Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

# PREVENTIVE EFFECT OF BIO-AQ ON CARDIAC MARKERS, LIPIDS, AND MEMBRANE BOUND ENZYMES IN ISOPROTERENOL - INDUCED MYOCARDIAL INFARCTION IN RATS

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*Received:23 January 2012, Revised and Accepted:22 April 2012*

**ABSTRACT**

Bio-Aq, is an Indian Ayurvedic herbomineral preparation of selected ingredients, which provides significant protection against ischaemia and hypertension. However, there is lack of information regarding the effect of Bio-Aq on the cardiac changes associated with isoproterenol (ISO)-induced myocardial infarction (MI).

Objectives: This manuscript reports the preventive effect of Bio-Aq on lipid profile, cardiac marker enzymes and membrane bound enzymes in ISO-induced cardiotoxic male Wistar rats.

Materials and Methods: Bio-Aq (25 and 50 mg/kg) was dissolved in dimethyl sulfoxide (DMSO) administered orally as pretreatment to Wistar rats daily for 28 days. After pretreatment, rats were induced with subcutaneous injection of ISO (85 mg/kg) at an interval of 24 h for two days to produce MI. Various biochemical markers of blood and tissue origin were estimated.

Results: ISO-induced rats showed a significant increase in the activities of marker enzymes in serum and there by subsequent decrease in the heart. ISO-induced rats also showed significant alterations in the activities of membrane bound ATPase in the heart of ISO-induced rats. In addition, increase in the levels of lipids in serum and heart. A rise in the levels of phospholipids (PL), LDL and VLDL and decreased HDL were also observed in the serum and decrease in the level of high density lipoprotein-cholesterol (HDL) in serum and PL levels, in the heart of ISO-induced rats was observed. Pretreatment with Bio-Aq (25 and 50 mg/kg) positively altered the activities of marker enzymes and other biochemical parameters in ISO-induced rats.

Conclusion: The present study concluded that Bio-Aq posses cardioprotective effect in ISO-induced MI in rats.

**Keywords:** Bio-Aq, cardiac markers, isoproterenol, lipids, lipoproteins, membrane bound enzymes, myocardial infarction.

## INTRODUCTION

In the 21st century, there is an ongoing effort to integrate complementary and alternative medications into the practice of conventional medicine, for the treatment of cardiovascular diseases (CVD) such as ischemic heart diseases (IHD). Epidemiological studies indicate that IHD, especially myocardial infarction (MI), will constitute the major disease-burden worldwide in the year 2020[1]. Myocardial ischemia occurs, when myocardial oxygen demand exceeds oxygen supply and as a result it causes cell injury known as MI, which is one of the most lethal manifestations of CVD[2].

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, has been reported to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle[3]. The induction of MI in experimental animals by ISO is probably due to its action on the sarcolemmal membrane, stimulation of adenylate cyclase, activation of $Na^+$ and $Ca^{2+}$ channels, exaggerated $Ca^{2+}$ inflow and energy consumption leading to cellular death[4]. ISO-induced MI serves as a well-standardized model to study the various effects of many drugs and heart function[5, 6].

Bio-Aq is a herbal medicine as well as herbal food enriched with protein, terpenoids, flavonoids, tannin, vitamins and minarals. It is only a herbal drug which acts on the endocrine system. Bio-Aq stimulates the pancreas to produce more insulin and reduce the high blood glucose level. Bio-Aq prevents acute manifestations of hyperglycemia and alleviates the symptoms of excessive thirst, frequent urination, weight loss, diabetes ketoacidos and long term complications such as retinopathy, neuropathy, nephropathy, macro vascular diseases and reduces dyslipidaemia. Since oxidative stress has been implicated in the development of CVD and Bio-Aq plays an important protective role against oxidative damage, we evaluated the preventive effect of Bio-Aq on cardiac markers, lipids and membrane bound enzymes in normal and ISO-treated MI in male albino Wistar rats.

## MATERIALS AND METHODS

### Experimental animals

All the experiments were carried out with male albino Wistar rats weighing 140–160 g , were obtained from Venkateshwara enterprises, Bangalore, India. They were housed in polypropylene cages (47 cm×34 cm×20 cm) lined with husk, renewed every 24 h under a 12:12 h light dark cycle at around 22◦C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.25% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen-free extract (carbohydrates). The diet provided metabolisable energy of 3600 kcal. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

### Drugs and chemicals

(±) Isoproterenol hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA. Bio-Aq was purchased from Agasthiar siddha Diabetes Research Centre, Nagercoil, Tamilndu, India. Creatine kinase-MB (CK-MB), creatin kinase (CK), Lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), triglycerides (TG) and HDL cholesterol kits were purchased from Agappe Diagnostics, Kerala, India. ATP was purchased from Himedia laboratory, Mumbai, India. All other chemicals used in the study were of analytical grade.

