Evaluation of Hepatoprotective and Antihyperlipidemic Activity of Bacopa monnieri Against Alcohol Induced Hepatic Toxicity in Rats

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Abstract:

The possible protective effect of Bacopa monnieriextract was examined for its hypolipidemic and hepatoprotective activityagainst alcohol-induced toxicity in rats. The experimental animals were intoxicated with alcohol (2 g/kg body weight, once daily for 30 days) via gastric intubation. Bacopa monnieri was administered at the dose of 200 mg/kg body weight along with the daily dose of alcohol for 30 days. In the present studywe observed that alcohol-induced rats showed a significant lowered the antioxidant defense systems, such as, reduced glutathione (GSH) and ascorbic acid and glutathione-S- transferase activity enhancedwhen compared to the controls. We observed the levels of triglycerides (TG), total cholesterol (TC), serum markers AST, ALT, AST activates increased and phospholipids (PL)were significantly decreased in the alcohol-induced group. Supplementation of Bacoapa monnieri improved the antioxidant status, serum markers enzymes and altering the lipid profiles to near normal. Thus, the findings of the present study indicated a significant hepatoprotective and ahypolipidemic activity of Bacopa monnieri which offered protection against alcohol-induced toxicity.

Key words: Alcohol, Bacopa monnieri, GSH, GST, lipid profiles, serum markers

Introduction

Alcohol, a primary psychoactive constituent in alcoholic beverages is one of the most worldwide abused drugs. Modest drinking may not have any remarkable health problems; however, chronic heavy drinking causes a variety of hepatic problems including additional fat in the liver alcoholic hepatitis and cirrhosis [Reynolds et al., 2003]. Alcoholic liver

diseasepathogenicity is mainly due to the excessive amount of reactive oxygen species (ROS) generation resulting in the damaging effects of the cellular antioxidant defence system,[Kaviarasan et al., 2008] as well as, improvement of the lipid peroxidation process.[Arulmozhi et al., 2010] By the taking chronic alcohol produces excess free radicals production where the antioxidant defenses are reduced, which results in consecutive degradation of cell membranes by a process known as lipid peroxidation. This process may abolish the veracity of the membranes both within and surrounding the cells, extremelyconceding cell function. consumption of chronic and additional alcohol may accelerate an oxidative mechanism directly or indirectly, which ultimatelyproducts tissue damage and cell death [Lieber et al., 2000].previous studies have established that the alcohol administration induces changes of the various lipid ingredients in the liver of both humans and animals. After alcohol consumption the lipid anomaliesnoticed include the alteration in the level of triglyceride,cholesterol, fatty acid and, particularly, fatty acid composition of membrane phospholipids [Remia et al., 1991&Wing et al., 1984]

Antioxidants of plant origin have been reported to either inhibit or prevent the development of fundamental cellular disturbances resulting from excessive alcohol consumption [Zhou et al., 2003]. Bacopa monnieri, commonly known as Brahmi. Bacopa monnieri may serve as a dietetic antioxidant, with numerous modes of action to protect the hepatic tissue against alcohol induced oxidative damage. Bioactive components of Brahmi belong to saponins, alkaloids, teriterpenes, flavonoids, and cucurbitacin. The aim of the present study was to evaluate the hepatoprotective effects of Bacopa monnieri on chronic alcohol administration, on the levels the lipidsprofile and antioxidant status in rat liver.

Materials and Methods

Animals

Malealbino rats (Wistar strain) weighing 180 ± 20 g were used in the current study.Total thirty animals used. The rats were kept on standard pellet diet and providing access to water ad libitum. The rats were housed in polypropylene cages and preserved under the temperature of 25.6 ±28^{o}C, in 12 h light/12 h dark situations.

Chemicals

In the present study we used all the chemicals used were Analar Grade (AR) and attained from the ensuing scientificcompanies: Fischer (Pitrsburg, PA, USA), Sigma

(St.Louis, ,MO,USA), Merck (Mumbai India),and Qualigens (Mumbai, India) and Ranbaxy(New Delhi, India),

Preparation of plant extract

The Bacopa monnieri leaves were collected from the plants grown in pots in the sri Venkateshwara University campus. They were washed methodically in running tap water toeradicate any dirt or soil units adhered and tarnished gently between tissue paper folds to remove any water droplets. Bacopa monnieri fresh leaves were collected and the methanolic extract of the leaves was prepared such that the final concentration was 200mg/ml. The methanol was vanished, and the remainder was resuspended in water for gavage feeding.

