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Neuroprotective effects of *Bacopa monnieri* on brain lipid peroxidation and Na⁺/K⁺, Mg²⁺ and Ca²⁺ ATPase activity against alcohol induced rats

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Abstract

Objective: The present study was investigated the Neuro-protective effect of *Bacopa monnieri* against alcohol-induced oxidative stress and tissue damage.

Design: This is a prospective animal study in which brain lipid peroxidation (MDA) and Na⁺/K⁺, Mg²⁺ and Ca²⁺ATPases(membrane bound enzymes) were demolished by alcohol consumption and restored with *Bacopa monnieri* administration. We supplemented rats with *Bacopa monnieri* for six weeks to evaluate the Neuroprotective effect against alcohol oxidative stress.

Methods: Twenty-four Wistar strain rats were divided into 4 equal groups: Normal control (NC), Alcohol treated (At), *Bacopa monnieri* treated (BM.t) and alcohol plus *Bacopa monnieri* treated (At + BM.t). *Bacopa monnieri* was given to the At group for six weeks and brain ascorbic acid, uric acid, MDA levels and membrane bound enzymes were assayed.

Results: Brain ascorbic acid, uric acid, Na⁺/K⁺, Mg²⁺ and Ca²⁺ATPases activities were significantly (P > 001) decreased, whereas malondialdehyde (MDA) levels were elevated in alcohol treated group. However, *Bacopa monnieri* extract supplementation to the alcohol treated rats reversed these effects and attained the ascorbic acid, uric acid, MDA levels and Na⁺/K⁺, Mg²⁺ and Ca²⁺ATPases activities to normal levels. Furthermore, degenerative changes in brain with alcohol treatment were minimized to nearness in architecture by *Bacopa monnieri* supplementation.

Conclusions: This study concludes that alcohol-induced neuro-toxicity was attenuated by *Bacopa monnieri* extract treatment, thus *Bacopa monnieri* can be used as a regular nutrient to protect the neurodegenerative diseases.

Keywords: alcohol, *Bacopa monnieri*, lipid peroxidation, membrane bound enzymes

1. Introduction

Consumption of alcoholic beverages is considered as a usual habit in most societies around the world. Chronic alcohol intake is associated with several degenerative and inflammatory processes in the central nervous system (CNS) (Pal *et al.*, 1993) [1]. Excess alcohol intake increases free radical or reactive oxygen species (ROS) production and causes oxidative stress by compromising the antioxidant defense system (Clemens *et al.*, 2004) [2]. The brain is deficient in oxidative defense mechanisms and hence is at great risk of damage mediated by reactive oxygen species (ROS) resulting in molecular and cellular dysfunction (Gupta *et al.*, 2003) [3]. The central nervous system (CNS) is vulnerable to free radical damage because of brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues (Skaper *et al.*, 1999) [4]. Moreover, brain has a high ratio of membrane surface area of cytoplasmic ratio, extended axonal morphology prone to injury, and neuronal cells are non-replicating. ROS can increase the permeability of blood brain barrier, after tubulin formation, and inhibit the mitochondrial respiration. If unchecked it can lead to a geometrically progressing lipid peroxidation (Gilman *et al.*, 1993) [5]. The central nervous system is vulnerable to oxidative stress, especially when a toxicant can modify the physiological balance between anti- and pro-oxidant mechanisms (Gonthier *et al.*, 2004) [6]. Brain has a high amount of polyunsaturated fatty acids (PUFAs) and high content of free ions. Specifically peroxidation of membrane lipids may cause impairment of membrane function, decreased fluidity, inactivation of membrane-bound enzymes (Na⁺/K⁺, Mg²⁺ and Ca²⁺ATPases), increased permeability to ions and possibly eventual membrane rupture (Edelfors *et al.*, 1990) [7]. The biochemical changes induced by alcohol consumption in the brain are not well understood, though some clinical and experimental have been focused on the effects of alcohol feeding on neuronal function including gross and microscopic morphology.

Bacopa monnieri (called Brahmi in Sanskrit) an ayurvedic medicinal plant have been used as a brain tonic, which contains a mixture of triterpenoid saponins designated as bacosides A and B (Chatterjee *et al.* 1963, 1965) [8]. Regular use of *Bacopa monnieri* leaf as a natural health supplement is beneficial in the treatment of neurological disorders associated with free radical induced damages (Stough *et al.*, 2001) [9]. This plant is also found to possess anticholinesterase activity (Venkatakrishnan *et al.*, 2012) [10] antioxidant activity (Subashri *et al.*, 2012) [11], antidepressant activity (Somoday *et al.*, 2012) [12], anti-inflammatory activity (Shabana Channa *et al.*, 2006) [13], anticancer activity (Ling Peng *et al.*, 2010) [14], antibacterial activity (Ravikumar *et al.*, 2005) [15].

