Ameliorative effect of *Bacopa monnieri* on alcohol induced hepatotoxicity and oxidative stress in albino rats

D Veera Nagendra Kumar, K Lakshmi Narasaiah and S Prakash Rao

**Abstract**

Alcoholic liver disease is one of the most serious consequences of chronic alcohol abuse and the oxidative stress plays an important role in the development of the disease. The current investigation has been conducted to investigate the influence of *Bacopa monnieri* on hepatic antioxidant status in alcohol treated rats. Administration of alcohol (2g/kg/day) for 6 weeks resulted in liver injury and tested animals were treated orally with plant extract (200mg/kg), prior to ethanol administration. Hepatic marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP) were analysed in serum. Hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPxs), glutathione reductase (GR) activities and reduced glutathione (GSH) content and malondialdehyde (MDA) level were studied. The extract produced significant (*p*<0.001) decreases AST, ALT, ALP activities, malondialdehyde levels and also increases liver antioxidant enzymes in alcohol treated rats. However, *Bacopa monnieri* extract supplementation to the alcohol treated rats reversed these effects and attained the antioxidant status to normal levels. The present study suggest that aqueous extract of *Bacopa monnieri* offers protection against oxidative stress and its ability might be attributed to its antioxidant potential.

**Keywords:** *Bacopa monnieri*, alcohol, antioxidant enzymes, ALT, ALP

**1. Introduction**

Chronic alcohol consumption leads to several metabolic disorders including hepatic and extra hepatic diseases [1]. Although excessive acute or chronic ingestion of alcohol represents a serious hazard to health, alcohol is still the second most widely used psychoactive substances in the world, after caffeine [2]. Alcohol is extensively metabolized in the liver, leading to the generation of acetaldehyde by the enzymatic activity in cytosol, microsomes, and peroxisomes. Acetaldehyde is further oxidized to acetate by acetaldehyde dehydrogenase in the mitochondria, which results in the generation of free radicals/reactive oxygen species (ROS) [3, 4]. Oxidation of ethanol by alcohol dehydrogenase generates NADH, and NADH-dependent production of ROS by various organelles increases after chronic ethanol treatment [5]. These free radicals in high amounts can diminish or impair the antioxidant homeostasis and leads to hepatic tissue damage.

*Bacopa monnieri* Linn. (Syn. *Herpestis monnieri* Linn. H.B. &K), family Scrophulariaceae (vernacular; Brahmi), is an annual creeping plant found throughout India, Nepal, Sri Lanka, China and Taiwan. It is also found in Florida and other southern states of the USA in wet, damp and marshy regions *Bacopa monnieri* has been used to promote memory enhancing activity [6] and intellect, to treat psycho neurological disorders and as a rejuvenator. This plant is also found to possess Antiparkinson activity [7], antidepressant activity [8], anticholinesterase activity [9] antioxidant activity [10], anti-ulcerogenic activity [11], anti-inflammatory activity [12], antibacterial activity [13], and anticonvulsant activity [14].

In view of the above importance of *Bacopa monnieri* it is much interested and practical importance to study the influence of long term *Bacopa monnieri* supplementation on antioxidative potential with reference to alcoholism. Keeping in view of medicinal value of *Bacopa monnieri*, the present study was designed to explore a possible new strategy to improve recovery from alcoholic liver injury in the rats. Hence, effects of *Bacopa monnieri* extract in alcoholics have been assayed by monitoring the activities of antioxidant enzymes and hepatotoxicity in the liver tissues of male albino rats.
2. Materials and Methods
2.1 Animal Care and Maintenance
Wistar strain male albino rats, aged 6 months and weighing 180 ± 20 g, were obtained from the Indian Institute of Science, Bangalore. The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (25 ± 2 °C) with a photo-period of 12 h light and 12 h dark cycle. The rats were fed with a standard rat pellet diet and water ad libitum. This study was approved by the Institutional Animal Ethics Committee and experiments were performed according to the regulations for the care and use of laboratory animals and its resolution number; 09 (ii)/a/CPCS/IAEC/07-08/SVU/Zool/ DVNK/ dated 26/6/08.

2.2 Preparation of plant extract
Fresh *Bacopa monnieri* plant was obtained from the Tirumala hills, Andhra Pradesh, India, and the whole plant was dried under shade dust-free conditions, and was ground into fine powder. 200g of powder has taken and macerated in 1000 ml under shade dust-free conditions, and was ground into fine powder. This juice was dried in rotary evaporator (Model: HS-2005V) and the whole plant was dried using the method of Ohkawa [20]. The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed by the method of Reitman and Frankel [21] and Serum alkaline phosphatase activity was performed using Bessay *et al.,* [22] method. All the enzyme activities were expressed per mg protein and the tissue protein was estimated according to the method of Lowry *et al.* [21] using bovine serum albumin (BSA) as a standard.

