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Beneficial Effects of *Rosmarinus Officinalis* on Isoproterenol Induced Myocardial-Infarction in Cardiotoxicity Rats

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ABSTRACT

The present study investigates protective effects of *Rosmarinus officinalis* on oxidative stress and cardiac markers in isoproterenol (ISO)-induced myocardial infarction in rats. METHOD: Male albino wistar rats were divided into four groups (n=6). Group I received 0.5% CMC treated as normal control group. Group II received isoproterenol (85 mg/kg body weight .s.c) for two consecutive days (29th and 30th days). Group III received combination of *Rosmarinus officinalis* (100 mg/kg) intragastric intubation for 30 days. Group IV rats received combination of *Rosmarinus officinalis* as in Group III and additionally isoproterenol was given for two consecutive days (29th and 30th days). Induction of rats with ISO resulted in a well-marked elevation in lipid peroxidation and cardiac marker enzymes whereas, antioxidant activities were significantly reduced. *Rosmarinus officinalis in resulted in* significant reduction in lipid peroxidation and cardic marker enzymes. In addition, significant elevation of antioxidant activities was also observed in *Rosmarinus officinalis* in treated rats. When compared to the individual treatment groups. These findings conclude that *Rosmarinus officinalis* exhibited the protective effect of ISO induced myocardial infarction in rats.

Keywords: *Rosmarinus officinalis*; Isoproterenol; Cardiac marker; Lipid peroxidation; Antioxidants

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INTRODUCTION

The major health challenge of 21st century is leading death cause in both developed and developing countries is cardiovascular disease (CVD). Myocardial infarction (MI) is a common evidence of cardiac ischemia, and happens when cardiac ischemia surpasses a crucial threshold, resulting in irreversible myocardial damage[1-2]. It is a life-threatening problem with high mortality and morbidity at the global level. Its toll will increase to 25 million people by 2030 mainly from heart diseases such as myocardial infarction (MI) and stroke [3]. Developing countries such as India are struggling to manage the impact of infectious diseases simultaneously with growing burden on society and health system caused by non-communicable diseases such as myocardial infarction. An increasing number of young Indians are succumbing to myocardial infarction [4]. Isoproterenol [L-b-(3,4-dihydroxyphenyl)-a-isopropyl aminoethanol hydrochloride; ISO] is a synthetic b-adrenergic agonist. When administered to animals in high doses, it is an important regulator of myocardial contractility and metabolism. ISO causes infarct-like necrosis of the heart muscle, which morphologically resembles MI in humans [5]. The rat model of isoproterenol (ISO) induced myocardial necrosis serves as a well accepted standardized model to evaluate several cardiac dysfunctions and to study the efficiency of various natural and synthetic cardioprotective agents [6]. The generation of highly cytotoxic free radicals through auto-oxidation of catecholamine plays an important role in ISO-induced cardiac damage [5,7]. Therefore, many investigators have tested the preventive effects of several antioxidants on ISO-induced AMI [8-10]. ISO induced necrosis is a multi factorial condition involving relative hypoxia, coronary insufficiency, alternations in metabolism, decreased level of high energy phosphate stores, intracellular Ca^{2+} overload, changes in electrolyte content and oxidative stress. 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). *Rosmarinus officinalis* Linn and mint (*Labiatae*) family are common house hold plant grown in many parts of the world. They are commonly used as a spice and flavoring agent in food processing [11]. *Rosmarinus officinalis* possess a variety of bioactivities including anti tumour [12] and anti-inflammatory actions [13]. Besides useful in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases [14], hepatotoxicity, atherosclerosis biliary upsets, as well as for tension headache, renal colic, heart disease and poor sperm motility [14-15]. As of our knowledge, there is no previous study on the cardioprotective role of *Rosmarinus officinalis* on ISO-induced MI in rats. Basically, this is the first study investigating the protective role of *Rosmarinus officinalis* the cardiac injury by

evaluating inflammatory markers in the rat model. The present study was undertaken to scientifically investigate the effect of *Rosmarinus officinalis* on cardiac markers and lipid profile in ISO-induced MI in Wistar rats.