Recently from our laboratory, we reported the Neuro-protective effect of *Bacopa monnieri*, which revealed an up-regulation in antioxidant status in alcohol-treated rats (Veera Nagendra Kumar *et al.*, 2016) [16]. However, the neuro-protective effect of *Bacopa monnieri* extract against alcohol-induced toxicity is not yet studied fully. Hence, in the present study has been under taken to evaluate the possible ameliorative effect of *Bacopa monnieri* extract against alcohol-induced oxidative stress and tissue damage.

2. Material and methods

2.1 Animals: The study involved young (3-4 months old; 200-220g) male albino rats of wistar strain purchased from Sri Venkateswara Traders Pvt. Limited, Bangalore, maintained in the animal house of the department in polypropylene cages. Standard conditions of humidity (50± 9% relative humidity), room temperature (25-28°C) and 12 h light/ dark cycle (6:00 AM to 6:00 PM) were maintained. A standard rodent diet (M/s Hindustan Lever Ltd., Mumbai) and water were provided *ad libitum*. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee RegdNo.438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 09 (iii)/a/CPSCA/IAEC/07-08/SVU/Zool/DVVK/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 macerate in 1000 ml of 95% ethanol for 12 h at room tempature, then filtered and squeezed with muslin cloth to obtain ethanol extract juice. This process was repeated three times and finally collection of this juice were dried in rotary evaporator (Model: HS-2005V) from this we had get jelly and then this jelly was converted to powder in lyodel freezer. We have done dose dependent studies by using, 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg and 300 mg/kg. Of this 200 mg/kg dose showed good antioxidant activity. So this study we selected dose of 200 mg/kg of ethanol extract of *Bacopa monnieri*.

2.3 Experimental design

The rats were divided into 4 groups, six rats in each group and treated as follows:

Group I: Normal control (NC): This group of rats (n = 6) were fed on normal diet and received Vehicle solution (2%, Tween-80) for equivalent handling.

Group II: Alcohol treatment (At): Alcohol treated (At): Six rats were received 20% alcohol (v/v) orally at the dose of 2 g/kg body weight via an orogastric tube everyday for a period of six weeks.

Group III: *Bacopa monnieri* treatment (BM.t): Rats received *Bacopa monnieri* extract (200 mg/kg body wt) orally for 6 weeks days

Group IV: Alcohol + *Bacopa monnieri* treatment (At+ BM.t): Rats received *Bacopa monnieri* for 6 weeks followed by alcohol (2g/kg) for 6 weeks.

2.4 Analytical Procedure

After completion of six weeks of treatment, the animals were sacrificed by cervical dislocation and the brain tissues were excised at 4°C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80°C for further biochemical analysis. The selected parameters such as MDA levels, Ascorbic acid, and Uric acid content were monitored by the methods of Ohkawa *et al.* (1979) [17], Omaye *et al.* (1971) [18], and Martinek (1970) [19] respectively. The activities of Na⁺/K⁺, Mg²⁺ and Ca²⁺ ATP ases were estimated in the brain by the method of Fritz and Hamrick (1966) [20]. The enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry, Rosebrough, Farr, and Randall (1951) [21], using bovine serum albumin (BSA) as a standard.

2.5 Chemicals

In the present study all chemicals used were of Analar Grade (AR) and purchased from the following scientific companies: Sigma (St.Louis, MO, USA), Fischer (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India).

2.6 Statistical analysis

The data has been analyzed by using SPSS (Version 16.0; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dennett's multiple comparison test and differences were considered significant at p < 0.05.

2.7 Histopathological Studies

The brain tissue was washed with ice-cold saline immediately after isolation and then fixed in 10% formalin solution. Sections of 3 mm thickness were stained with hematoxylin and eosin (HE) for histopathological examination.

