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THERAPEUTIC POTENTIAL OF *BACOPA MONNIERI* AGAINST ALCOHOL INDUCED RENAL DAMAGE IN MALE ALBINO RATS

D. Veera Nagendra Kumar*[1]

[1*]Department of Zoology, Government Degree College, Porumamilla, Kadapa, Andhra Pradesh-516193, India.

ABSTRACT

Objective: This study was to evaluate the potential of successive *Bacopa monnieri* extract against nephrotoxicity induced by Alcohol in rats. The evaluation was done through measuring kidney antioxidant status and renal damage markers. **Materials and Methods:** For this study, the rats were divided into four groups (n = 6 in each group): normal control (NC), *Bacopa monnieri* treated (Bm.t), alcohol treated (Al.t), and alcohol plus *Bacopa monnieri* treated (Al.t + Bm.t). *Bacopa monnieri* was given to the Alcohol treated group for 30 days and renal antioxidant enzymes were assayed. **Results:** Renal antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities, the levels of glutathione and urea were significantly ($P < .001$) decreased, whereas malondialdehyde levels and creatine, urea, uric acid were elevated in Alcohol treated group. However, *Bacopa monnieri* extract supplementation to the Alcohol treated rats reversed these effects and attained the antioxidant status and renal damage markers to normal levels. **Conclusion:** This study concludes that alcohol-induced nephro-toxicity was attenuated by *Bacopa monnieri* extract treatment, thus *Bacopa monnieri* can used as a regular nutrient to protect the renal cells.

KEYWORDS

Alcohol, *Bacopa monnieri*, Antioxidant enzymes, MDA and Renal damage markers.

Author for Correspondence:

Veera Nagendra Kumar D,
Department of Zoology,
Government Degree College, Porumamilla,
Kadapa, Andhra Pradesh-516193, India.

Email: veeranagendrakumar@gmail.com

INTRODUCTION

Alcoholic beverages are used universally and alcohol is the world's most widely used psychoactive drug, but chronic, excessive alcohol consumption leads to permanent organ damage or death[1]. Liver is the primary organ responsible for the oxidation of ingested alcohol, but other tissues, including the kidney, may contribute to alcohol metabolism as well[2].

The alcohol oxidation by kidney is favored in alcohol-treated rats, thereby suggesting a pathogenic role for acetaldehyde in the nephrotoxic effect of alcohol ingestion. Regular alcohol consumption raises the blood pressure, which is a risk factor for renal damage[3]. Also, increased ROS, partly generated from acetaldehyde oxidation, may contribute to the occurrence of oxidative stress in kidney tissue[4]. Oxidative stress and ROS-mediated toxicity have been considered as the primary routes of alcohol induced ultra structural changes in kidney[5] and play a central role in the development of alcoholic related diseases[6].

Earlier studies reported that chronic alcohol consumption can deplete the antioxidant enzyme status in particular superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and increased the peroxidative damage in the kidney of rats. ROS-induced altered antioxidant system further continued to damage the vital bimolecular, and this condition ultimately impaired the kidney function[7,8].

However, treatment of alcohol-induced renal oxidative injuries by antioxidant drugs has not received a wide recognition. In spite of the tremendous advances made in allopathic medicine, no effective renal protective medicine is yet available. Drugs of plant origin are known to play a vital role in the management of kidney diseases, and have protective effect against oxidative stress in rats.

Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. *Bacopa monnieri* is one of the world's best known spices, used since time immemorial for its health benefits. *Bacopa monniera* Linn. Is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as insomnia, anxiety, epilepsy, hysteria etc[9]. Preclinical and clinical studies have shown that *Bacopa monnieri* improves memory and mental function[10,11]. The plant has been shown as a potent free radical scavenger and antioxidant[12]. Besides it also exhibits vasodilatory[13], calcium antagonistic[14] properties. Preliminary studies indicated that bacosides, the major saponins are responsible for the facilitatory and modulatory effects of *Bacopa monnieri*[15]. Recently from our laboratory, we reported the hepato-protective effect of *Bacopa monniera*, which revealed an up-regulation in antioxidant status and a decrease in malondialdehyde (MDA) levels in alcohol-treated rats. However, the nephroprotective effect of *Bacopa monnieri* extract against alcohol-induced toxicity is not yet studied fully. Hence, in the present study we made an attempt to explore the nephro-protective effect of *Bacopa monnieri* extract against alcohol-induced oxidative stress. The protective effects of *Bacopa monnieri* extract have been monitored by assaying the antioxidant enzymes and MDA levels in alcohol ingested rats.

