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EFFICACY OF BACOPA MONNIERI ON OXIDATIVE STRESS AND NA^+/K^+, CA^{2+} ATPASEACTIVITY AGAINST ALCOHOL INDUCED MYOCARDIAL DAMAGE

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Abstract:

The myocardium, like other tissues, has enzyme and non-enzyme systems to neutralize free radicals.The present study was investigated the efficacy of *Bacopamonnieri* in alcohol-induced oxidative stress. In this study we assayedcardiacantioxidantenzymesand Na^+/K^+, Ca^{2+}ATPases (membrane bound enzymes). Rats were divided into four equal groups: Normal control (NC), Alcohol treated (Al), *Bacopamonnieri*treated (Bm) and alcohol plus *Bacopamonnieri*treated (Al+Bm). *Bacopamonnieri* was given to the At group for six weeks and cardiacSOD, ascorbic acid, MDA levels and membrane bound enzymes were assayed. Cardiac superoxide dismutase,ascorbic acid, Na^+/K^+, Mg^{2+} and Ca^{2+}ATPases activities were significantly (P >001) decreased, whereas malondialdehyde(MDA) levels were elevated in alcohol treatedgroup. However, *Bacopamonnieri*extract supplementation to the alcohol treated rats reversed these effects and attained the SOD, ascorbic acid, MDA levels and Na^+/K^+, Ca^{2+}ATPases activitiesto normal levels. This study concludes that alcohol-induced cardiac toxicity was attenuated by *Bacopamonnieri*extract treatment,thus *Bacopamonnieri* can used as a regular nutrient to protect the cardiovascular diseases.

Key words: Alcohol, *BacopaMonnieri*, SOD, MDA.,Na^+/K^+, Ca^{2+}ATPases

Introduction:

Oxidant balance in the heart has a very important role in protecting the heart and in allowing normal cardiac contractile performance. In general the amount of antioxidants is sufficient to protect the heart from any oxidant production that might occur under normal circumstances (Kukreja et al., 1992).The response of the body to chronic or acute administration of ethanol has been shown to result in generation of oxygen derived free radicals in many tissues and to cause alterations in cardiac muscle (Whellan et al., 2005) However, the occurrence of myocardial damage is mainly due to hyperlipidemia, loss of plasma membrane integrity and membrane peroxidation (Krushna et al., 2009).Reynolds et al (2003) reported a strong correlation between alcohol intake and sudden cardiac death. Na^+ /K^+ -ATPase is implicated in metabolic energy production as well as in the uptake, storage, and metabolism of catecholamines, serotonin, and glutamate (Carageorgiou et al. 2007). Ca^{2+}-ATPase activity is associated with neuronal excitability, cellular depolarization and fine tunning of Ca^{2+}-channel activity (Lees, 1991).Thebiochemical changes induced by alcohol consumption in the cardiac tissue are not well understood, though some clinical and experimental studies have been focused on the effects of alcohol feeding on renal function including gross and microscopic morphology Mg^{2+}-ATPase activity associated with mitochondrial membrane bound enzyme which is involved in turnover of ATP synthesis in conjugation with oxidative phosphorylation (Brzoska et al., 2003). Despite great progress made in the field, the development of suitable drugs for the treatment of alcoholism remains a challenging goal for alcohol research. In general, plants contain some biologically active compounds, which are responsible for the prevention and detoxification of free radicals thereby protecting themselves from oxidative stress and the subsequent consequences (You et al., 2010).

Plant derived products have been used for medicinal purposes for centuries and also being used in our daily *food* intake. 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.*Bacopamonnieri* anayurvedic medicinal plant have been used as a brain tonic, which contains a mixture of triterpenoidsaponins designated as bacosides A and B (Chatterjee et al. 1963, 1965). This plant

is also found to possess anticholinesterase activity(Venkatakrishnanet al., 2012) antioxidant activity (Subashrietal., 2012), anti-inflammatory activity(ShabanaChannaet al., 2006), anticancer activity (LingPenget al., 2010), antibacterial activity (Ravikumaret al., 2005).However, cardio-protective effect of *Bacopamonnieri* extract against alcohol-induced toxicity is not yet studied fully. Hence, in the present study has been under taken to evaluate the effficacy of *Bacopamonnieri* extract against alcohol-induced cardiac oxidative stress.

Material and methods:

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 (25-28ºC) 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.

Preparation of plant extract

Fresh *Bacopamonnieri*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 *Bacopamonnieri.*

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 (Al): Alcohol treated (Al): 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: *Bacopamonnieri* treatment (Bm). Rats received Bacopa*monnieri*extract(200 mg/kg body wt) orally for 6 weeks days

Group IV: Alcohol + *Bacopamonnieri*treatment (Al+ Bm): Rats received *Bacopamonnieri*for 6 weeks followed by alcohol (2g/kg) for 6 weeks.