### Induction of experimental myocardial infarction

Isoproterenol (85 mg/kg) dissolved in normal saline was injected subcutaneously to rats at an interval of 24 h for two days to induce experimental MI[7].

### Experimental design

A total of 30 rats divided in to 5 groups of 6 rats in each. Bio-Aq was dissolved in 0.2% dimethyl sulfoxide (DMSO) and administered to rats orally using an intragastric tube daily for a period of 28 days.

Group 1: Normal control rats
Group 2: Normal + Bio-Aq (50 mg/kg)
Group 3: ISO-control rats (85 mg/kg)
Group 4: Bio-Aq (25 mg/kg) + ISO

Group 5: Bio-Aq (50 mg/kg) + ISO

At the end of the experimental period, after 12 h of second ISO-injection, all the rats were anesthetized with sodium pentobarbital (35 mg/kg, *i.p.*) and sacrificed by cervical decapitation. Blood was collected; plasma was separated and used for various biochemical estimations. The heart tissue was excised immediately from the animals, washed off blood with ice-chilled physiological saline and used for various biochemical estimations. A known weight of the heart tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

**BIOCHEMICAL ASSAYS**

**Assay of cardiac marker enzymes**

Creatine kinase and creatine Kinase-MB levels were estimated by Witt and Trendelenburg (1982) [8] methods using a commercial kit (Product No. 11405002 and Product No. 11404002). Lactate dehydrogenase activities was estimated by Wei Bhaar (1975) [9] method using a commercial kit (Product No. 11407002). Aspartate amino transferase and Alanine amino transferase were assayed by the methods of Clim (1976) [10] and Thefeld et al. (1994)[11] respectively (Product No. 11408001 and 11409001) were obtained from Agappe Diagnostics, Kerala, India.

**Estimation of lipids and lipoprotein**

Lipids were extracted by the method of Folch et al.[12] The levels of total cholesterol and triglycerides (TGs) were estimated by the methods of Zlatkis [13] and Schettler and Nussel (1975) [14] method using a commercial kit (Product No. 11409001) obtained from Agappe Diagnostics, Kerala, India.

The levels of free fatty acids (FFAs) and phospholipids (PLs) in serum and heart tissue were estimated according to the methods of Falholt et al.[15] and Zilversmit and Davis[16] respectively. HDL levels were estimated by Assmann (1979) [17] method using a commercial kit (Product No. 11010001). Serum lowdensity lipoproteins (LDL) and very lowdensity lipoproteins (VLDL) were calculated as VLDL=triglycerides/5 and LDL=total cholesterol–(HDL cholesterol+VLDL cholesterol) respectively.

**Assay of membrane bound enzymes**

The activity of $Na^+/K^+$-ATPase was assayed according to the procedure of Bonting (1970) [18]. The content of phosphorus liberated was estimated as described by Fiske and Subbarow (1925) [19]. The activity of $Ca^{2+}$-ATPase was assayed according to the method of Hjerken and Pan (1983) [20].The activity of $Mg^{2+}$-ATPase was assayed by the method of Ohnishi et al. (1982) [21]. Total Protein levels were determined by the method of Lowry et al. (1951) [22].

**Statistical analysis**

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± S.D. from six rats in each group. $p$ values <0.05 were considered as significant.

**RESULTS**

**Effect of Bio-Aq on cardiac marker enzymes**

Fig. 1 (a,b), 2 (a,b) &3 (a,b) represent the effect of Bio-Aq on heart weight and cardiac marker enzymes such as (CK,CK-MB, LDH, AST and ALT) in serum and heart of normal and ISO-induced rats. Rats induced with ISO, showed a significant increase in heart weight and the activities of these cardiac marker enzymes in serum with subsequent decrease in the heart, when compared with normal rats. Pretreatment with Bio-Aq (25 and 50 mg/kg) for a period of 28 days significantly decreased the heart weight and the activities of these marker enzymes in serum with significant increase in the heart of ISO-induced rats.