METERIAL AND METHODS

Animals

Male Wistar albino rats aged 3 months and weighing 180 ± 20g were obtained from Indian Institute of Science, Bangalore, Karnataka, India. The rats were housed in clean polypropylene cages having six rats per cage and maintained in a temperature controlled room (27 ± 2°C) with a photoperiod of a 12-h light and 12-h dark cycle. The rats were given standard pellet diet (Lipton Rat Feed, Ltd., Pune, and Maharashtra, India) and water ad libitum throughout the experimental period. The experiments were carried out in accordance with CPCSEA guidelines and the protocol was approved by the Institutional Animal Ethics Committee (regd.no. 09 (ii) / a / CPCSCA/IAEC/07-08/SVU/Zool/ DVNK/dated 26/6/08.

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).

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 1000ml 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, 50mg/kg, 100mg/kg, 150mg/kg, 200mg/kg, 250mg/kg and 300mg/kg. Of this 200mg/kg dose showed good antioxidant activity. So this study we selected dose of 200mg/kg of ethanol extract of *Bacopa monnieri.*

Grouping of animals

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

Group No.1

Normal control (NC): This group of rats received vehicle solution (2% of Tween 80).

Group No.2

Alcohol treatment (Al.t): Rats received alcohol orally at a dose of (2 g/kg body weight orally for 30 days as per the method of Mallikarjuna et al.

Group No.3

Bacopa monnieri treatment (Bm.t): Rats received ethanolic extract of *Bacopa monnieri* (200 mg/kg body weight orally for 30 days).

Group No.4

Alcohol treatment + *Bacopa monnieri* treatment (Al+ Bm.t): This group of rats received both alcohol and *Bacopa monnieri* as described in group 2 and group 3 for 30 days.

Alcohol was given first, and then *Bacopa monnieri* was administered orally to the same rats within 5 minutes.

After 24 hours of the last treatment, all the animals were euthanized and kidney 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.

Biochemical assays

The selected antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and the content of glutathione (GSH) and MDA (lipid peroxidation) levels were estimated by employing the methods of Misra and Fridovich[16], Aebi[17], Flohe and Gunzler[18] Carlberg and Mannervik[19], Theodorus *et al*[20] and Ohkawa *et al*[21], respectively. Non-protein nitrogen constituents were determined by the methods of Patton and Crouch (1977) for urea, Fossati *et al*, (1980) for uric acid and Bartels and Bohmer (1972) for creatinine.

Statistical analysis

The data were analyzed by using SPSS (Version 13.5; 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 were compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at $P < 0.001$.

RESULTS AND DISCUSSION

In this study, crucial defense mechanism against alcohol-induced oxidative stress has been evaluated by determining the antioxidant enzyme activities. It was found that renal SOD enzyme

Activity was significantly (P<.001) decreased with alcohol consumption and increased with *Bacopa monnieri* alone treatments (Figure No.1). However, the decreased SOD activity due to alcohol treatment was ameliorated with *Bacopa monnieri* extract combination. Here, we observed that SOD enzyme activity in combination treated group was restored to near normal ones.

Figure No.2 shows that renal CAT enzyme activity in alcohol-fed rats was significantly decreased compared with normal controls. Whereas, the CAT activity in *Bacopa monnieri* plus alcohol received group was significantly increased than that of alcohol alone treated group. The elevation in CAT activity with combination treatment was lower than that of *Bacopa monnieri* alone treatment.

A significant decrease in renal GPx activity was noticed in alcohol treated rats when compared with the normal control rats. No significant change in GPx activity with *Bacopa monnieri* alone treatment

was observed. However, GPx activity was increased with *Bacopa monnieri* plus alcohol combination treatment compared with alcohol alone treated rats (Figure No.3).

Figure No.4 represents the effect of *Bacopa monnieri* extract, alcohol intoxication and their combination on renal GR enzyme activity alterations. GR activity was significantly (P<.001) lower in alcohol treated rats when compared with the normal controls. Whereas, in group IV a significant increase in GR activity was noticed when compared with alcohol alone treated rats.