3. Results

3.1 Effect of *Bacopa monnieri* extract on MDA, ascorbic acid and uric acid levels in alcohol induced rats

Statistical analyses clearly indicated a negative impact of Alcohol intoxication on the MDA, ascorbic acid and uric acid levels status of the brain. Significant ($p < 0.001$) decreases in ascorbic acid and uric acid levels and high level of MDA were observed in the alcohol rats compared with normal control rats. Alcohol rats with *Bacopa monnieri* treatment, showed significant ($p < 0.01$) increases in ascorbic acid and uric acid levels and decrease in MDA level, which reflects restoration of the antioxidant enzyme systems and MDA levels to near-normal values (Table. 1)

Table 1: Effect of *Bacopa monnieri* extract on lipid peroxidation (MDA), uric acid and ascorbic acid levels in rats with alcohol induced oxidative stress in rat brain

ENZYMES	MDA Ψ	Ascorbic acid Ψ Ψ	Uric acid Ψ Ψ Ψ
Normal Control (NC)	174.833±6.82	2.481±0.015	19.674±0.352
Alcohol treated (At)	233.041±7.133* (+33.29)	1.553±0.013* (-37.4)	12.712±0.308* (-54.7)
Bacopa treated (BM.t)	174.791±5.107* (-0.081)	2.516±0.018* (+1.41)	19.743±0.0424* (+0.416)
Alcohol Plus Bacopa (At+BM.t)	206±5.11** (+16.95)	2.047±0.146** (-17.49)	16.383±0.311** (+28.87)

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).

Ψ The values are expressed in μ moles of Malondialdehyde formed/ gm wet weight of the tissue.

Ψ Ψ The values are expressed in mg of Ascorbic acid/ gm wet weight of the tissue.

Ψ Ψ Ψ The values are expressed in mg of Uric acid/ gm wet weight of the tissue.

3.2 Effects of *Bacopa monnieri* on membrane bound enzymes in alcohol-induced rats

Fig1-3 shows the effect of *Bacopa monnieri* extract on the activities of Na^+/K^+ , Mg^{2+} and Ca^{2+} ATP ases (membrane bound enzymes) in different experimental groups. In alcohol treated rats, The activities of Na^+/K^+ , Mg^{2+} and Ca^{2+} ATP ases, were Significantly ($p < 0.001$) lower than normal rats. Treatment with *Bacopa monnieri* 200 mg/kg/day resulted in higher the activities of Na^+/K^+ , Mg^{2+} and Ca^{2+} ATP ases as compared to alcohol treated rats. The protective effect of *Bacopa monnieri* extract against alcohol oxidative damage was evidenced by increased enzymes and decreased MDA levels in the brain of rats.

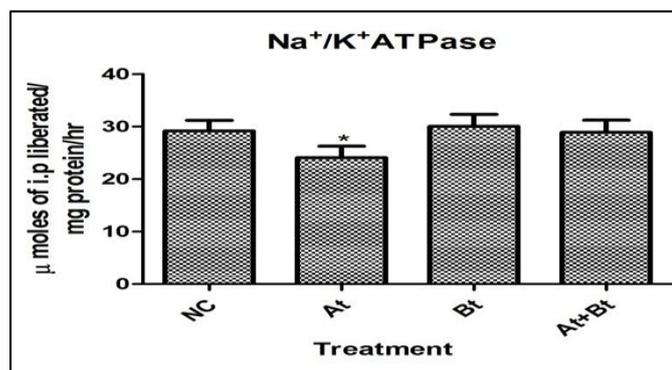


Fig 1: Effect of Alcohol (At), *Bacopa monnieri* treatment (BM.t) and the combination (At + Bt) on brain Na^+/K^+ ATPase enzyme activity. Values are significantly different ($P < 001$) compared with *normal control (NC).

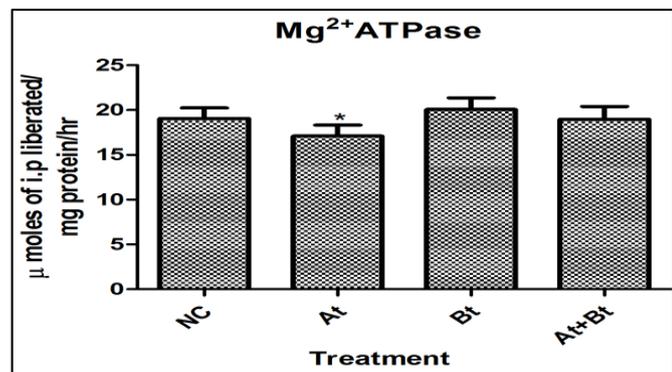


Fig 2: Effect of Alcohol (At), *Bacopa monnieri* treatment (BM.t) and the combination (At + BM.t) on brain Mg^{2+} ATPase enzyme activity. Values are significantly different ($P < 001$) compared with *normal control (NC).