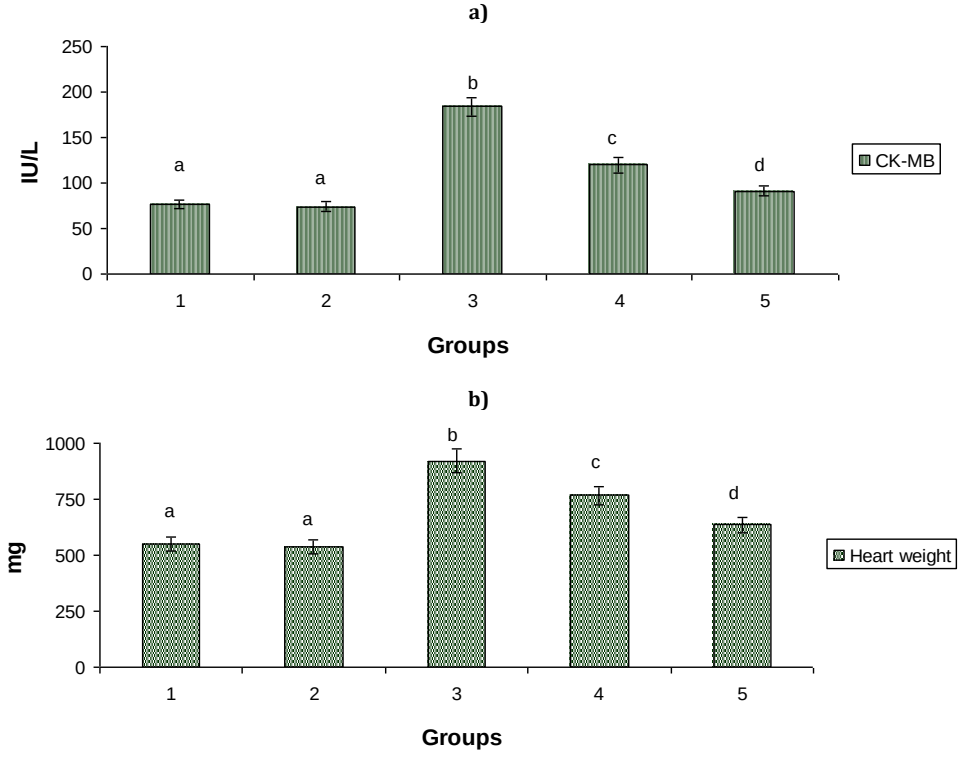



**Figure 1 (a, b). Effect of Bio-Aq on the activity of serum creatine kinase-MB (CK-MB) and heart weight in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

