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Research Article

CARDIOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *MEDICAGO SATIVA* STEM ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN WISTAR ALBINO RATS

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ABSTRACT

Objective: To evaluate the cardioprotective effect of ethanolic extract of *Medicago sativa* stem (EMsS) on isoproterenol induced myocardial infarction in Wistar Albino rats.

Methods: Myocardial infarction was induced by interaperitonial injection administration of isoproterenol (ISO) 85 mg/kg in experimental rats. *M.sativa* stem extract was administered intraperitoneally at a dose of 100 mg/kg and 150 mg/kg for a period of 28 days. On 29th and 30th day the rats were induced with ISO (twice at an interval of 24 hours). The biochemical estimations serum lipid profile, liver marker enzymes, serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxaloacetate transaminases (SGOT), cardiac marker enzymes such as CK-MB and lactate dehydrogenase (LDH) were measured in the experimental rats. The antioxidants such as super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidase (LPO) were measured in the heart homogenate of experimental animals.

Results: Biochemical estimations revealed a significant fall in the levels of antioxidants in ISO induced groups. ISO induced group showed an increased levels of lipid profile. But HDL-C level decreased. In ISO induced rats, the pretreatment of plant extract reversed the lipid profile level to near normal than ISO induced control rats. Liver marker enzymes and cardiac marker enzymes were increased significantly in ISO induced rats. These enzyme levels were reverted to near normal level in the plant extract pretreated groups.

Conclusion: EMsS possesses significant cardioprotective activity in ISO induced rats.

Keywords: Cardioprotective, *Medicago sativa*, ISO, SGPT, SGOT, CK-MB, LDH, SOD, CAT, GPx, LPO and HDL-C.

INTRODUCTION

Cardiovascular diseases (CVDs) such as hypertension and myocardial infarction (MI) are the most important cause of mortality in developing countries due to changing lifestyles [1]. MI is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demands [2].

Myocardial infarction is one of the main causes of death from cardiovascular diseases. Myocardial ischemia occurs while myocardial oxygen demand exceeds oxygen supply causing cell injury known as myocardial infarction, which is one of the most fatal manifestations of cardiovascular diseases [3].

Nature is the lifeline of our health as it provides all necessary things for survival. Medicinal plants are nature's gift to human beings to make disease free healthy life and play a vital role to preserve our health [4].

Nowadays research has been focused on medicinal plants and food products derived from medicinal plants that have been found to have certain preventive measures in the treatment of cardiovascular disease (CVD) [5]. Use of herbs for the treatment of cardiovascular diseases in Ayurveda, Chinese and Unani system of medicine has given a new lead to understand the pathophysiology of these diseases. Therefore, it is rational to use the formulations which were prepared using natural resources for identifying and selecting inexpensive and safer approaches for the management of cardiovascular diseases along with the current therapy [6]. Hence the present study was carried to find out the cardioprotective effect of ethanolic extract of *Medicago sativa* stem in isoproterenol induced rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *M.sativa* was collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, and authenticated(BSI/SRC/5/23/201213/Tech/1686) by the authority of the botanical survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of plant extract

The whole plant was washed with running water and different parts of the plant were separated and dried under shade. Dried stem were coarsely powdered. Then, 20g of the powered sample was extracted with 90% ethanol using soxhlet apparatus. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder. They were stored in tight containers for further analysis.

Drugs usage

Isoproterenol drug was used for the study. Drug solution was freshly prepared in saline before each experiment.

Induction of myocardial infarction

Myocardial infarction was induced by intraperitoneal (i.p.) injection of isoproterenol (85 mg/kg body weight, dissolved in saline, for two consecutive days (29th and 30th day).

Experimental animals

Thirty six male wistar albino rats weighing 150-200 g were selected for the study. They were bought from a Central Animal Breeding Station, Thrissur, Kerala. The rats were maintained with standard laboratory conditions. The animals were kept in neat cages, bottomed with sterile and fed with standard pellet diet and water *ad libitum*. The rats were acclimatized to laboratory conditions for 15 days before the commencement of the experiments. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee (Reg no: 623/02/b/CPCSEA).

Experimental design

The experimental rats were divided into six groups, each group consisted of six rats.

