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D Veera Nagendra Kumar
Assistant Professor,
Division of Physiology and Ethnopharmacology,
Department of Zoology,
Government Degree College,
Porumamilla, Andhra Pradesh,
India.

K Lakshmi Narasaiah
Assistant Professor
Department of Botany,
Government Degree College,
Porumamilla, Andhra Pradesh,,
India.

S Prakash Rao
Assistant Professor
Department of Chemistry,
Government Degree College,
Porumamilla, Andhra Pradesh,
India.

Correspondence:
D Veera Nagendra Kumar
Assistant Professor,
Division of Physiology and Ethnopharmacology,
Department of Zoology,
Government Degree College,
Porumamilla, Andhra Pradesh,
India.

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 (GPx), 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, Srilanka, 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], antiulcerogenic 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/CPCSCA/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 macerate in 1000 ml of 95% ethanol for 12 h at room temparture, 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 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 at 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 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*. [23] using bovine serum albumin (BSA) as a standard.

2.5 Chemicals
All the chemicals used in the present study were analar Grade (AR) and obtained from the following significant companies: Sigma (St.Louis, MO, USA), Fischer (Pitrsburg, PA, USA), Merk (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (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* Ethanolic 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 Ethanolic extract of *Bacopa monnieri* on Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) in alcohol induced oxidative stress in rat liver

Enzymes	SOD Ψ	CAT Ψ Ψ	GPx Ψ Ψ Ψ
Normal control (NC)	13.432±0.396	0.332±0.019	0.796±0.039
Alcohol treated (At)	7.969±0.486* (−30.860)	0.256±0.049* (−24.594)	0.506±0.028* (−53.31)
Bacopa treated (Bt)	16.666±.940* (+55.875)	0.535±0.102* (+46.355)	1.565±0.088* (+68.836)
Alcohol plus Bacopa (At+Bt)	14.303±0.531** (+19.466)	0.516±0.026** (+33.310)	1.144±0.243** (+42.410)

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),
alcohol treated(** < 0.01) (Dunnett's multiple comparison test).
Ψ Values are expressed in units of superoxide anion reduced/mg protein/min.
Ψ Ψ Values are expressed in µ moles of H2O2 degraded/mg protein/min.
Ψ Ψ Ψ 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

Enzymes	GR Ψ	GSH Ψ Ψ	MDA Ψ Ψ Ψ
Normal control (NC)	0.698±0.042	104.925±1.214	64.635±2.013
Alcohol treated (At)	0.35±0.018* (−51.977)	53.086±2.445* (−51.112)	113.24±3.602 * (+88.365)
Bacopa treated (Bt)	0.812±0.039* (+27.067)	166.853±2.635 * (+52.823)	42.55±3.317* (−21.521)
Alcohol plus Bacopa (At+Bt)	1.112±0.164 ** (+67.696)	138.484±3.333 * * (+45.567)	97.906±3.576 ** (+62.804)

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).
Ψ Values are expressed in µmol of NADPH oxidized/mg protein/min.
Ψ Ψ Values are expressed in µmol of glutathione/g wet weight of the tissue.
Ψ Ψ Ψ Values are expressed in µmol of malondialdehyde/g wet weight of the tissue.

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

Enzymes	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control (NC)	72.02 ± 14.22	41.36 ± 4.57	66.04±2.56
Alcohol treated (At)	128.22± 5.10* (+62.333)	76.54 ±11.12* (+74.265)	132.6±6.66* (+82.286)
Bacopa treated (Bt)	66.25±2.043* (-46.061)	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)

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 $O_2^{\cdot -}$, H_2O_2 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 treatment 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 hepato protective 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 iswell 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 [48]. 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 methanol 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