The oxidative stress markers such as, GSH and MDA values were represented in Figures 5 and 6.

The changes in GSH and MDA content with different treatments are inverse proportional to each other in comparison. Individually, a depletion in GSH and elevation in MDA levels were observed with alcohol alone treatment compared with normal control rats. Nevertheless, these adverse effects of alcohol in renal tissue were reversed with *Bacopa monnieri* supplementation in group 4 rats. The protective effect of *Bacopa monnieri* extract against alcohol oxidative damage was evidenced by increased GSH content and decreased MDA levels in the kidney of rats.

Kidney function markers

As shown in Table No.1, the alcohol produced significant increase in the levels of plasma creatinine, urea and uric acid when compared with normal group, while, administration of ginger extract to the alcoholic rats significantly reduced the levels of plasma creatinine, urea and uric acid when compared with the alcoholic group, but no significant changes were observed when compared with the normal rats. This indicates that, treatment with *Bacopa monnieri* extract normalized the plasma creatinine, urea and uric acid.

Discussion

The etiology of free radicals in alcohol kidney disease is well established (Befrits *et al*, 1995). The results of the present study show that supplementation of *Bacopa monnieri* may protect the renal cells and reduces the severity of damage due to alcohol toxicity.

In the current investigation, we observed a decreased activity of SOD in the alcohol-treated group. Mallikarjuna *et al*[22], reported that alcohol administration depleted the SOD activity in the kidney and liver tissues of albino rats. Several studies have reported that alcohol consumption decreases the SOD activity in the liver, heart, brain, kidney muscle, and serum[1]. The reduced activity of SOD in the presence of alcohol may cause the accumulation of $O2^{\bullet}$, H_2O_2, or the products of its decomposition. This may be due to the excessive production of free radicals and superoxide radicals, so the SOD activity was decreased to counter the same. However, *Bacopa monniera* supplemented to alcohol treated group exhibits increased SOD activity. 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[23].

Catalase (CAT) plays a vital role in decomposition of hydrogen peroxide (H_2O_2) into water and

Oxygen thus protects the cells from oxidative damage caused by H_2O_2. In this study, we observed that renal CAT activity was significantly decreased in alcohol ingested rats than that of control rats. Balasubramaniyan *et al*[24], reported that the CAT activity in kidney and liver was decreased in the alcohol-treated group. The decreased CAT activity indicates inefficient scavenging of hydrogen peroxides, due to oxidative inactivation of enzyme[25]. Decrease in both SOD and CAT activities with alcohol consumption may cause the accumulation of O_2 and H_2O_2 which results in oxygen intolerance and triggers a number of deleterious reactions. 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[12]. Hence, alcohol-induced oxidative stress might be countered effectively by ginger and maintain the higher CAT activities in renal cells.

The present study showed that also the activity of GSH-Px was significantly decreased in alcohol treated rats, which may disturb the glutathione homeostasis in the renal cells and ultimately leads to the damage of kidney. It was indicates impaired scavenging of H_2O_2 and lipid hydro peroxides. Decreased GPx activity may be due to either inactivation of enzyme by free radicals[26] or depletion of its co-substrates (GSH and NADPH) availability in alcohol treated rats[27]. However, decreased renal GPx activity due to alcohol was increased and reached to control level with *Bacopa monnieri* extract treatment. The activity of GSH-Px was significantly increased with Alcohol and *Bacopa monnieri* and combination treatment group which indicates that Bacopa could inhibit and/or scavenge the free radicals in rat renal tissue. Furthermore, *Bacopa monniera* possesses antioxidant compounds namely bacosides A, B and flavonoids, Battacharya *et al*[28], which may be involved in the antioxidant defense mechanism and protect the kidney tissue against alcohol induced free radicals damage.

Renal GR activity was also decreased significantly in alcohol treated rats. Decrease in GR activity after alcohol ingestion attributed to either increased oxidation or decreased synthesis of GSH[29]. Although GR activity decreased with alcohol treatment, we observed the increased values with alcohol plus *Bacopa monnieri* combination treatment. This might be a *Bacopa monniera* mediated adaptive response from the renal cells to counter the alcohol-induced free radicals. Previous studies also confirmed that increased GR activity with ginger treatment takes place in coping the oxidative damage[30]. 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[31].