2.3 Experimental design
The rats were divided into four groups and treated as described below.

**Group I:** Normal controls: rats received only 0.9% saline.

**Group II:** Alcohol-treated rats: rats received 2.0 g/kg body weight/day for 6 weeks

**Group III:** *Bacopa monnieri* treated: rats received 200mg/ kg body weight/ day for 6 Weeks

**Group IV:** Alcohol and *Bacopa* treated: as described in group II and group III for a period of 6 weeks

2.4 Analytical procedure
After 24 hours of the last treatment, all the animals were euthanized and liver tissues were excised. The tissue was washed with ice cold saline, immediately immersed in liquid nitrogen and stored at −80 °C for further biochemical analysis. Hepatic SOD activity was assayed by the method of Misra and Fridovich [15] at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein. CAT activity was determined at room temperature by using the modified version of Aebi [16] and absorbance of the sample was measured to 240 nm for 1 min in a UV-spectrophotometer. Activity of GPx was determined by the method of Flohe and Gunzler [17] in the presence of NADPH and absorbance was measured at 340 nm using cumene hydrogen peroxide. GR enzyme activity was determined according to the method of Carlberg and Mannervik [18]. The concentration of reduced GSH was measured as described by Akerboom and Sies [19]. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product MDA by using the method of Ohkawa *et al.* [20]. The activities of serum

2.5 Chemicals
All the chemicals used in the present study were analar Grade (AR) and obtained from the following significant companies: Sigma (StLouis, MO, USA), Fischer (Pitsburg, PA, USA), Merk (Mumbai, India), Ranbaxy (New Delhi, India), Qualigen (Mumbai, India).

2.6 Statistical analysis
The results were expressed as mean ± SEM of six rats per group and the statistical significant was evaluated by one-way analysis of variance (ANOVA) using the SPSS (version 15.0) program followed by LSD. Values were considered statistically significant when (*p<0.01).

3. Results
3.1 Effect of *Bacopa monnieri* Ethanol extract on antioxidant enzyme activities and MDA levels in alcohol-induced rats
Significant (*p<0.001) decreases in SOD, CAT, GPx, GR activities and GSH level and a high level of MDA were observed in the alcohol rats compared with normal control rats. Alcohol rats with *bacopa* treatment, showed significant (*p<0.01) increases in SOD, CAT, GR, GPx activities and GSH level, and a decrease in MDA level, which reflects restoration of the antioxidant enzyme systems to near-normal values (Table. 1–2).

Effect of Ethanol extract of *Bacopa monnieri* on Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) in alcohol induced oxidative stress in rat liver

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>SOD**</th>
<th>CAT***</th>
<th>GPx****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>13.432±0.396</td>
<td>0.332±0.019</td>
<td>0.796±0.039</td>
</tr>
<tr>
<td>Alcohol treated (At)</td>
<td>7.969±0.486*</td>
<td>0.256±0.049*</td>
<td>0.506±0.028*</td>
</tr>
<tr>
<td>(−30.860)</td>
<td>(−24.594)</td>
<td>(−53.31)</td>
<td></td>
</tr>
<tr>
<td>Bacopa treated (Bt)</td>
<td>16.666±.940*</td>
<td>0.535±0.102*</td>
<td>1.565±0.088*</td>
</tr>
<tr>
<td>(+55.875)</td>
<td>(+46.355)</td>
<td>(+68.836)</td>
<td></td>
</tr>
<tr>
<td>Alcohol plus Bacopa (At+Bt)</td>
<td>14.303±0.531**</td>
<td>0.518±0.026**</td>
<td>1.144±0.243**</td>
</tr>
<tr>
<td>(+19.466)</td>
<td>(+33.310)</td>
<td>(+42.410)</td>
<td></td>
</tr>
</tbody>
</table>

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), alcohol treated(** < 0.01) (Dunnett’s multiple comparison test).

Values are expressed in units of superoxide anion reduced/mg protein/min.

Values are expressed in μ moles of H2O2 degraded/mg protein/min.

Values are expressed in μmol of NADPH oxidized/mg protein/min.
Effect of Ethanolic extract of *Bacopa monnieri* on glutathione reductase (GR), glutathione peroxidase (GPx) and lipid peroxidation (MDA) in rats with ethanol induced oxidative stress in rat liver

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>GR</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>0.698±0.042</td>
<td>104.925±1.214</td>
<td>64.635±2.013</td>
</tr>
<tr>
<td>Alcohol treated (At)</td>
<td>0.35±0.018*</td>
<td>53.086±2.445*</td>
<td>113.24±3.602*</td>
</tr>
<tr>
<td>Bacopa treated (Bt)</td>
<td>0.81±0.039*</td>
<td>166.853±2.635*</td>
<td>42.55±3.317*</td>
</tr>
<tr>
<td>Alcohol plus Bacopa (At+Bt)</td>
<td>1.11±0.164**</td>
<td>138.44±3.333**</td>
<td>97.906±3.576**</td>
</tr>
</tbody>
</table>

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<0.01), Alcohol treated (** < 0.01) (Dunnett’s multiple comparison test).