**a)**

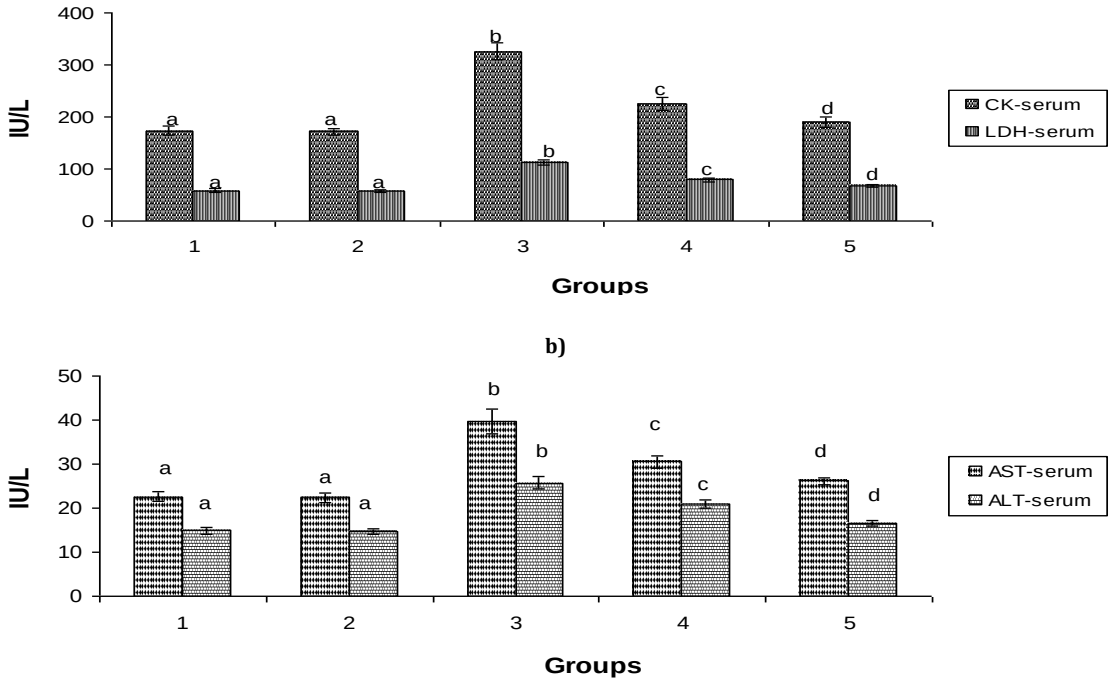



**Figure.2. Effect of Bio-Aq on the activity of serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

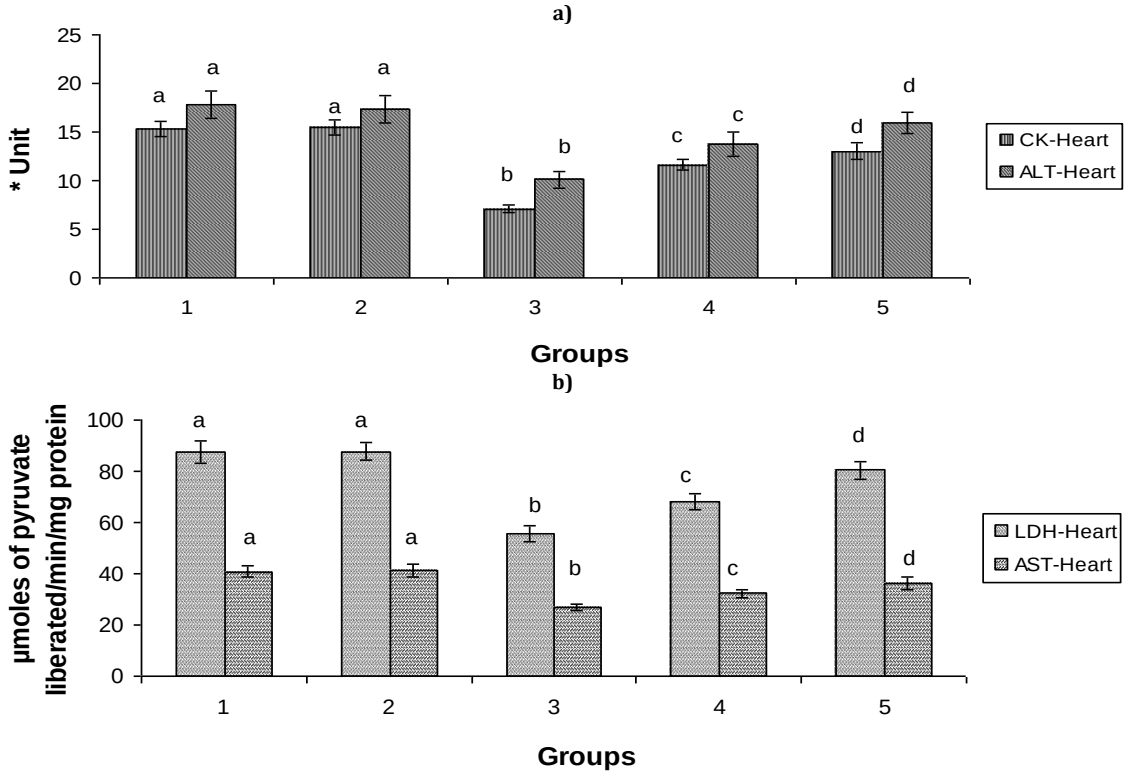



**Figure 3 (a,b). Effect of Bio-Aq on the activity of heart creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

***CK activity: µmol of phosphorus liberated/min/mg protein, *ALT: nmol of pyruvate liberated/min/mg protein.**
**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