Glutathione (GSH) is a foot marker of oxidative stress, and plays a crucial role in maintaining the proper defense against oxidants. In our present study, the GSH levels were decreased in the kidney 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[32] 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 kidney. Hepatoprotective and nephroprotective effect of *Bacopa monnieri* following increased GSH levels have been reported[33].

In the current study, MDA levels were increased in alcohol rats. 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[34]. Unstable ROS that were generated during alcohol metabolism can react with membrane lipids and cause lipid peroxidation. 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 30 days. It was also demonstrated that the major pungent constituent in bacosides and saponins exhibits antioxidative effect against peroxidation of phospholipids and scavenge the various free radicals. Kishore *et al*[35], bacosides and bacopas aponins exhibits antioxidative effect against peroxidates. This result suggests that Bacopa extract can protect the renal cells from alcohol-induced peroxidative damage.

In the current study the effect of *Bacopa monnieri* on the kidney functions was assessed by the determination of the levels of plasma creatinine, urea and uric acid, and the study revealed that post-administration of *Bacopa monnieri* extract to the alcoholic rats reduced and normalized the levels of plasma creatinine, urea and uric acid. Moreover, the

study of Swaroopa *et al*[36], demonstrated that ethanol extract of ginger rendered significant protection against induced nephrotoxicity, which was evident from the lowered serum urea, and creatinine levels in the mice that pre-treated with *Bacopa monnieri* extract, and this study concluded that *Bacopa monnieri* extract significantly protected the elevation of serum creatinine and urea levels. The presence of saponins, and flavonoids in *Bacopa monnieri* extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels.

Table No.1: Effects of *Bacopa monnieri* extract on renal damage Markers of rat serum or plasma during chronic Alcohol administration

S.No	Renal damage markers mg/dl	Normal Control (NC)	Alcohol treated (Al.t)	*Bacopa monnieri* treated (Bm.t)	Alcohol and *Bacopa monnieri* treated (Al+Bm.t)
1	Albumin	3.758±0.45	4.616±0.41*	3.789± 0.56	3.631±0.52**
2	Creatine	0.310±0.026	0.451±0.074*	0.315±0.032	0.342±0.064**
3	Urea	19.383±1.97	16.451±0.074*	19.562±1.98	18.118±1.37**
4	Uric Acid	1.167±0.18	1.955±0.37*	1.175±0.21	1.090±0.24**

Values are expressed as mean ± S.D of 6 animals. Alcoholic control is compared with normal.
Withdrawal groups are compared with both normal and Alcoholic control.
* Values are statistically significant at P* <0.001 when compared with normal.
#Values are statistically significant at P** <0.001 when compared with Alcoholic control.

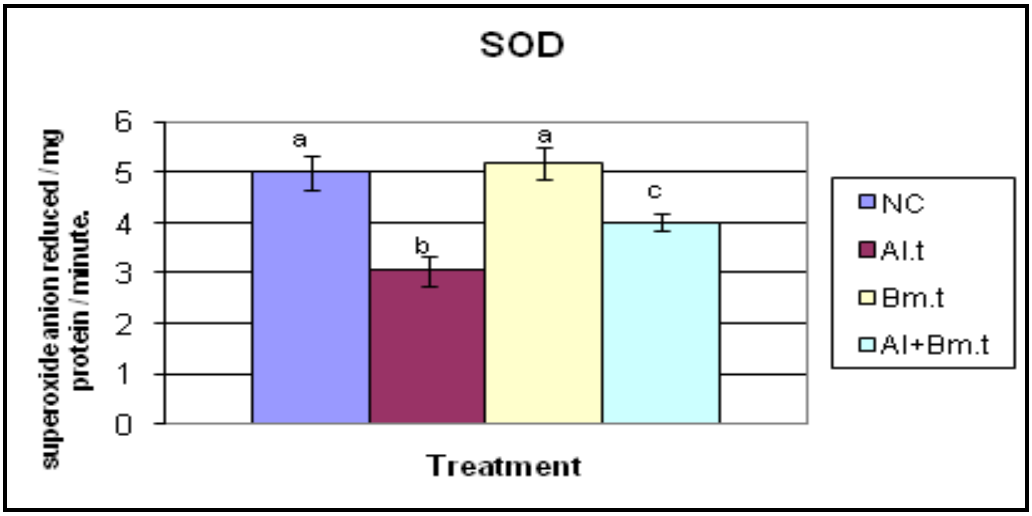

Figure No.1: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney superoxide dismutase (SOD) activity. Values are significantly different ($^{b}P< 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

