this rise in MDA level (Table 3).

There was a marked decrease in the level of GSH and the activities of SOD and CAT in ethanol treated group when compared with normal control group. The GSH level and activities of SOD and CAT were significantly increased (p<0.05) in EBM and silymarin treated groups (Table 3).

DISCUSSION

It has been found that EBM effectively could prevent ethanol-induced biochemical changes of liver toxicity. The hepatoprotective activity of the ethanolic extract was monitored by estimating serum transaminases, serum alkaline phosphatase and bilirubin, which give a good idea about the functional state of the liver (27). The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminases and ALP was the clear indications of cellular leakage and loss of functional integrity of cell membrane (28).

There was a significant decrease in mean liver weight of the animals in ethanol-induced group, which could be blunted significantly by EBM. This decrease in liver weight by ethanol-induced hepatotoxic rats and its recovery by EBM, suggests that EBM possibly has a positive anabolic effect.

Formation of ROS, oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic ethanol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors (11). Additionally, alcohol-induced liver injury has been associated with increased amount of lipid peroxidation (29). Indeed, EBM supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that EBM possibly has antioxidant property to reduce ethanol-induced membrane lipid peroxidation and thereby to preserve membrane structure. It may thus be plausible that in our study, loss of membrane structure and integrity because of lipid peroxidation was accompanied with an elevated level of activities of SGOT, SGPT, ALP and bilirubin.

Our study further revealed that chronic exposure to ethanol decreased the activities of the ROS scavenging enzymes, viz. SOD and CAT. This is in line with assumption suggested earlier by Sandhir and Gill (1), that decrease in the activity of antioxidant enzymes SOD, CAT and GSH following ethanol exposure may be due to the damaging effects of free radicals, or alternatively could be due to a direct effect of acetaldehyde, formed from oxidation of ethanol, on these enzymes. In our studies, it reveals that EBM could restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals and hepatocellular damage.

GSH is a naturally occurring antioxidant important in the antioxidant defence of the body. It has been reported that determination of GSH, can serve as a key to know the amount of antioxidant reserve in the blood and probably in the organism and also, contribute in evaluating the possibilities available for the recuperation of alcoholic patients (30, 31). Therefore, the levels of glutathione are of critical importance in liver injury caused by toxic substances such as ethanol. It has been claimed that binding of acetaldehyde, a metabolite of ethanol, with GSH may contribute to reduction in the levels of GSH (1). Our results are in line with this earlier report because we found that after EBM -supplementation, elevated GSH level in rats with ethanol could be blunted to normal level. This ability of EBM to protect the liver from ethanol-induced damage might be attributed to its ability to restore the activity of antioxidative enzymes. Thus, results of these studies together with those of earlier ones, suggest that EBM has an ability to protect the liver from ethanol-induced damage through its direct antioxidative effect.

In summary, we demonstrate that EBM prevents ethanol-induced oxidative stress and hepatic injury. Since these models of hepatic damage in the rat simulate many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in EBM might be effective as plant hepatoprotectors and thus may have some obvious therapeutic implications. Therefore, it seems logical to infer that EBM, because of its antioxidant property, might be capable of protecting the hepatic tissue from ethanol-induced liver injury. As *B. monnieri* contains large amounts of saponins it might be suspected that the hepatoprotective activity might be due to the presence of saponins in the extract. The activity may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in ethanol-induced hepatotoxicity. Further studies regarding the isolation and characterisation of the active principles responsible for hepatoprotective activity is currently under progress.

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