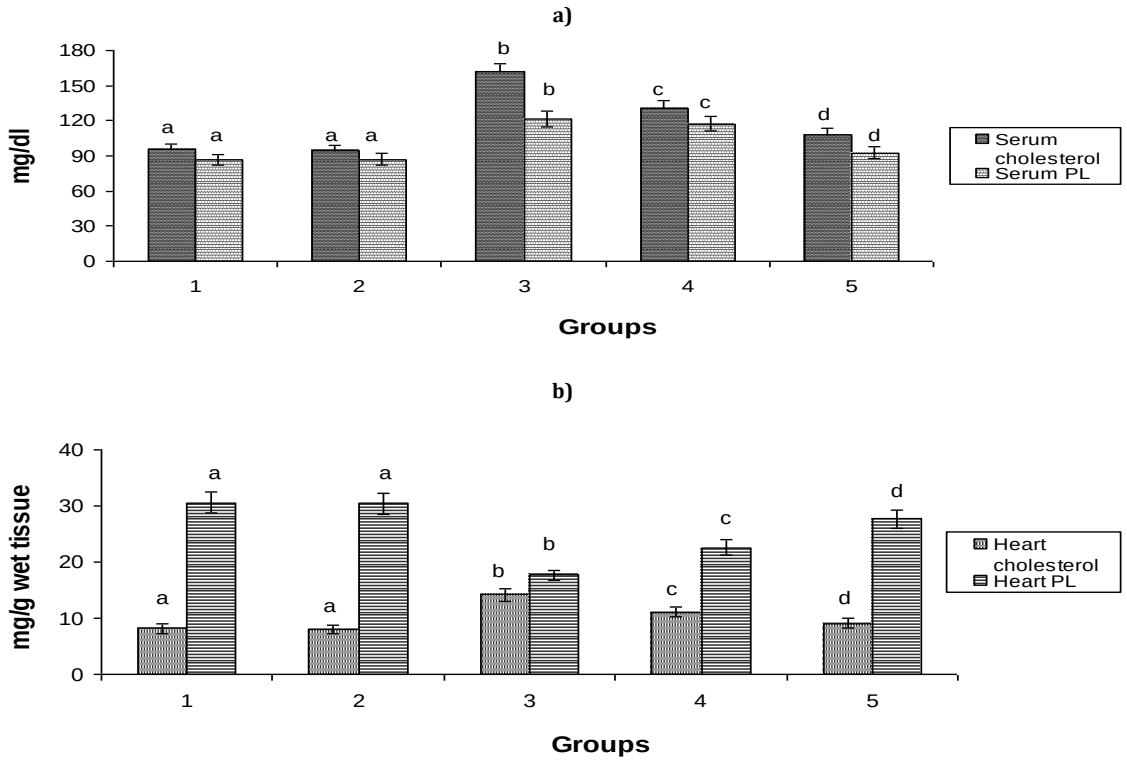
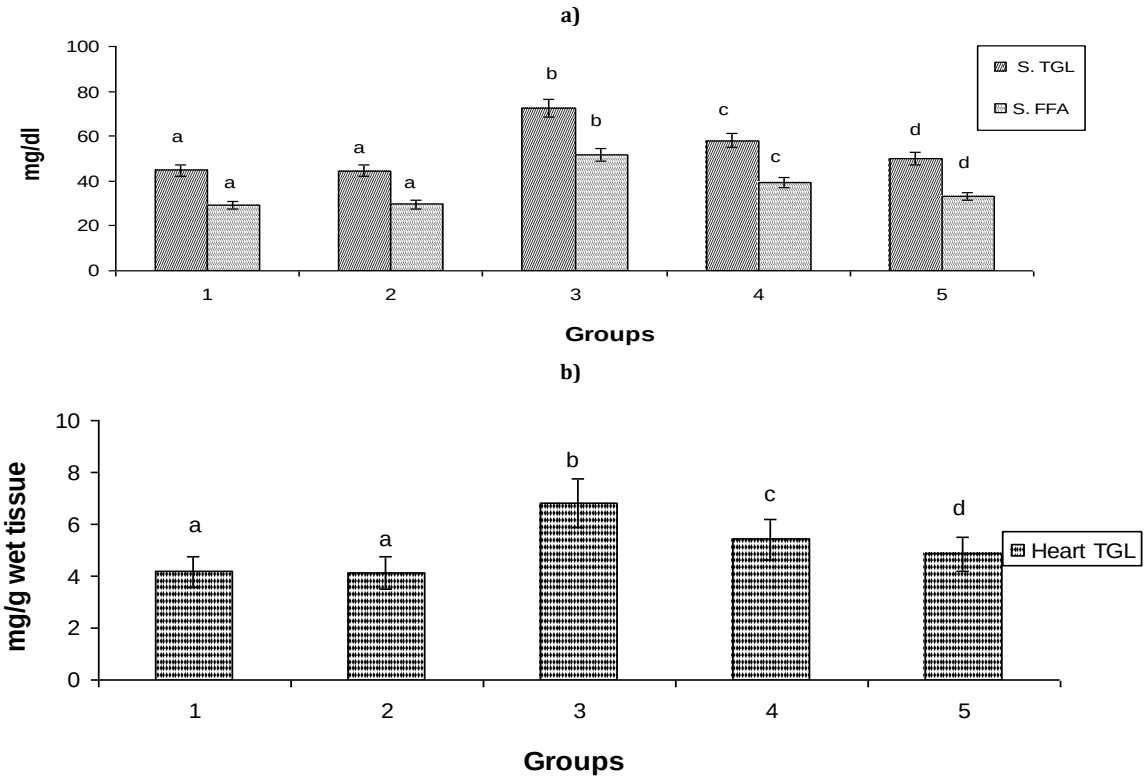