Analytical Procedure:

After completion of six weeks of treatment, the animals were sacrificed by cervical dislocation and the heart tissues were excised at 4^0C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80^0C for further biochemical analysis. The selected parameters such as SOD MDA levels, Ascorbic acid,and content were monitored by the methods Misra H P &Fridovich (1972), Ohkawa et al. (1979), Omaye et al. (1971), respectively. The activities of $Na+/K^+$, Mg^{2+} and Ca^{2+} ATP ases were estimated in the heart by the method of Fritz and Hamrick (1966). 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), using bovine serum albumin (BSA) as a standard.

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).

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 $p < 0.05$.

Results:

Effect of *Bacopamonnieri*extract on SOD, ascorbic acid and MDA levels in alcohol inducedrats

Statistical analyses clearly indicated a negative impact of Alcohol intoxication on the MDA, ascorbic acid and uric acid levels status of the heart. Significant (p<0.001) decreases in SOD activity, ascorbic acid and high level of MDA wereobserved in the alcohol rats compared with normal control rats. Alcohol rats with *Bacopamonnieri*treatment, showed significant(p<0.01) increases in SOD, ascorbic acid and decrease in MDA level, which reflectsrestoration of the antioxidant enzyme systems.(Table. 1)

Effects of *Bacopamonnieri*on membrane bound enzymesin alcohol-induced rats

Fig1-2shows the effect of *Bacopamonnieri*extract on the activities of Na+/K+, and Ca^{2+} ATPases (membrane bound enzymes) in different experimentalgroups. In alcohol treated rats, The activities of Na^{+}/K^{+}, and Ca^{2+} ATP ases, were Significantly (p<0.001) lower than normal rats.Supplementation with *Bacopamonnieri*200 mg/kg/day resulted in higherthe activities of Na^{+}/K^{+}, Ca^{2+}ATPases as compared to alcohol treatedrats. The protective effect of Bacopamonnieri extract against alcohol oxidative damage was evidenced by increased enzymes and decreased MDA levels in thecardiac tissue of rats.

4. Discussion:

Alcohol induced changes in heart for a period of time can damage the antioxidant homeostasis, membrane bound enzymes(Na^{+}/K^{+}andCa^{2+}ATPases)produce structural derangement in cardiac tissue that may contributes to the development of cardiovascular diseases. In this study, we demonstrated that *Bacopamonniera*was able to attenuate the alcohol induced increased MDAlevels and restored the decreased activities of the antioxidantsSOD, ascorbic acidlevels in the heart of rats.

The enzymatic antioxidant system plays a frontline defense against ROS toxicity in cardiac cells and other tissues. In the present study, SOD activity were deceased in the heart tissue of alcohol ingested rats. Similar decrease in SOD activity was also reported by previous studies in the heart of rats with chronic ethanol feeding [Pushpalatha et al., 2002]. The significant decrease in SOD activity due to alcohol indicates inefficient scavenging of reactive oxygen species (ROS) which might be implicated to oxidative inactivation of enzymes .The reduced activity of SOD in presence of alcohol may cause the accumulation of $O2 \bullet^{-}$, H2O2 or the products of its decomposition. Recently Abhishekhmathur et al., (2010)indicated that phenolic compounds Bacoside-A of *Bacopamonnieri*. These compounds may be responsible to scavenge the superoxide anion radicals and thereby maintain the high activity of SOD even in alcoholics.

We have found that the level of ascorbic acid in the heart were lower in alcohol administrated rats than in the control. The earlier reports have also been demonstrated ascorbic acid was decreased during ethanol intoxication (Svensson et al., 1992).Subir Kumar Das et al.,(2007)reported similar results in the alcoholic rat brain respectively. The decrease level of heart ascorbic acid in alcohol treated rats only could be as a result of increased utilization ofantioxidant in scavenging the free radicals generated during acute alcohol intoxication. In the present study with *Bacopamonnieri* treatment in alcohol treated rats, ascorbic acid level was increased. This may be due to the influence of *Bacopamonnieri* compounds on the reactive oxygen species which were produced during alcohol metabolism. Thus, *Bacopamonnieri* may exert a beneficial effect in countering the toxic free radicals in the heart.

Lipid peroxidation represents excessive production of the free radicals, which attack cellular biomolecules (Sarrafzadeh et al., 2000). Malondialdehyde (MDA), a marker of lipid peroxidation was significantly elevated with alcohol intoxication in the heart tissue. Earlier reports have also shown an increase in MDA levels following exposure to alcohol (Shailendersinghchuhan et al., 2013). In the present study, we found a significant reduction in MDA levels in group 4 rats, which received *Bacopamonnieri* along with alcohol for a period of 6 weeks. This result suggests that *Bacopamonnieri* extract can protect the neuronal cells from alcohol-induced peroxidative damage. It was also demonstrated that the major pungent constituent in *Bacopamonnieri*, bacoside-A exhibits antioxidative effect against peroxidation of phospholipids and scavenge the various free radicals.