MATERIALS AND METHODS

Chemicals

Isoproterenol hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Preparation of Rosmarinus officinalis plant extract

The plant was collected from Kolli hills, Nmakkal, TamilNadu, India. The plant was identified at the taxonomy department TamilNadu Agricultural University, Coimbatore. Extraction of *Rosmarinus officinalis* was carried out according to the method of Dorman *et al.* (2003) [16]. Briefly, 50 g fine powdered herb were mixed with 500 ml distilled water in a quick fit flask round-bottom flask which connected to a hydrodistillation apparatus and the water was left to boil slowly for 120 minutes. The water from the flask was removed and another 300 ml of fresh distilled water were added and was boiled another 60 minutes. Water fractions were combined and filtered through qualitative Whatman filter. The filtrate was then subjected to lyopholyzation process through freeze drier under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at 4°C until used.

Animals

Male albino wistar rats weighing about 150g were obtained from Sri Venkateshwara Enterprises Bangalore, India. After one week of acclimatization all animals were housed six per polypropylene plastic cage covered with metal grids and a hygenic bed of husk in a specific-pathogen free animal room under controlled conditions of a 12h light/12 hour dark cycle, and provided with standard food pellets (Diet Composition- wheat broken-moisture 9.0%, crude protein, 11.5% crude fat, 1.9% crude fibre 4% ash 0.2%, nitrogen-free extract 73.4% supplied by Hindustan Lever Ltd, Mumbai, India) and tap water *ad libitum*. The study was conducted after obtaining a clearance from the Institutional animal ethical committee (IAEC) (Reg .no P.Col/52/2010/IAEC/VMCP) of Vinayaka Mission College of Pharmacy, Salem,Tamil Nadu.

Experimental design

The rats were divided into four groups comprising of six animals in each groups as given below.

Group I: Rats received 1.0ml of 0.5% carboxymethyl cellulose (CMC) throughout the experimental period and served as the untreated control.

Group II: Rats received ISO (85 mg/kg body weight) subcutaneously twice at an interval of 24 hours on the 29th and 30th days.

Group III: Received *Rosmarinus officinalis* via intra gastric intubation at a daily dose of (220 mg/kg body weight) respectively for a period of 30 days.

Group IV: Rats received *Rosmarinus officinalis* as in group III for 30 days and at the last of the experimental period on 29th and 30th day rats received ISO injections via subcutaneously twice at an intravel of 24 hours [17].

At the end of experimental period, the animals were sacrificed, heart was immediately removed and placed in ice-cold saline to remain in diastole to remove blood and the blood vessels and atrial tissue were trimmed off. Heart tissue was homogenized in 0.1 M Tris for biochemical analysis. Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances (TBARS) in tissues via the method of Niehaus and Samuelson [18] and plasma or erythrocyte via the method of Yagi [19]. Reduced glutathione (GSH) content was determined via the method of Moron et al [20]. Glutathione peroxidase (GPx) activity was assayed via the method of Rotruck [21] with a modification: a known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time period. The amount of H_2O_2 utilized was determined via the method of Habig et al [22].The values are expressed as µmoles of GSH utilized/min/mg Hb or protein. Superoxide dismutase (SOD) was assayed using the method of Marklund and Marklund [23]. The activity of catalase (CAT) was determined via the method of Sinha [24]. The levels of cTnT and cTnI in serum were estimated using standard Kit by chemiluminenscence immunoassay (Roche Diagnostics, Switserland) was expressed as ng/mL for serum. The AST and ALT enzymes were assayed by the method of Reitman [25]. The activity of lactate dehydrogenase (LDH) was assayed in serum using a commercial kit purchased from Qualigens Diagnostics, Mumbai, India. Creatine kinase activity was assayed by the method of Okinada [26].

Statistical analysis

The results presented here are the means ± SD of 6 rats in each group. The results were analyzed using one-way analysis of variance (ANOVA) and the group means were compared using Duncan's multiple range tests using SPSS version 12 for Windows. The findings were considered as statistically significant if $P<0.05$ [27].