Experimental design

Thirty rats divided into five groups of six rats in each group and treated as follows:

1. Normal control (NC): This group of rats received saline (0.9%), for a period of 30 days.

2. Alcohol treatment (At): This group of rats received absolute alcohol orally with 2 g/kg body weight via orogastric tube for thirty days.

3. Bacopa monnieri treatment (Bt): Rats treated with Bacopa monnieri extract (200mg/kg body weight.

4. Alcohol treatment + Bacopa treatment (At+Bt): This group of rats received both alcohol and Bacopa monnieri as described in group 2 and group 4 for Six weeks.

5. Alcohol + Silymarin (Standard drug): Rats treated with alcohol and Silymarin (25mg/kg body weight/day).

Tissue collection and Analytical procedures

After twenty-four hours the animals were sacrificed of the last treatment by cervical dislocation. At 4°C the liver was excised, washed with ice cold saline and blotted. the liver was immediately immersed after the atria and blood vessels were trimmed, in liquid nitrogen and stored at -80ºC for further biochemical analysis. The selected lipid metabolic profiles such as phospholipids (PL),triglycerides (TG), total cholesterol (TC), and levelsweremonitored by the methods ofZilversmidth and Davis (1950), Liebermann Bernhard reaction as described by Natelson (1971), respectively. Ascorbic acid, GSH and GST activity were monitored by the methods of Omaye et al., (1971), Theodorus et al., (1981). Serum markers enzymes were assayed by Moss and Handerson (1999). by the

experiments were carried out in accordance with the guidelines and protocol approved by the Institutional Animal Ethics Committee.

Statistical analysis:

The results expressed were expressed as means six rats per group for control and experimental animals. The data were analysed using one-way analysis of variance (ANOVA) on SPSS/PC and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were statistically considered significant the P value was less than 0.001.

Results

In the present study there was a significant decrease in GSH, Ascorbic acid and GST activity enhanced in alcohol treated rats, (Table.1). These parameters were reversed back to normal levels followingBacopa monnieri treatment. However, with Bacopa monnieritreatment the activities came back to normal levels, indicating that treatment with Bacopa monnierinormalized the altered liver antioxidant enzyme levels as well.

The data in Tables 2 depict the changes in the levels of lipid profile in the liver of the control and experimental animals. The levels of total cholesterol, triglycerides, and the phospholipids were significantly increasedin alcohol treated rats. Co-administration of Bacopa monnieri progressively enhanced the lipid profile toward normal when compared to silymarin.

In the current study we observed hepatic stress marker ALT, AST and ALP were increased in alcohol exposure rats compared to normal rats. After pretreatment with Bacopa monnierialcohol rats showed all these marker enzymes significantly decreased [Table 3].

Discussion

In the currentstudy, we observed GSH was decreased in alcohol treated rat group. In the liver, the enduring alcohol exposure enzyme activities were increasesassociated to the and utilization and recycling of glutathione[Kode et al., 2004].Previous studies also stated that glutathione level was decreased in liver tissue of alcohol treated rats[Rodrigo et al.,2002). Alcohol induces lipid peroxidation and reducesGlutathioneassets, then there are measures that occur after the alcohol metabolites creations. During the metabolism of alcohol leads to GSH oxidation the ROS intermediates produced[Bilanda et al., 2004], resulting in the diminution of GSH. However, alcohol ingested rats treated with GSH levels were increased.

Previous studies explained that ginger exerts an antioxidant effect by decreasing lipid peroxidation, increasing GSH level and maintaining normal levels of antioxidant enzymes[Ahmad et al 2000]. Treatment of Bacopa monnieri extract showed protective effect in alcohol rats by enhancing the antioxidant enzyme activities including GSH level.