1. Lieber CS. Alcohol and the liver: Metabolism of alcohol and its role in hepatic and extra hepatic diseases. Mt Sinai J Med. 2000; 67**:**84-94.
2. Puzziferri I, Signorile A, Guerrieri F, Papa S, Cuomo V, Steard O. Chronic low dose ethanol intake: biochemical characterization of liver mitochondria in rats. Life Sci. 2000; 66**:**477-484.
3. Reddy SK, Husain K, Schlorff EC, Scott RB, Somani SM. Dose response of ethanol ingestion on antioxidant defense system in rat brain subcellular fractions. Neurotoxicology. 1999; 20:977-87.
4. Husain K, Mejia J, Lalla J, Kazim S. Dose response of alcohol induced changes in BP, nitric oxide and antioxidants in rat plasma. Pharmacol Res. 2005; 1:337-43.
5. Zima T, Fialova L, Metsek O, Janebova M, Crkovska J, Malbohan I *et al*. Oxidative stress, metabolism of ethanol and alcohol-related diseases. Journal of Biomedical Science. 2001; 8:59-70.
6. Stough C, Lloyd J, Clarke J, *et al.* The chronic effects of an extract of *Bacopa monnieri* (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology. 2001; 156:481-484.
7. Jadiya P, Khan A, Sammi SR, Kaur S, Mir SS, Nazir A. Anti-Parkinsonian effects of *Bacopa monnieri*: insights from transgenic and pharmacological Caenorhabditis elegans models of Parkinson's disease. Biochemical Biophysical Research Communications. 2012; 413(4):605-610.
8. Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized extract of *Bacopa monnieri* in experimental models of depression in rats. Phytomedicine. 2002; 9:207-211.
9. Uabundit N, Wattanathorn J, Mucimapura S, Ingkaninan K. Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease model. Journal of Ethnopharmacology. 2010; 127:26-31
10. Bhattacharya SK. Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *Bacopa monnieri* in rat frontal cortex, striatum and hippocampus. Phytotherapy Research. 2000; 14(3):174-179.
11. Rao CHV, Sairam K, Goel RK. Experimental evaluation of *Bacopa monnieri* on rat gastric ulceration and