**Figure 4 (a,b). Effect of Bio-Aq on the levels of serum and heart total cholesterol, and phospholipids (PL) in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**



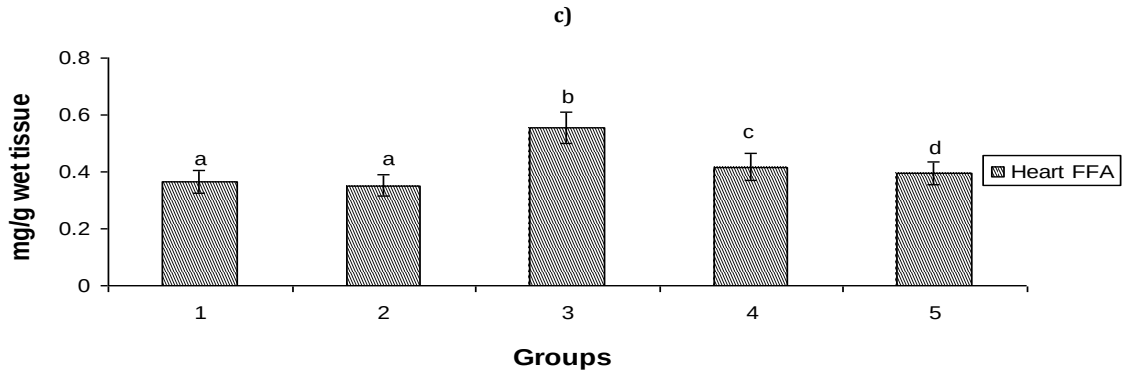



**Figure 5 (a,b,c). Effect of Bio-Aq on the levels of serum and heart triglycerides (TG), free fatty acids (FFA) in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