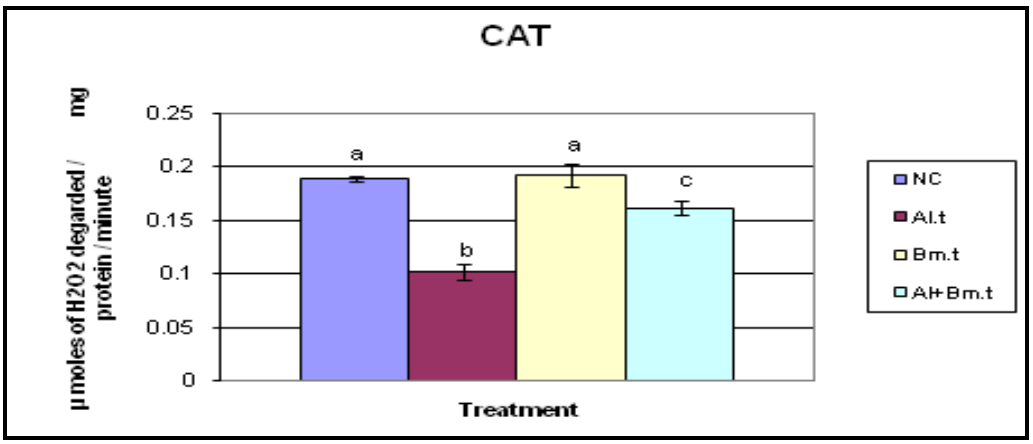

Figure No.2: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney superoxide dismutase (SOD) activity. Values are significantly different ($^{b}P < 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

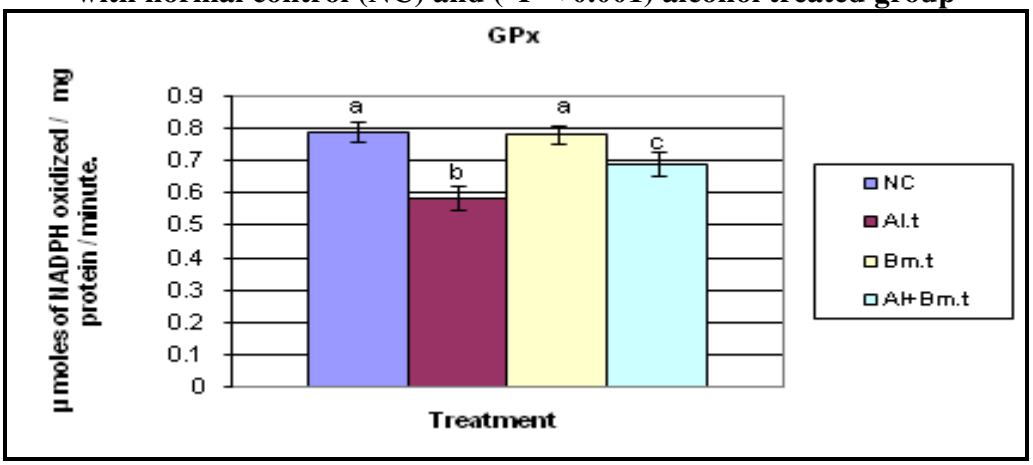

Figure No.3: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney Glutathione peroxidase (GPx) activity. Values are significantly different ($^{b}P < 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