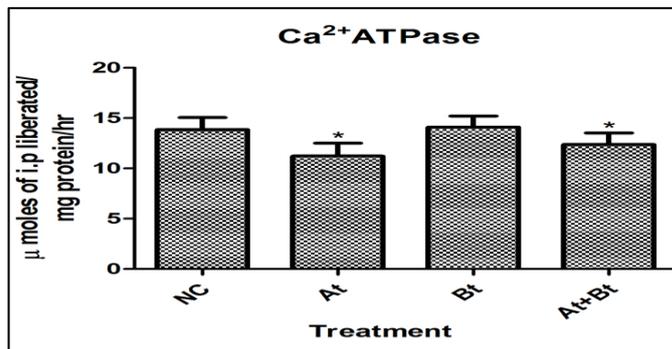


Fig 3: Effect of Alcohol (At), *Bacopa monnieri* treatment (BM.t) and the combination (At + BM.t) on brain Ca^{2+} ATPase enzyme activity. Values are significantly different ($P < 001$) compared with *normal control (NC).

3.3 Effect of *Bacopa monnieri* Extract on Histopathological Changes

In figure 4 At photomicrograph illustrates the progressive derangement of neuronal cell with alcohol feeding, and the fourth image showed recovery from the damage, by alcohol plus *Bacopa monnieri* combination treatments (At + BM.t). We observed diffused cellular infiltration, degenerative changes in neurons, edema and separation of cells in the brain of alcohol intoxicated rats. The damaged cells were appeared to restore with *Bacopa monnieri* plus ethanol treatment, which indicates protective effect of *Bacopa monnieri* on neuronal cells.

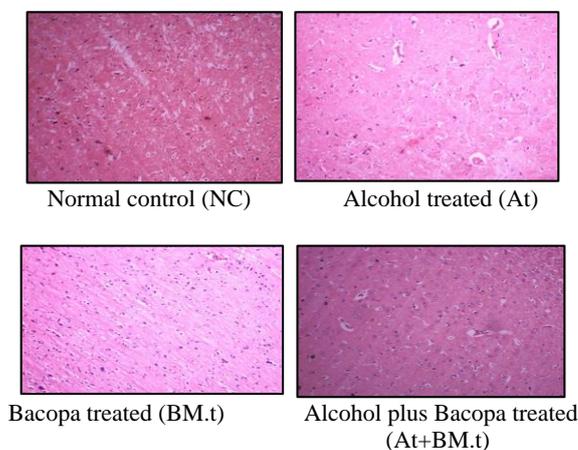


Fig 4: Effect of Alcohol (At), *Bacopa* (BM.t) and combination of both Alcohol plus *Bacopa* (At + BM. t) on histopathological changes in rat brain.

4. Discussion

Alcohol induced changes in brain for a period of time can ruin the antioxidant homeostasis, membrane bound enzymes (Na^+/K^+ , Mg^{2+} and Ca^{2+} -ATPases) produce structural derangement in brain that may contribute to the development of neurodegenerative diseases. In this study, we demonstrated that *Bacopa monniera* was able to attenuate the alcohol induced increased lipid peroxidation and restored the decreased activities of the antioxidants uric acid, ascorbic acid levels in the brain of rats. Strong supportive evidences from histopathological study further indicated the tissue protective properties of *Bacopa monniera* as the rescued mild edema and slight vacuolation were observed. Since neurodegenerative diseases could be influenced by structural damage the brain and preventing brain damage by the supplementation of *Bacopa monniera* plant extract implies beneficial effects of *Bacopa monniera* against alcohol toxicity.

Lipid peroxidation represents excessive production of the free radicals, which attack cellular biomolecules (Sarrafzadeh *et al.*, 2000) [22]. Brain contains high concentrations of polyunsaturated fatty acids, and has high oxygen demand which makes it more prone to damage by free radicals (Halliwell *et al.*, 1992) [23]. Malondialdehyde (MDA), a marker of lipid peroxidation was significantly elevated with alcohol intoxication in the brain tissue. Earlier reports have also shown an increase in MDA levels following exposure to alcohol (Shailender Singh Chauhan *et al.*, 2013) [24]. In the present study, we found a significant reduction in MDA levels in group 4 rats, which received *Bacopa monniera* along with alcohol for a period of 6 weeks. This result suggests that *Bacopa monniera* extract can protect the neuronal cells from alcohol-induced peroxidative damage. It was also demonstrated that the major pungent constituent in *Bacopa monniera*, bacoside-A exhibits antioxidative effect against peroxidation of phospholipids and scavenges the various free radicals.