RESULTS AND DISCUSSIONS

Effect of Rosmarinus officinalis on lipid peroxidation, antioxidants in serum and tissue on myocardial infracted rats and control rats. Cardiovascular disease is major global health problem

reaching epidemic proportion in Indian subcontinent[28] and low and middle income countries accounting for 78% of all death [29]. Myocardial infarction remains a major cause of morbidity and mortality worldwide. Important treatment of a heart attack is necessary to prevent permanent damage and to save lives. New therapies are needed to treat ischemia because current treatment has only a limited impact on survival and annual cost[30]. ISO-induced cardiac lesions are morphologically similar to those of 'coagulative myocytolysis' or myofibrillar degeneration, which is one of the important finding described in acute MI and sudden death in man [31]. Various mechanism have been proposed to explain the toxic role of ISO on myocardium, including increased cAMP [30], increased intracellular Ca^{2+} overload [32], depletion of high energy phosphate and oxidative stress [33]. Excessive generation of cellular toxic free radicals, due to the auto-oxidation metabolic products (ISO undergo auto-oxidation produces quinones, which react with oxygen to produce superoxide anion (O^{2-}) and H_2O_2)[33]. Table 1 depicts the effect of *Rosmarinus officinalis* on circulatory and tissue levels of TBARS in the control and experimental rats. The levels of circulatory and tissue TBARS were significantly ($P<0.05$) increased in the ISO alone induced rats (group II) as compared with control rats (group I). *Rosmarinus officinalis* combinational administration to the ISO induced rats (group IV) significantly ($P<0.05$) decreased the levels of TBARS in the circulatory and tissue as compared with ISO induced rats (group II). *Rosmarinus officinalis* supplementation (group III) the levels of circulatory and tissue TBARS were decreased when compared to control rats. 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 [34].

Table 1 Effect of *Rosmarinus officinalis* on circulatory and plasma lipid peroxidation in control and experimental rats

Groups	Group I	Group II	Group III	Group IV
Plasma TBARS(nmoles ml)	1.91 ± 0.04^{a}	3.44 ± 0.12^{d}	1.86 ± 0.06^{b}	2.66 ± 0.06^{c}
Tissue TBARS(nmoles/mg of protein)	2.31 ± 0.40^{b}	3.66 ± 0.69^{c}	1.92 ± 0.05^{a}	2.52 ± 0.07^{b}

Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT).

In the present study elevation of lipid peroxidation (TBARS) in ISO induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes [35]. The decreased level of TBARS in *Rosmarinus officinalis* treated rats when compared to control and isoproterenol induced rats. This might be due to enhanced activities of antioxidant enzymes for *Rosmarinus officinalis*. Oxidative stress plays a major role in the

development of myocardial infarction through generation of free radicals and depletion of endogenous antioxidant enzymes. The most commonly recognized antioxidants are SOD, Catalase and SOD changes the structure of oxidants and breaks them into hydrogen peroxide. CAT is an endogenous antioxidant enzyme present in the cytoplasm that detoxifies the free radical hydrogen peroxide into water and oxygen molecules. The equilibrium between these enzymes is an important process for the effective removal of ROS in intracellular organelles [36]. Table 2 illustrates the effect of *Rosmarinus officinalis* on circulatory antioxidants (SOD, CAT, GSH and GPx) in the control and experimental rats. The serum antioxidants levels were significantly (*P<0.05*) reduced in the ISO induced myocardial infarcted rats (group II) as compared with control rats (group I). *Rosmarinus officinalis* administration to the ISO induced rats (group IV) significantly (*P<0.05*) elevated the levels of antioxidants when compared to the ISO induced rats (group II). On *Rosmarinus officinalis* supplementation (group III) the levels of antioxidants were increased when compared to control rats. Table 3 depicts the combinational treatment of *Rosmarinus officinalis* on heart tissue antioxidants (SOD, CAT, GPx and GSH) of control and experimental rats. The antioxidant levels of myocardial tissue was significantly (*P<0.05*) reduced in ISO induced myocardial infarcted rats (group II) when compared with control rats (group 1). Administration of *Rosmarinus officinalis* to the ISO induced rats (group IV) indicates significantly *(P<0.05)* increased levels of tissue antioxidants as compared to the ISO induced rats (group II). On *Rosmarinus officinalis* supplementation (group III) the levels of antioxidants were increased when compared to control rats. In the present study SOD activity decreased significantly in the ISO treated group of animals, which might be due to an excessive formation of superoxide anions. These excessive superoxide anions might inactivate SOD and decrease its activity, decline in SOD activity may be explained by the fact that excessive superoxide anions may inactivate SOD enzyme [37]. *Rosmarinus officinalis* treatment improved unsignificantly SOD activity. The increase in the enzyme activity was 24% and this rise is really significant because it is greater than 20% [38]. In the absence of adequate SOD activity, superoxide anions are not dismuted into H_2O_2, which is the substrate for the H_2O_2 scavenging enzymes CAT & GPx. As a result, there is an inactivation of the H_2O_2 scavenging enzymes CAT and GPx, leading to a decrease in their activities. Administration of *Rosmarinus officinalis* to ISO challenged rats effectively prevented the depletion of SOD, CAT & GPx activities, which can be correlated to the scavenging of free radicals by *Rosmarinus officinalis*, resulting in the protection of these enzymes. GSH is one of the major antioxidant enzymes to scavenge the free radicals during tissue damage [39]. GSH scavenges singlet oxygen, superoxide, and peroxy radicals to form oxidized GSH and other disulfides. Also,