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 cardiac tissue.. 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).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]. Any alteration in the membrane lipid components results in the inactivation of these membrane-bound enzymes (Barriviera et al., 1996). 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).Administration

of *Bacopamonnieri*plant extract to alcoholic rats reverted the diminished Na^+/K^+-ATPase, Ca^{2+}ATPase and Mg^{2+}ATPases activities to near normal. This result suggest that the inhibition of drug on reactive species and lipid peroxidation may restore membrane fluidity and hence the functional ability of associated enzymes.

5. Conclusion:

From the results in the present study, it is concluded that alcohol-induced oxidative stress in rat cardiac tissue is amenable to attenuation by *Bacopamonnieri*extract. The protective effect of *Bacopamonnieri*extract can be correlated directly with its ability to reduce the rate of lipid peroxidation as well as it restored the enhancecardiacmembrane bound enzymes. The findings of this study suggest that *Bacopamonnieri*can be used as a safe, cheap, and effective alternative chemo preventive and protective agent in the management of alcohol-related cardiovascular diseases.

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Table. 1. Effect of *Bacopamonnieri*extract on SOD, ascorbic acid, MDA in rats with alcohol induced oxidative stress in rat cardiac tissue.

Groups	SODΨ	Ascorbic acidΨ Ψ	MDAΨΨΨ
Normal control (NC)	5.456±6.82	2.481±0.015	174.833±6.82
Alcohol treated (Al)	3.251±7.133* (+13.29)	1.553±0.013* (-21.4)	233.041±7.133* (+19.29)
Bacopa treated (Bm)	5.021±5.107* (-0.081)	2.516±0018* (+1.41)	174.791±5.107* (-0.081)
Alcohol plus Bacopa (Al+Bm)	4.230±5.11** (+11.95)	2.047±0.146** (-8.49)	206±5.11** (+9.95)

Values in the parenthesis denote percent change over normal control.
The values are significant compared to the following: control
(*p<0.001),
Alcoholtreated (** < 0.01) (Dunnett's multiple comparison test).
Ψ the values are expressed in µ moles of superoxide anioin protein per minute
Ψ Ψthe values are expressed in mg of Ascorbic acid/ gm wet weight of the tissue.
Ψ ΨΨthe values are expressed in µ moles of malondialdelyde formed/ gmwet weight of the tissue.
Figure 1. Effect of Alcohol (Al), Bacopa*monnieri* treatment (Bm) and the combination (Al + Bm) on cardiacNa^+/K^+ATPase enzymeactivity. Values are significantly different (P < 001).

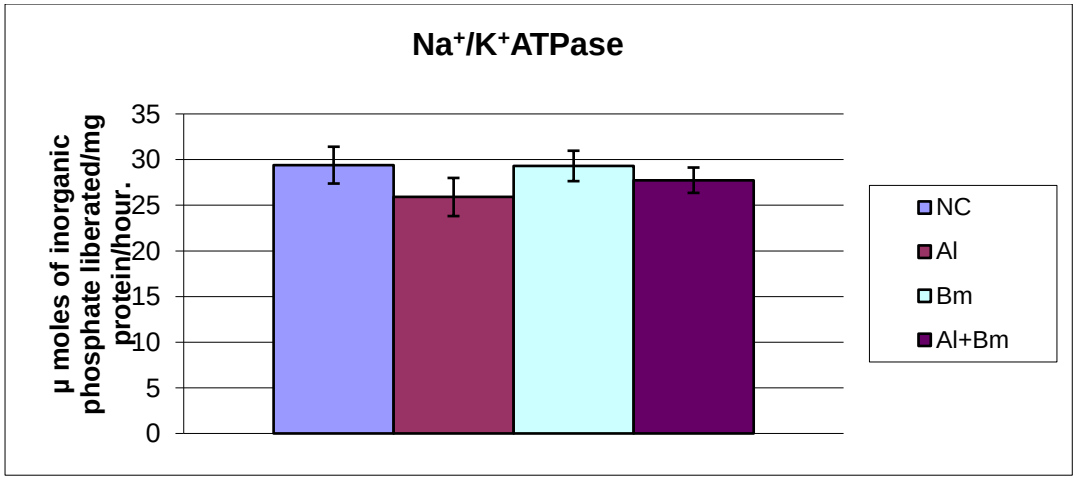

Figure 2. Effect of Alcohol (Al), Bacopa*monnieri* treatment (Bm) and the combination (Al + Bm) on cardiac Ca^{2+} ATPase enzymeactivity. Values are significantly different (P < 001).