The increase inthe GST activity inthe liveras aresponse tothealcoholconsumption suggestsits activation due to oxidative stress. The increased glutathionecontribution in conjugation response mediated by augmented GST activity seems to be a reasonable model for the reduced GSH level due to alcohol exposure30. Increased GST activity suggests its activation due to oxidative stress[Iizuka et al.,1991, Das and Vasudevan] in their dose dependent alcohol studies observed increased GST activity. The stimulation of GST due to Bacopa monnieri feeding in liverindicates that Bacopa monnieri feeding can confer protection against the toxic effect of xenobiotics. The increase in GST activity in all these tissues further support the hypothesis that regular intake of Bacopa monnieri can enhance the activity of phase II detoxification enzymes.

As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide anion radical, hydrogen peroxide, the hydroxyl radical, and singlet oxygen[Dinu et al., 2005]. In the present study we observed decrease in the level of liver ascorbic acid in alcohol treated group only could be because of increased utilization of this antioxidant in scavenging the free radicals generated during acute alcohol intoxication[Balasubramanian et al., 2003]. In the current study with bacopa monnieri treatment in alcohol treated group, ascorbic acid level was increased. This may be due to the Bacopa compoundsflavonoids, bacosides influence on the Reactive oxygen species which were formed during alcohol metabolism. Thus, Bacopa monnieri may exert atherapeutic effect in disputing the toxic free radicals in the hepatic tissue.

Alcohol feeding is known to increase the biosynthesis and to decrease the catabolism of fatty acid and cholesterol, resulting in hypertriglyceridemia and hypercholesterolemia [Kumar et al., 2002].Previous studies confirmed that chronic alcohol feeding could rise the lipolytic activity and elevate Free fatty acids concentration. The increased FFAs with alcohol consumption may rise the circulating triglycerides concentrations, which may be due to enhanced phosphatide phosphohydrolase activity [Drago et al., 2002].Increased Triglycerides levels after alcohol absorption may be due to the increased availability of glycerophosphates, free fatty acids decreased Triglycerides lipase activity, and decreased fatty oxidation. These

increased TG levels may lead to increased accessibility of Free fatty acids for esterification. Phospholipids are the vivacious components of a bio membrane and mainly act as membrane-bound enzymes regulators important in influential the alcoholism pathology. [El-Hilaly etal., 2006]. Hence, the alteration in the membrane composition may be the reason for the toxic defects caused by alcohol.The decreased phospholipid levels in the liver may be due to the increased activity of phospholipases in the hepatic tissue. Earlier studies have demonstrated that chronic exposure to ethanol may lead to a progressive increase in membrane phospholipase activity [Zhihong et al., 1996]. Hence, the alteration in the membrane composition may be the reason for the toxic defects caused by alcohol. Thus, the Bacopa monnieri extract consumption could result in accumulation of active ingredients within the cells, as well as in the cell plasma membrane receptors may reduce the plasma TG by increasing pancreatic lipase and amylase, which inhibit lipid hydrolyse in intestinal tract reducing lipid peroxidase [Liu et al., 2003].The pretreatment of Bacopa monnieri extract was active in counteracting the oxidative stress induced damage by decreasing the hepatic lipid levels of rats.The significant increase levels of cholesterol, phospholipids triglycerides, and free fatty acids in the kidney caused by the administration of streptozotocin in rats were brought down to normalcy on treatment with ginger [Shanmugam et al., 2022].

The current study found that supplementation with Bacopa could improve oxidative status in liver tissue so that it increased the level of its enzymes. Reactive oxygen species and nitric oxide are responsible for the induction of hepatocyte apoptosis. The excess ingesting of alcohol has been well related with partialinjuryand livermetabolism alongwith leakagecytoplasmic hepatic enzymes enzymes into the blood. [Atta et al., 2010]. AST, ALT and ALP are considered among the most sensitive markers of hepatocellular injury. ALP, which is secreted from the lysosomes, is also a marker enzyme for assessing liver damage [Singha et al., 2007]. Previous reports have shown that exposure of hepatocytes to ethanol perturbs the membrane structure and functions thereby increasing the leakage of AST [Rajakrishnan, et al., 2001]. Supplementation with the Bacopa monnierisignificantly decreased levels of serum enzyme markers, thus telling that the extract possessed compounds such as Bacosides A and B that protected the hepatocytes from alcohol induced liver injury and subsequent leakage of the enzymes into the circulation.