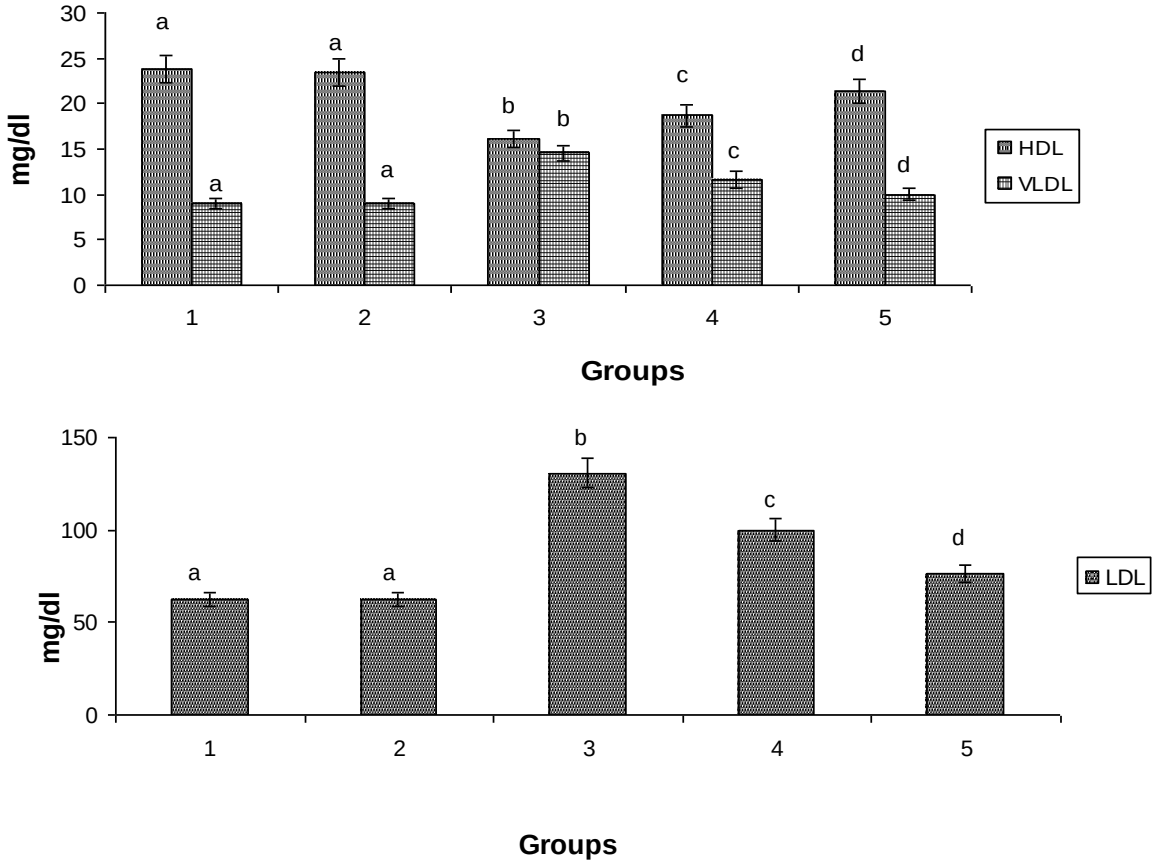



**Figure 6 (a,b). Effect of Bio-Aq on the levels of serum high-density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

**Each value is mean ± S.D. for 6 rats in each group**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

**Effect of Bio-Aq on lipoproteins**

Figures 6 (a,b) shows the levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) in serum of normal and ISO-induced rats. Rats treated with ISO, showed a significant increase in LDL and VLDL levels with a significant decrease in HDL levels when compared to normal control rats. Pretreatment with Bio-Aq to ISO-induced rats showed a significantly minimized the alterations in the lipoproteins levels.

**Effect of Bio-Aq on membrane bound enzymes in tissues**

Table 1 illustrates the effect of Bio-Aq on the activities of sodium potassium-dependent adenosine triphosphatase ($Na^+/K^+$-ATPase), calcium-dependent adenosine triphophatase ($Ca^{2+}$-ATPase) and magnesium-dependent adenosine triphophatase ($Mg^{2+}$-ATPase) in normal and ISO-induced rats. The activity of $Na^+/K^+$-ATPase was decreased and the activities of $Ca^{2+}$ and $Mg^{2+}$-ATPases were increased significantly in the heart of ISO-induced rats. Bio-Aq

pretreatment to ISO-induced rats significantly minimized the alterations in the activities of these membrane bound enzymes in myocardium when compared to ISO-alone induced rats.

For all the parameters studied Bio-Aq at a dose of 25 and 50 mg/kg to ISO-induced rats showed significant effects. However the higher dose (50 mg/kg) giving a better effect than lower dose (25 mg/kg). Bio-Aq at 50 mg/kg to normal rats didn't show any significant effect.

**Table 1: Effect of Bio-Aq on the activities of sodium potassium dependent adenosine triphosphatase ($Na^+/K^+$-ATPase), calcium dependent adenosine triphosphatase ($Ca^{2+}$-ATPase) and magnesium dependent adenosine triphophatase ($Mg^{2+}$-ATPase) in the heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

| Groups | $Na^+/K^+$-ATPase Units*/mg protein | $Ca^{2+}$-ATPase Units*/mg protein | $Mg^{2+}$-ATPase Units*/mg protein |
|---|---|---|---|
| Normal control | $0.61 \pm 0.061^a$ | $1.57 \pm 0.098^a$ | $5.83 \pm 0.49^a$ |
| Normal + Bio-Aq (50 mg/kg) | $0.63 \pm 0.060^a$ | $1.59 \pm 0.099^a$ | $5.76 \pm 0.48^a$ |
| ISO (85 mg/kg) control | $0.35 \pm 0.027^b$ | $2.63 \pm 0.142^b$ | $10.12 \pm 0.70^b$ |
| Bio-Aq (25 mg/kg) + ISO | $0.47 \pm 0.033^c$ | $1.94 \pm 0.129^c$ | $8.05 \pm 0.62^c$ |
| Bio-Aq (50 mg/kg) + ISO | $0.54 \pm 0.047^d$ | $1.76 \pm 0.101^d$ | $6.15 \pm 0.51^d$ |

*** Activity expressed as units: µmol of phosphorus liberated/min/mg protein**
**Each value is mean ± S.D. for 6 rats in each group.**
**Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).**

## DISCUSSION

Isoproterenol is well known cardiotoxic agent due to its ability to destruct myocardial cells. In this study, significant decline was shown in the activities of cardiac markers such as creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in the heart of ISO-treated rats, which is in consistent with earlier report[23]. Decreased activities of theses enzymes in heart could be due to the release of the enzyme from damaged myocardium into circulation by ISO. This could be also due to damage of the myofibril degeneration and myocyte necrosis.