### 3.2 Effects of *Bacopa monnieri* Ethanolic Extract in Serum

#### Levels of ALT, AST, ALP in alcohol-induced rats

Table 3 shows the effect of *Bacopa monnieri* Ethanolic Extract on serum ALT, AST, ALP, in different experimental groups. In alcohol treated rats, the levels of ALT, AST, ALP, were significantly (*p<0.001) higher than normal rats. Treatment with Bacopa 200 mg/kg/day resulted in lower serum level of these enzymes as compared to alcohol treated rats.

Effect of Ethanolic extract of *Bacopa monnieri* on hepatic markers in the serum of control and ethanol-administered rats

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>72.02±14.22</td>
<td>41.36±4.57</td>
<td>66.04±2.56</td>
</tr>
<tr>
<td>Alcohol treated (At)</td>
<td>128.22±5.10*</td>
<td>76.54±11.12*</td>
<td>132.6±6.66*</td>
</tr>
<tr>
<td>Bacopa treated (Bt)</td>
<td>66.25±2.043*</td>
<td>68.75±2.726*</td>
<td>54.25±3.576*</td>
</tr>
<tr>
<td>Alcohol plus Bacopa (At+Bt)</td>
<td>89.23±7.96**</td>
<td>60.72±5.24**</td>
<td>83.6±8.10**</td>
</tr>
</tbody>
</table>

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), Alcohol treated (** < 0.01) (Dunnett’s multiple comparison test).

### 4. Discussion

Chronic alcohol consumption demonstrates significant increase in free radical production and decreases antioxidant status in the liver of rat [24]. Earlier studies established that *Bacopa monnieri* constituents can improve the antioxidant capacity under various drug-induced oxidative stress conditions in various tissues, [25, 26]. As a major finding of the present study, we demonstrated that alcohol induced detrimental effects in hepatic marker enzymes were recovered with *Bacopa monnieri* ethanolic extract treatment. In addition to these findings, alcohol-induced decrease in antioxidant status and increase in MDA content were significantly attenuated by 6 weeks *Bacopa monnieri* supplementation in the liver of rats.

In the present study, liver SOD activity was significantly decreased with alcohol administration. Similar decrease in SOD activity in hepatic tissue [27] has also been reported during alcohol intoxication. The reduced activity of SOD in presence of alcohol may cause the accumulation of O2·−, H2O2 or the products of its decomposition [28]. The SOD activity was elevated with the administration of *Bacopa monnieri* extract in alcohol treated rats. This data indicates that *Bacopa monnieri* can effectively counteract the superoxide radicals during alcohol-induced stress condition. This elevation may be due to the presence of antioxidant bioactive compounds in *Bacopa monnieri*. The antioxidants compounds like are bacosides A and B and the phenolic compounds of *Bacopa monnieri* were responsible for scavenging the superoxide anion radicals [29].

We also found that liver CAT activity was significantly decreased in alcohol ingested rats than that of control rats. Mallikarjuna et al. [30] reported a similar decrease in CAT activity in the liver of alcohol treated rats. The decreased CAT activity indicates inefficient scavenging of hydrogen peroxides, due to oxidative inactivation of enzyme [31]. However, *Bacopa monnieri* supplemented to alcohol treated group showed significant increased CAT activity in the hepatic tissue which indicates the antioxidant property of *Bacopa monnieri*. In this *Bacopa monnieri* is known to suppress reactive oxygen species and enhance these enzymes activities. Thus the ameliorated activities of SOD and CAT in alcohol exposed rats on *Bacopa monnieri* supplementation may be due to the antioxidant constituents which can scavenge free radicals [32].

The present study showed that GPx activity was significantly decreased in alcohol treated rats, which may disturb the glutathione homeostasis in the liver cells and ultimately leads to the damage of hepatocytes. Decreased GPx activity may be due to either inactivation of enzyme by free radicals [33] or depletion of its co-substrates (GSH and NADPH) availability in alcohol treated rats. The reduced GPx activity may also be due to reduced availability of GSH as observed in the current investigation. Upon *Bacopa monnieri* treatment the GPx activity was increased in alcohol treated rats. In our earlier studies we demonstrated that alcohol-induced decrease in brain GPx activity was reversed by *Bacopa monnieri* supplementation [34]. The activity of GSH-Px was significantly increased with ethanol and *Bacopa monnieri* and combination treatment group which indicates that Bacopa could inhibit and/or scavenge the free radicals in rat hepatic tissue.