Conclusion:

From the above results we conclude that the levels of serum marker enzymes and lipid metabolic profiles were elevated in alcohol treatment and decreased antioxidant status. The supplementation of Bacopa monnieri extract caused a significant decrease in the activities of serum marker enzymes, lipid profiles indicating its protective effect, wherein no toxic effects were observed. Similar observations were made in the antioxidants also. The results of the present study prove the hepatoprotective effect of Bacopa monnieri extract protection against alcohol induced hepatic toxicity.

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Table. 1 Effect of Bacopamonnieriextract on glutathione (GSH), Ascorbic acid (AA) and Glutathione -S- transferase (GST) in rats with alcohol induced oxidative stress in rat liver.

Groups	GSH $^{\Psi}$	Ascorbic acid $^{\Psi\Psi}$	GST $^{\Psi\Psi\Psi}$
Normal control (NC)	12.432±0.396	0.332±0.019	28.76±0.039
Alcohol treated (At)	7.969±0.486* (−29.860)	0.256±0.049* (−24.594)	45.987±0.028* (−53.31)
Bacopa treated (Bt)	12.666±.940* (+43.875)	0.535±0.102* (+46.355)	27.56±0.088* (+68.836)
Alcohol plus Bacopa (At+Bt)	10.303±0.531** (+12.466)	0.516±0.026** (+33.310)	39.654±0.243** (+42.410)
Alcohol plus silymarin	10.345±0.531** (+15.466)	0.612±0.026** (+31.310)	40.654±0.243** (+39.410)

All the values are mean±SD of six individual observations.

Values in the parenthesis denote percent change over normal control.

The values are significant compared to the following: control (*p < 0.001),

alcoholtreated(**< 0.01) (Dunnett's multiple comparison test).

$^{\Psi}$Values are expressed in µmol of glutathione/g wet weight of the tissue.

$^{\Psi\Psi}$ Values are expressed in mg ascorbic acid/wet weight of tissue

$^{\Psi\Psi\Psi}$ Values are expressed in µmol of glutathione/mg protein/min.

Table 2. Effect of Bacopa monnierion hepatic lipid profiles in control and alcohol-administered rats

Groups	Triglycerides mg/g of tissue	Phospholipids mg/g of tissue	Total cholesterol mg/g of tissue
Normal control (NC)	4.76 ± 6.22	20.54 ± 4.57	5.67±2.56
Alcohol treated (At)	8.22± 5.10* (+14.333)	13.54 ±11.12* (+35.265)	7.98.6±6.66* (+76.286)
Bacopa treated (Bt)	4.01.25±2.043* (-46.061)	21.09.75±2.726* (-56.859)	5.93.25±3.576* (−23.711)
Alcohol plus Bacopa (At+Bt)	7.23 ± 7.96** (+23.245)	17.72 ± 5.24** (+23.365)	6.78.6±8.10** (+65+.435)
Alcohol plus silymarin	8.45 ± 7.96** (+12.245)	18.72 ± 5.24** (+45.365)	7.78.6±8.10** (+62+.435)

All the values are mean±SD of six individual observations.

Values in the parenthesis denote percent change over normal control.

The values are significant compared to the following: control (*p < 0.001),

Alcoholtreated (**< 0.01) (Dunnett's multiple comparison test).

Table 3. Effect of Ethanolic extract of Bacopa monnierion hepatic markers in the serum of control and alcohol-administered rats

Groups	AST (U/L)	ALT(U/L)	ALP(U/L)
Normal control (NC)	74.02 ± 13.22	43.36 ± 4.57	61.04±2.56
Alcohol treated (At)	121.22± 5.10* (+62.333)	76.54 ±11.12* (+74.265)	129.6±6.66* (+82.286)
Bacopa treated (Bt)	66.25±2.043* (-41.061)	68.75±2.726* (-56.859)	54.25±3.576* (−23.711)
Alcohol plus Bacopa (At+Bt)	89.23 ± 7.96** (+55.245)	60.72 ± 5.24** (+63.365)	83.6±8.10** (+74+.435)
Alcohol plus silymarin	91.87.23 ± 7.96** (+54.245)	64.54.72 ± 5.24** (+34.365)	87.6±8.10** (+54+.435)

All the values are mean±SD of six individual observations.

Values in the parenthesis denote percent change over normal control.

The values are significant compared to the following: control (*p < 0.001),

Alcoholtreated (**< 0.01) (Dunnett's multiple comparison test).