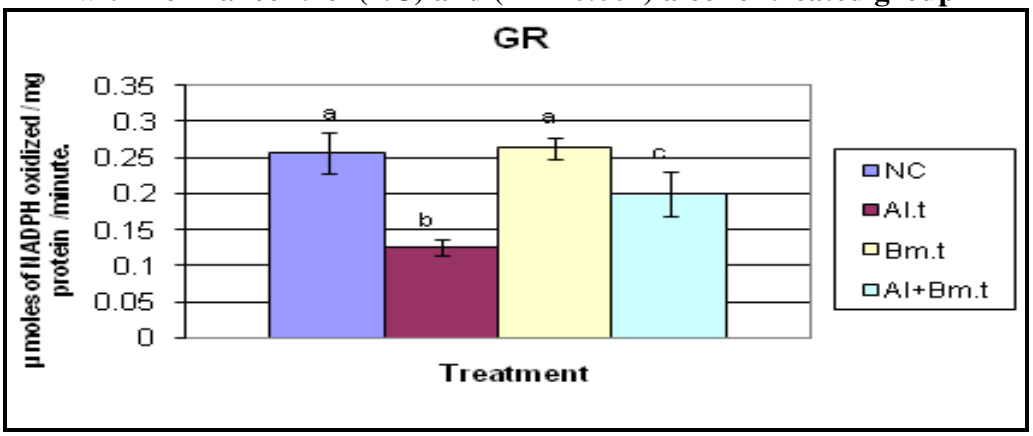

Figure No.4: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney Glutathione redoctase (GPx) activity. Values are significantly different ($^{b}P < 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

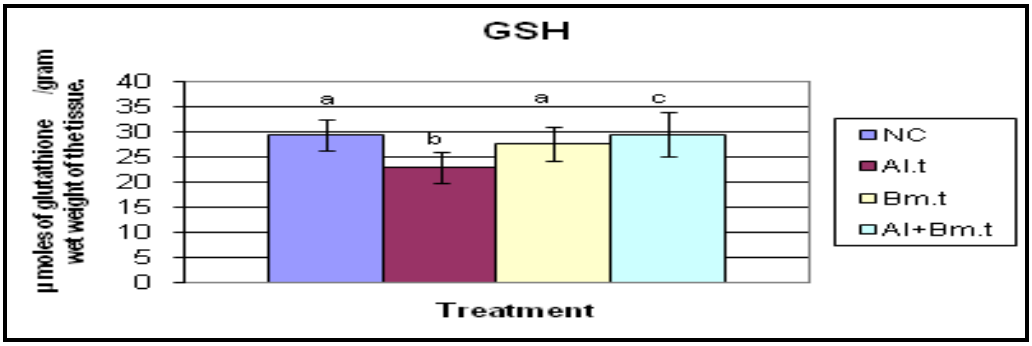

Figure No.5: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney Glutathione level. Values are significantly different ($^{b}P < 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

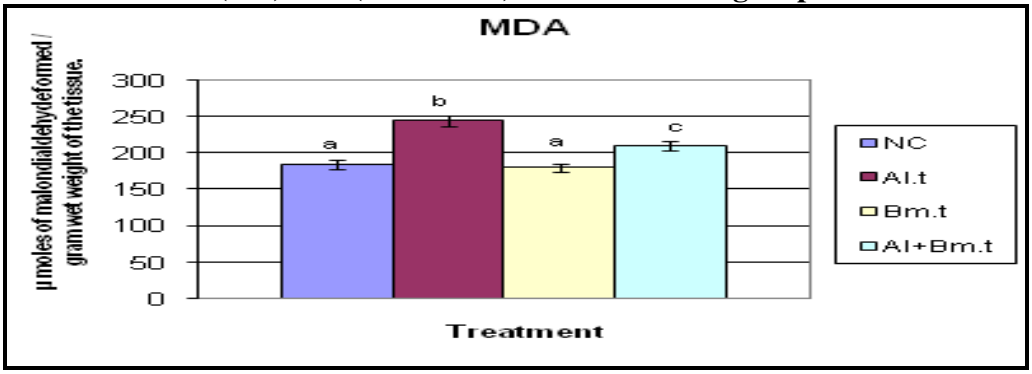

Figure No.6: Effect of Bacopa treatment (Bm.t), Alcohol (Al.t), and the combination (Al + Bm.t) on Kidney MDA level. Values are significantly different ($^{b}P < 0.001$) compared with normal control (NC) and ($^{c}P < 0.001$) alcohol treated group

CONCLUSION

These results confirmed the alcohol-induced nephro-toxicity in terms of decreased antioxidant status, increased lipid peroxidation and damaged renal cells. However, all these adverse effects were reversed by *Bacopa monnieri* supplementation. Thus, this data suggests that *Bacopa monniera* can be used as a nephro-protective nutrient and antioxidant supplement to protect the kidney from alcohol induced oxidative damage.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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