secretion. Indian J Physio Pharmacol. 2000; 44:435-441.
12. Channa S, Dar A, Anjum S, Yaqoob M. Antiinflammatory activity of *Bacopa monnieri* in rodents. Journal of Ethnopharmacology. 2006; 104(1-2):286-289.
13. Sundriyal A, Rawat DS. Singh AK. Tissue culture, phytochemical and pharmacological study of *Bacopa monnieri*. Asian Journal of Biochemical and Pharmaceutical Research. 2013; 1(3):243-260.
14. Reas SK, Amee K, Paulose CS. Glutamate receptor gene expression and binding studies in pilocarpine induced epileptic rat: neuroprotective role of *Bacopa monnieri* extract. Epilep Behav. 2008; 12:54-60.
15. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170-3175.
16. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984; 105:125-126.
17. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol.1984; 105:114-121.
18. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol. 1985; 113:484-490,
19. Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. Methods Enzymo. 1981; l 77:373-382.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351-358.
21. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28:56-63.
22. Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum. J Biol Chem. 1946; 164:321-9.
23. Lowry OH, Rosebrough NC, Farr AL *et al.* Protein measurement with the folin-phenol reagent. J Biol Chem. 1951; 193:265-275.
24. Galazyn-Sidorczuk M, Brzoska MM, Jurczuk M, *et al.* Oxidative damages to protein and DNA in rats exposed to cadmium and/or ethanol. Chem Biol Interact. 2009; 180:31-38.
25. Sumathy T, Subramanian S, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monnieri* on morphine induced hepatotoxicity in rats. Phytotherapy Research. 2001; 15:643-645.
26. Radhika Kapoor, Saurabh Srivastava, Poonam Kakkar. *Bacopa monnieri* modulates antioxidant responses in brain and kidney of diabetic rats. Environmental Toxicology and Pharmacology. 2009; 27:62-69.
27. Jurczuk M, Brzoska MM, Moniuszko-Jakoniuk J, *et al.* Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. Food Chem Toxicol. 2004; 42:429-438.
28. Dinu D, Nechifor MT, Movileanu L. Ethanol-induced alterations of the antioxidant defense system in rat kidney. J Biochem Mol Toxicol. 2005; 19:386-395.
29. Hou, CC, Lin SJ, Cheng JT, Hsu FL. Bacopaside III, bacopasaponin G, and bacopasides A, B, and C from *Bacopa monnieri*. J Nat Prod. 2002; 65:1759-1763.
30. Mallikarjuna K, Sahitya Chetan P, Sathyavelu Reddy K, Rajendra W. Ethanol toxicity: Rehabilitation of hepatic antioxidant defense system with dietary ginger. Fitoterapia. 2008; 79:174-178.
31. Somani SM, Husain K, Diaz-Phillips L, *et al.* Interaction of exercise and ethanol on antioxidant enzymes in brain regions of the rat. Alcohol. 1996; 13:603-610.
32. Tripathi YB, Chaurasia S, Tripathi E, Upadyay A, Dubey GP. Bacopa *monnieri* Linn. As an antioxidant: mechanism of action. Indian Journal of Experimental Biology. 1996; 34:523-526.
33. Pigeolet E, Corbisier P, Houbion A, *et al.* Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Dev. 1990; 51:283-297.
34. Veera Nagendra Kumar D, Shanmugam KR, Ramakrishna CH, Venkata Subbaiah G. Sathyavelu Reddy K. *Bacopa monnieri* modulates antioxidant enzymes responses against alcohol-induced oxidative stress in rat brain. Indo American journal of pharmaceutical research. 2016; 6:4569-4575.
35. Ulusu NN, Sahilli M, Avci A, Canbolat O, Ozansoy G, Ari N. *et al.,* Pentose Phosphate Pathway, Glutathione-Dependent Enzymes and Antioxidant Defense during Oxidative Stress in Diabetic Rodent Brain and Peripheral Organs: Effects of Stobadine and Vitamin E. Neurochemical Research. 2003; 28:815-823.
36. Dey A, Lakshmanan J. The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver. Food Funct. 2013; 4:1148-1184.
37. Swaroopa M. Remediation of hepatic antioxidant defense system during ethanol withdrawal stress with dietary ginger. European Journal of Experimental Biology. 2013; 3(5):262-267.
38. Chakravarty AK, Garai S, Masuda K, Nakane T, Kawahara N. Bacopasides III-IVP; three new triterpenoid glycosides from *Bacopa monnieri*. Chem Pharm Bull. 2003; 51:215-217.
39. Mahapatra SK, Das S, Dey SK, Roy S. Smoking induced oxidative stress in serum and neutrophil of the university students. Al Ameen J Me Sci. 2008; 1: 20-31.
40. Konukoglu D, Serin O, Kemerli DG, Serin E, Hayiroglu A, Oner B. A study on the carotid artery intima-media thickness and its association with lipid peroxidation. Clin Chim Acta. 1998; 277:91-98.
41. Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J Trace Elem Med Biol. 1995; 9:144-149.
42. Rohini G, Sabitha KE, Devi CSS. *Bacopa monnieri* Linn. Extract modulates antioxidant and marker enzyme status in fibrosarcoma bearing rats. Indian Journal of Experimental Biology. 2004; 42:776-780.
43. Nordmann R. Alcohol and antioxidant systems. Alcohol and Alcoholism. 1994; 29:513-522.
44. Das SK, Vasudevan DM. Effect of ethanol on liver antioxidant defense system: a dose dependent study. Indian J Clin Biochem. 2005; 20:80-84.
45. Sudha, Srinivasan P, Manikandaselvi S, Thinagarbabu R. Protective effect and antioxidant role of *achyranthus aspera* l. Against ethanol-induced oxidative stress in rats. International journal of pharmacy and pharmaceutical sciences. 2012, 485-92.
46. Kishore K, Singh M. Effect of bacosides, an alcoholic extract of *Bacopa monnieri* Linn. (brahmi), on experimental amnesia in mice. Indian J Exper Biol. 2005; 43:640-645.

47. James WPT. Alcohol: Its metabolism and effects. In: Garrow JS, James WPT. (Eds.), Human Nutrition and Dietetics, Churchill Livingstone, London. 1993, 103-118.
48. Rajagopal SK, Manickam P, Periyasamy V, Namasivayam N. Activity of Cassia auriculata leaf extract in rats with alcoholic liver injury. J Nutr Biochem. 2003; 14:452-458.
49. Singha PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees against ethanol-induced toxicity in mice. J Ethnopharmacol. 2007; 111:13-21.
50. Rajakrishnan V, Menon VP. Potential role of antioxidants during ethanol induced changes in the fatty acid composition and arachidonic acid metabolites in male Wistar rats. Cell Biol Toxicol. 2001; 17:11-22.
51. Adewusi EA, Afolayan AJ. Effect of *Pelargonium reniforme* roots on alcohol induced liver damage and oxidative stress. Pharm Biol. 2010; 48:980-987.
52. Pradeep K, Raj Mohan CV, Gobianand K, Karthikeyan S. Protective effect of *Cassia fistula* Linn. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biol Res. 2010; 43(1):113-25.