Group I: served as a control

Group II: Rats were administered with ISO dissolved in normal saline (85mg/ kg body weight) on 29th and 30th day with 24 hour interval between applications.

Group III: Rats were treated with standard drug metoprolol dissolved in normal saline (100 mg/kg body weight) intraperitoneally for a period of 30 days.

Group IV: Rats were administered with EMsS extract control (150mg/kg body weight) for a period of 30 days.

Group V: Rats were treated with EMsS dissolved in normal saline (100 mg/kg body weight) for a period of 28 and then ISO was administered intraperitoneally on 29th and 30th day.

Group VI: Rats were treated with EMsS dissolved in normal saline (150 mg/kg body weight) for a period of 28 and then ISO was administered intraperitoneally on 29th and 30th day.

At the end of the experimental tenure the rats were kept for overnight fasting and then they were mild anaesthetized with diethyl ether. Blood was by cardiac puncture and then animals were sacrificed by cervical decapitation; serum was separated immediately by cold centrifugation and used for various biochemical estimations. The heart tissue was excised immediately from the animals, washed with ice-cold physiological saline and were placed in 10% for histopathological studies. A known weight of the heart tissue was homogenized with PBS (Phosphate Buffer Saline) solution. The heart homogenate was centrifuged and the supernatant was analyzed for antioxidant activities.

RESULTS

Lipid profiles

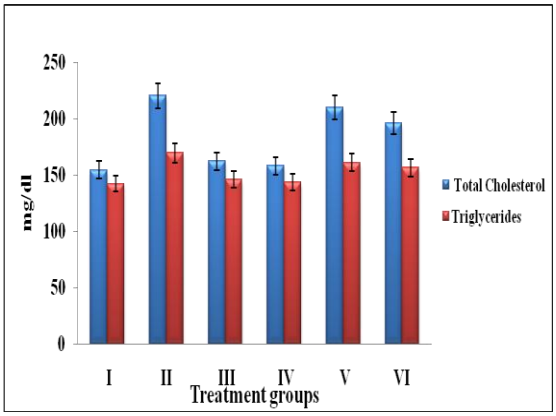

Fig. 1: Levels of total cholesterol and triglycerides

Biochemical analysis

The Serum total cholesterol [7], triglycerides [8], HDL- C [9], LDL- C [10] were measured in the experimental animals. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured spectrophotometrically by utilizing the method of Reitman and Frankel [11]. CK-MB [12] and lactate dehydrogenase (LDH) [13] was estimated in the different experimental animals. VLDL-C level was calculated using formula. Antioxidant enzymes activity such as catalase (CAT) [14], super oxide dismutase (SOD) [15], glutathione peroxidase (GPx) [16] and lipid peroxidase (LPO) [17] were analysed in the heart homogenate of different experimental animals.

Statistical analysis

Results were expressed as the mean ± SD. Statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (17.0) and the individual comparisons were obtained by the Duncan's multiple range test (DMRT) (Duncan, 1957). A value of $p<0.05$ was considered to indicate a significant difference between groups. For antioxidant activities comparison was made using student "t" test ($p<0.05$).

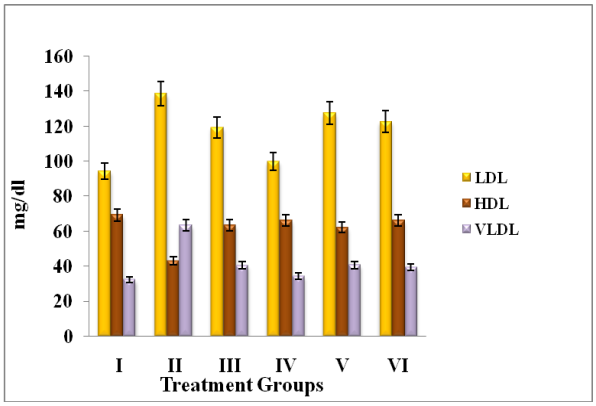

Fig. 2: Levels of HDL, LDL and VLDL

Figure 1 and 2 shows the levels of TC, TG, LDL-C, HDL-C and VLDL-C in the serum of experimental animals. The ISO induced rats showed significant ($p<0.05$) increased in serum lipid profiles except HDL-C when compared with normal rats. The ethanolic extract of *Medicago sativa* stem treated rats (100mg/kg body weight and 150mg/kg body weight) showed decreases in the content of lipid profiles when compared with ISO induced rats. Similarly HDL-C level significantly decreased in ISO induced rats when compared with normal rats. The administration of plant extract alone treated group showed a decrease in the levels of lipid profiles except HDL-C when compared with standard drug treated group.