Prior administration of Bio-Aq (25 and 50 mg/kg) for a period of 28 days, significantly prevented the ISO-induced elevation in the activities of diagnostic marker enzymes in serum, and significantly increased the activities of these markers in the heart. Bio-Aq on the myocardium, reducing the cardiac damage thereby restricting the leakage of these enzymes. Bio-Aq is a complex herbal preparation and some of the ingredients like *Terminalia arjuna, Curcuma longa, Phyllanlhus niruri, Withania sominifera* and *Emblica officinalis* has been reported to prevent cardiovascular disorders in Indian system of medicine[24].

The observed increase in the body weight In ISO-induced rats is possibly due to the accumulation of water content in the intramuscular space and by necrosis of cardiac muscle fibres. Pretreatment with Bio-Aq significantly decreased the heart weight in ISO-induced rats; this could be due to reduction in the process of necrosis offered by Bio-Aq.

Lipids play an important role in the pathogenesis of MI. ISO-treated cardiotoxicity is associated with increased levels of circulatory lipids. Hypercholesterolemia and hypertriglyceridemia are the risk factor for the development of MI. In this study, we observed increased levels of total cholesterol, TG, FFA in the serum and myocardium of ISO-control rats.

Increased levels of blood cholesterol and their accumulations in the heart are well associated with myocardial damage[25]. The main target available for ROS for attack is polyunsaturated fatty acids (PUFA), which is the precursor for lipid peroxide formation. Lipid peroxide elevation could be attributed to the accumulation of lipids in the heart[26]. The observed increase in TG after MI may be due to elevated flux of fatty acids and impaired removal of very low density lipoprotein (VLDL) from the serum. Pretreatment with Bio-Aq decreased the levels of TG and cholesterol in MI rats.

ISO-induced rats also showed an increase in the levels of PL and FFA in serum. The FFA liberated from adipose tissue also enters into the myocardium, and the process is proportional to the FFA concentration in the coronary sinus. Though the heart can utilize FFA for its energy requirements, the excess FFA may be used for the synthesis of TG, resulting in hypertriglyceridemia. PL is essential components for the integrity of cellular membrane and subcellular organelles. Many fatty acids are substrates for the biosysthesis of PL[27]. Pretreatment with Bio-Aq decreases the levels of serum PL and FFA in ISO-treated rats. This could be due to lipid lowering property of the drug.

Alterations in lipid metabolism directly reflect the composition of lipoproteins in ISO-induced cardiotoxic rats. HDL and LDL-cholesterols are significant variables for CHD. Prior treatment with Bio-Aq decreased the increased levels of serum LDL-cholesterol, VLDL-cholesterol and increased the lowered levels of serum HDL-cholesterol in ISO- treated cardiotoxic rats. Increased levels of serum HDL cholesterol observed in ISO-induced rats pretreated with Bio-Aq, facilitate the transport of cholesterol from peripheral tissues to the liver for catabolism and excretion from the body. Experiments conducted with the bark of Arjuna have been shown to possess hypolipidemic, hypocholesterolemic, hypotensive, activities [28].

Alterations in the activities of membrane bound enzymes affect the function of the heart. Vajreswari and Narayanareddy,[29] (1992) have shown that the failure of the cell membrane to maintain normal transmembrane ionic distribution through ion pumps is considered to be a major event in the pathogenesis of ischemia and arrhythmia. A significant decreased activity of $Na^+/K^+$-ATPase and significant increased activities of $Mg^{2+}$ and $Ca^{2+}$-ATPase in the heart were observed in ISO-induced rats. Decreased activity of $Na^+/K^+$-ATPase could be due to enhanced lipid peroxidation by ISO[5]. Decreased activity of $Na^+/K^+$-ATPase can lead to a decrease in sodium efflux, thereby altering membrane permeability[30]. $Ca^{2+}$-ATPase regulates the calcium pump activity[31]. Enhanced $Ca^{2+}$-ATPase activity observed in ISO-treated rats is due to the activation of adenylate cyclase by ISO. $Mg^{2+}$-ATPase activity is involved in other energy requiring process in the cell and its activity is sensitive to lipid peroxidation.

Pretreatment with Bio-Aq normalized the activities of these membrane bound enzymes in ISO-induced rats. Restoration of $Na^+/K^+$-ATPase activity due to Bio-Aq pretreatment in ISO-induced rats could regulate the intracellular $Ca^{2+}$ levels, thereby protecting the myocardium from excess damage by maintaining the membrane integrity.

## CONCLUSION

In conclusion, oral pre-treatment with Bio-Aq for the period of 28 days significantly minimized the alterations in heart weight, cardiac marker enzymes, lipid profile, and membrane bound enzymes. Thus our study demonstrates the cardioprotective role of Bio-Aq in ISO-induced oxidative stress in rats.

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