Hepatic GR activity was decreased in the alcohol treated rats. GR serves to regenerate reduced GSH from oxidized GSSG by the activation of GPx. The decrease in GR activity may reflect the decline of the production and availability of GSH to overcome H2O2 [35]. This may be due to over production of hydrogen peroxides which can inactivate the GPx activity [36] and finally it can lead to disturb the GSH/GSSG ratio. The previous reports have also been demonstrated that GR activity was decreased in the liver of alcohol rats [37]. GR activity was elevated in *Bacopa monnieri* plus alcohol treated rats. This elevation may be due to *Bacopa monnieri* bioactive compounds such as bacosides A and B, Alkaloids, saponins, and sterols, flavonoids and other phytochemicals antioxidant activity [38].

Glutathione being an important cellular reductant, involved in
protection against free radicals, peroxides and toxic compounds [39]. GSH depletion is one of the chief factors that lead to lipid peroxidation [40]. In our present study, the GSH levels were decreased in the liver of rats exposed to alcohol as compared to control rats. The decreased GSH level may be due to increase level of lipid oxidation products which may be associated with the less availability of NADPH required for the activity of glutathione reductase (GR) to transform oxidized glutathione to GSH [41] due to the increased production of ROS at a rate that exceeding the ability to regenerate GSH for long term ethanol exposure. The decreased GSH level in association with decreased GR activity may support the explanation as evidence. Administration of Bacopa monnieri increased glutathione levels in the liver. Hepatoprotective and nephroprotective effect of Bacopa monnieri following increased GSH levels have been reported Rohini et al., [42]. Similarly, an increase in GSH levels against alcohol induced depletion in the liver indicates hepatoprotective role of Bacopa monnieri.

Lipid peroxidation is a complex process that damages the cell structure and function. Peroxidation of membrane lipids initiates the loss of membrane integrity; membrane bound enzyme activity and cell lysis [43]. Malondialdehyde (MDA), a marker of lipid peroxidation was significantly elevated with alcohol intoxication in the liver tissue. It is well known that chronic alcohol ingestion elevates the MDA levels, which reflect extensive lipid peroxidation process in liver, heart, and kidney of rats [44]. In the present study, we found a significant reduction in MDA levels in group 4 rats, which received Bacopa monnieri along with alcohol for a period of 6 weeks. This result suggests that Bacopa extract can protect the hepatic cells from alcohol-induced peroxidative damage. Recently Sudha et al., [45] reported that Achyranthus aspera extract ameliorated the alcohol-induced hepatotoxicity, and this protection was mediated either by preventing the drug-induced decline of hepatic antioxidant defense system or by direct free radical scavenging activity of Bacopa monnieri. It was also demonstrated that the major pungent constituent in bacosides and bacopasaponins exhibits antioxidative effect against peroxidation of phospholipids and scavenge the various free radicals. Kishore et al., [46] bacosides and bacopasaponins exhibits antioxidative effect against peroxidation of phospholipids and scavenge the various free radicals.

The excess consumption of alcohol has been well associated with distorted damage and metabolism in liver along with leakage of cytoplasmic liver enzymes into the blood. [47]. AST, ALT and ALP are considered among the most sensitive markers of hepatocellular injury. If injury involves organelles, such as mitochondria then the soluble enzymes such as AST compartmented will also be similarly released indicating membrane [49]. ALP, which is secreted from the lysosomes, is also a marker enzyme for assessing liver damage [49]. Previous reports have shown that exposure of hepatocytes to ethanol perturbs the membrane structure and functions thereby increasing the leakage of AST [50]. The increased levels of AST, ALT enzymes in the serum have been observed in alcohol administered rats, which indicate increased permeability, damage and necrosis of hepatocytes [51]. Pretreatment with the extract of Bacopa monnieri significantly decreased levels of serum enzyme markers, thus suggesting that the extract possessed compounds that protected the hepatocytes from alcohol induced liver injury and subsequent leakage of the enzymes into the circulation.

Decreased levels of serum AST, ALT and ALP in rats pretreated with methanolic extract of Cassia fistula prior to Alcohol induced toxicity had been reported by Pradeep et al., [52].

5. Conclusion

In the present study the reversal of altered antioxidant enzymes status and peroxidative damage in hepatic tissues by Bacopa monnieri extract suggests its antioxidant and anti-peroxidative property and hence reveals its potential to play a crucial role in defense against free radicals. From Our results confirm that Bacopa monnieri could be responsible for the restoration of metabolic activities and according protection against alcohol-induced oxidative stress. The mechanism could be related to scavenging activity of the extract. However, possible involvement of other mechanisms cannot be ignored at this stage. Thus, Bacopa monnieri appears to have potential as adjunct therapy to possibly inhibit the liver complications due to alcohol induced hepatotoxicity.

6. References

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