Levels of liver marker enzymes

The liver marker enzymes were analysed in the different experimental groups. The ISO induced rats showed a significant ($p<0.05$) increase in the level of SGOT and SGPT when compared to normal rats. The group V and VI showed decrease in the level of liver marker enzymes when compared with ISO induced rats. The administration of EMsS extract alone treated group showed a decrease in the level of liver marker enzymes when compared with standard drug treated group.

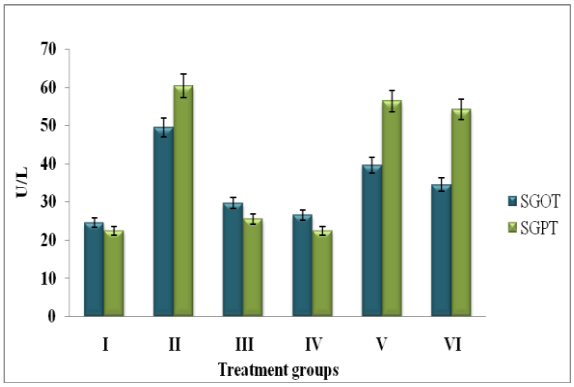

Fig. 3: SGOT and SGPT levels in the experimental animals

Cardiac marker enzymes

Figure 4 shows the levels of LDH and CK-MB in the different experimental groups. The ISO induced rats showed a significant ($p<0.05$) increase in the level of LDH and CK-MB when compared to normal rats. The group V and VI showed decrease in the level of cardiac marker enzymes when compared with ISO induced rats. The administration of EMsS extract alone treated group showed a decrease in the level of cardiac marker enzymes when compared with standard drug treated group.

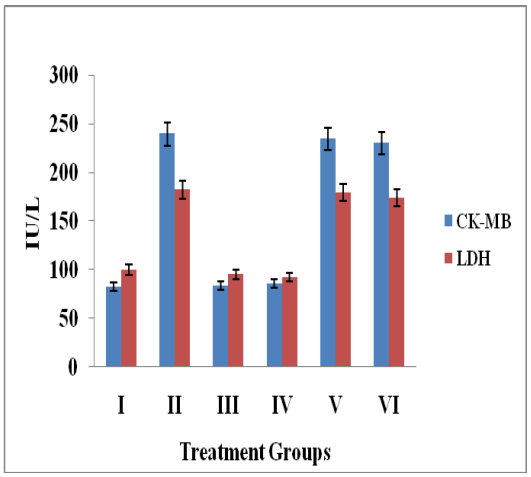

Fig. 4: Changes in cardiac marker enzymes in different experimental rats

Activities of enzymic antioxidants

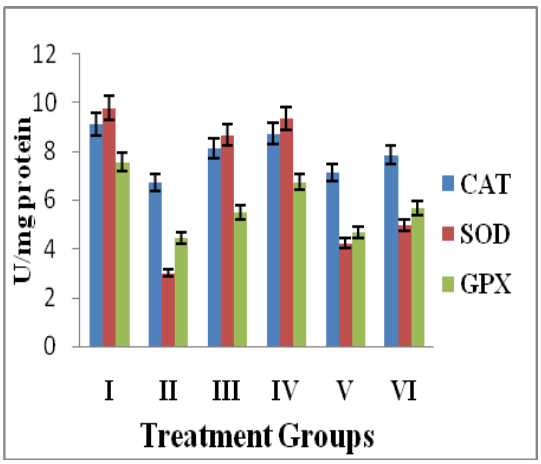

Fig. 5: Activities of CAT, SOD and GPX in the experimental rats

Figure 5 shows the levels of enzymic antioxidants such as CAT, SOD and GPx in the heart homogenate of different experimental groups. The ISO induced rats showed a significant (p<0.05) decrease in the level of enzymic antioxidants when compared to normal rats. The group V and VI showed a significant increase in the level of CAT, SOD and GPx when compared with ISO induced rats. The administration of EMsS extract alone treated group showed an increase in the level of enzymic antioxidants when compared with standard drug treated group.