The enzymatic antioxidant system plays a frontline defense against ROS toxicity in brain cells and other tissues. In the present study, uric acid levels were decreased in the brain tissue of alcohol ingested rats. This may be due to inhibition of adenine nucleotide turnover. Another possibility that high amount of uric acid may have been utilized for scavenging the free radicals which are generated during alcohol intoxication. Reactive oxygen species react with lipids and because peroxidative changes that result in elevated lipid peroxidation. The increased lipid peroxidation with alcohol may be an indication of a decrease in non enzymatic antioxidants of defense mechanisms. (Shanmugham *et al.*, 2010) [25] reported that with alcohol treatment renal uric acid levels were decreased. The decreased uric acid levels in the alcohol treated group may be due to alternations in the metabolism of purines. Serum uric acid level is known to be increased by alcohol via alcohol induced activation of adenine nucleotide turnover which was triggered by the acetate formed from alcohol (Puig *et al.*, 1984) [26]. However, with administration of *Bacopa monniera* to alcoholic group uric acid level was increased. This may be due to the reduction of ROS and free radicals deleterious effect by *Bacopa monniera* in alcohol treated rats.

We have found that the level of Ascorbic acid in the brain were lower in alcohol administered rats than in the control. The earlier reports have also been demonstrated ascorbic acid was decreased during ethanol intoxication (Svensson *et al.*, 1992) [27]. Subir Kumar Das *et al.*, (2007) [28] reported similar

results in the alcoholic rat brain respectively. The decrease level of brain ascorbic acid in alcohol treated rats only could be as a result of increased utilization of antioxidant in scavenging the free radicals generated during acute alcohol intoxication. In the present study with *Bacopa monniera* treatment in alcohol treated rats, ascorbic acid level was increased. This may be due to the influence of *Bacopa monniera* compounds on the reactive oxygen species which were produced during alcohol metabolism. Thus, *Bacopa monniera* may exert a beneficial effect in countering the toxic free radicals in the brain.

In our present study, we observed that there was a significant decrease in Na^+/K^+ -ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase activities in alcohol induced rat brain. This may be due to free radical induced lipid peroxidation and protein oxidation in cell membrane followed by the alteration of the membrane fluidity, enzyme properties and ion transport (Hall *et al.*, 1989) [29]. The membrane-bound ATPases are integral proteins responsible for the maintenance of ion homeostasis through active transport and control of delicate chemical gradient that is necessary for the optimal function of the central nervous system (Dzhafaroz *et al.*, 1989) [30]. Any alteration in the membrane lipid components of brain results in the inactivation of these membrane-bound enzymes (Barriviera *et al.*, 1996) [31]. 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 lyses (Romero *et al.*, 1998) [32]. Administration of *Bacopa monniera* plant extract to alcoholic rats reverted the diminished Na^+/K^+ -ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase activities to near normal. This result suggests that the inhibition of drug on reactive species and lipid peroxidation may restore membrane fluidity and hence the functional ability of associated enzymes.

Another novel finding from this study is that Alcohol-induced architectural changes in brain were modulated by six weeks of treatment with *Bacopa monniera* extract. Continuous exposure to Alcohol alone produced damages in the brain as seen by mild edema, excesses of lymphocytes, and division of cells through microscopic images. These structural derangements in the brain may lead to tissue damage and impair cognitive functions. Therefore, there may be more Alcohol induced structural damages in the brain. However, co-administration of *Bacopa monniera* extract along with Alcohol showed its protective effects against Alcohol-induced architectural damage. The lower extents of formation of cavities, less lymphocytes and no partition of cells indicated tissue protective properties of the *Bacopa monniera* extracts. Since ROS plays a key role in tissue damage, the beneficial outcome of *Bacopa monniera* could be associated with enhanced antioxidant defense system in the brain. Several naturally occurring compounds, including flavonoids, alkaloids and saponins (bacoside A and B), were identified in *Bacopa monniera* extracts. These compounds have been claimed to be the responsible factors for most of the pharmacological efficacies of the extracts, such as antioxidant and neuroprotective properties and enhancing the memory and learning skills (Russo *et al.*, 2005) [33].

5. Conclusion

On the basis of the results obtained in the present study, it is concluded that alcohol-induced oxidative stress in rat brain is amenable to attenuation by *Bacopa monniera* extract. The

protective effect of *Bacopa monnieri* extract can be correlated directly with its ability to reduce the rate of lipid peroxidation as well as it restored the enhance brain membrane bound enzymes. The findings of this study suggest that *Bacopa monnieri* can be used as a safe, cheap, and effective alternative chemo preventive and protective agent in the management of alcohol-related brain diseases.

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