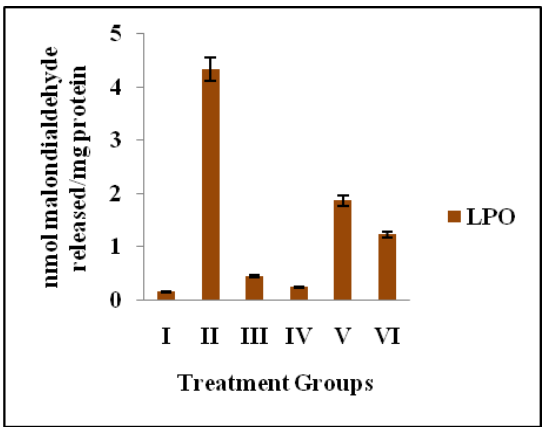

Fig. 6: Level of lipid peroxidation in different experimental rats

Lipid peroxidation

Figure 6 shows the levels of lipid peroxides in the different experimental groups. The ISO induced rats showed a significant (p<0.05) increase in the level of lipid peroxides when compared to normal rats. The group V and VI showed a significant decrease in the level of lipid peroxides when compared with ISO induced rats. The administration of EMsS extract alone treated group showed a decrease in the level of lipid peroxides when compared with standard drug treated group.

DISCUSSION

ISO-induced MI serves as a well standardized model to study the beneficial effects of many drugs and cardiac function since it mimics the clinical conditions of myocardial infarction due to ischemia in humans [18]. Lipids play an important role in cardiovascular disease, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure, and stability of cellular membranes. Excess lipids in the blood are considered to accelerate the development of arteriosclerosis and are the major risk factor in myocardial infarction. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage [19].

Isoproterenol administration has been reported to increase adenylate cyclase activity resulting in enhanced cAMP formation. The significant increase observed in the lipid profile in rats treated with isoproterenol alone could be due to enhanced lipid biosynthesis by cardiac cAMP on isoproterenol administration. HDL is known to be involved in the transport of cholesterol from the tissues to the liver for catabolism. Increase in the myocardial cholesterol on isoproterenol administration could be due to increased direct uptake of LDL from the blood by the tissues [20].

Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound enzymes. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with mediated atherosclerosis [21]. Elevation of lipid peroxides in ISO induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes [22].

Free radical scavenging enzymes such as SOD, CAT & GSHPx are the first line of cellular defense against oxidative injury decomposing superoxide and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. The equilibrium between these enzymes is an important process for the effective removal of oxygen free radicals [23] [24]. The fall in the activity of GSHPx in the ISO group might be correlated to decrease availability of its substrate, reduced GSH. Reduction in myocardial SOD and GPx activities strongly suggest overwhelming superoxide radical generation and hydrogen peroxide formation following isoproterenol administration [25]. The metabolic damage of myocardium results in increase in the concentration of the marker enzymes like LDH and CK-MB [26].

The serum enzymes creatine kinase (CK), AST and ALT serve as sensitive indices to assess the severity of myocardial damage [27]. Myocardial damage was estimated by measuring the activity of cardiac marker enzymes. The tissue specificity of enzymes makes them the markers of tissue damage. During myocardial infarction leakage of the enzymes such as CK-MB, AST and ALT results. This accounts for the increased activity of these enzymes in serum and decreased activity in heart tissue of rats induced with myocardial infarction [28].

CONCLUSION

The cardio protective effect of ethanolic extract of *Medicago sativa* stem is probably related to its ability to reduce the elevated levels of cardiac enzyme markers, and lipid profile. The activity might be due to the presence of phytochemicals and antioxidants in the ethanolic extract of *Medicago sativa* stem. Further studies are needed to prove the actual mechanism of action of *Medicago sativa.*

The present results clearly emphasize the beneficial action of ethanolic extract of *Medicago sativa* stem proved to be effective in reducing the myocardial damage. Hence this would be an easy and inexpensive way of preventing the incidence of myocardial infarction.

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