antioxidant compounds have been shown to increase GSH reductase activity that maintains GSH in a reduced state [40-41]. Decrease in the level of GSH in ISO-treated animals indicated that the depletion of GSH resulted in enhanced lipid peroxidation, and excessive lipid peroxidation caused increased GSH consumption [40]. GSH levels depleted by ISO were significantly restored by *Rosmarinus officinalis*, either due to increased synthesis of GSH or by stimulation of GR activity.

Effect of Rosmarinus officinalis on Cardiac markers

Table 2: Effect of Rosemary on circulatory antioxidants in control and experimental rats

Groups	Group I	Group II	Group III	Group IV
SOD(Units/min/mg of Hb)	11.27 ± 0.17^{b}	9.81 ± 0.47^{a}	13.23 ± 0.41^{b}	10.81 ± 0.45^{c}
CAT (µmoles of H_2O_2 decomposed/min/mg of Hb)	16.18 ± 0.73^{b}	13.24 ± 0.77^{a}	17.65 ± 0.65^{c}	15.16 ± 0.59^{b}
GSH(µmoles/mg of Hb)	1.77 ± 0.04^{b}	1.01 ± 0.04^{a}	2.45 ± 0.09^{d}	2.05 ± 0.05^{c}
GPx(µmoles of GSH consumed /min/mg of Hb)	15.41 ± 0.63^{c}	11.01 ± 0.60^{a}	18.08 ± 0.60^{c}	16.31 ± 0.63^{b}

Units SOD - Superoxide dismutase CAT - Catalase
GSH - Glutathione GPx - Glutathione Peroxidase

Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT)

Table 3 Effect of Rosemary on tissue antioxidants in control and experimental rats

Groups	Group I	Group II	Group III	Group IV
SOD(Units/mins/mg of protein)	12.10 ± 0.53^{b}	9.90 ± 0.51^{a}	13.65 ± 0.50^{c}	10.71 ± 0.53^{c}
CAT (µmoles of H_2O_2 decomposed/min/mg of protein)	15.03 ± 0.61^{b}	13.05 ± 0.56^{a}	17.18 ± 0.66^{c}	14.08 ± 0.69^{b}
GSH(mmoles/100g of tissue)	1.71 ± 0.04^{c}	1.09 ± 0.04^{a}	2.55 ± 0.88^{d}	1.49 ± 0.56^{b}
GPx(µmoles of GSH consumed/min/mg protein)	14.95 ± 0.62^{c}	11.65 ± 0.59^{a}	18.85 ± 0.74^{d}	13.21 ± 0.67^{b}

Units SOD - Superoxide dismutase CAT - Catalase
GSH - Glutathione GPx - Glutathione Peroxidase

Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT)

Table 4 Effect of Rosemary on cardiac marker enzymes in control and experimental rats

Groups	Group I	Group II	Group III	Group IV
Troponin I (ng/mL)	0.24 ± 0.06^{a}	0.79 ± 0.40^{c}	0.19 ± 0.11^{a}	0.42 ± 0.13^{b}
Troponin T (ng/mL)	0.43 ± 0.17^{a}	2.34 ± 0.44^{c}	0.32 ± 0.12^{a}	1.44 ± 0.36^{b}
CK(IU/L)	162.45 ± 1.23^{a}	215.8 ± 1.07^{c}	145.67 ± 1.74^{b}	161.45 ± 1.35^{d}
CK-MB(IU/L)	103.11 ± 1.98^{a}	173.88 ± 1.88^{c}	97.68 ± 1.39^{b}	127.87 ± 1.24^{d}
LDH(IU/L)	284.30 ± 1.26^{a}	352.63 ± 1.25^{c}	237.18 ± 1.29^{b}	327.97 ± 1.22^{d}
AST(IU/L)	123.93 ± 0.87^{a}	249.13 ± 0.97^{d}	110.63 ± 0.73^{b}	169.48 ± 1.06^{c}
ALT(IU/L)	86.16 ± 1.06^{a}	155.48 ± 0.97^{c}	77.61 ± 1.07^{b}	109.03 ± 1.06^{d}

Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT)

Myocardium contains an abundant concentration of diagnostic marker enzyme of myocardial infarction viz., AST, ALT, LDH, CK-MB and TROPONIN once myocardium is damaged, releases of its content into the extra cellular fluid serves as the diagnostic enzyme marker of myocardial damage tissue [42]. Cardiac Troponins T and I are the cardiac specific proteins and highly sensitive, precise and released when myocardial cell damage occurs. Hence, Troponins are the ideal biomarkers for MI, which are present at high concentration in the myocardium, whereas absent in non-cardiac tissues. The release of cardiac troponin has been associated with the severity of infarction [43]. CK-MB has greater than 95% sensitivity and specificity for myocardial injury when measured between 24-36 hours. Estimation of elevated serum enzymes is a useful guide for necrosis of myocardium [44]. Table 4 depicts the effect of *Rosmarinus officinalis* on cardiac markers (Troponin I and T, CK, CK-MB, LDH, AST and ALT) in the control and experimental rats. The level of these cardiac markers were significantly ($P<0.05$) increased in the isoproterenol induced rats (group II) as compared to the control rats (group 1). Administration of *Rosmarinus officinalis* to the ISO induced rats (group IV) significantly ($P<0.05$) decreased the levels of cardiac markers as compared to the ISO induced rats (group II). On *Rosmarinus officinalis* supplementation (group III) the levels of cardiac markers were decreased when compared to control rats. In this study, we have observed elevated levels of cTnT and cTnI in ISO-induced rats. This could be due to action of quinine, which produces free radicals. It is well accepted that when ISO have biotransformation, it produces quinine as metabolite, which is involved is cardiac damage. Pretreatment with *Rosmarinus officinalis* for a period of 21 d significantly decreased these troponins levels in ISO-induced rats. The decline in the troponin levels indicates the reduction in cardiac injury. ISO causes significant damage to myocardium and a significant increase in the levels of serum marker enzymes such as AST, ALT, CK and LDH. In the current study, there was an increase in activities of the marker enzymes CK, LDH, AST and ALT in the isoproterenol induced myocardial infarcted in rats [45]. These findings confirm the onset of myocardial necrosis and leaking out of the marker enzymes from heart to blood [46]. The amount of marker enzymes is directly proportional to ISO-induced necrotic lesions present in the myocardium. In the present study, *Rosmarinus officinalis* treatment to the MI-induced rats reduce the cardiac damage and restrict the leakage of enzymes as evident from a significant reduction in the activities of cardiac marker enzymes in serum.

CONCLUSION

In the current study, we can conclude that *Rosmarinus officinalis* has potential myocardial action. The present results show significant effect of *Rosmarinus officinalis* lipid peroxidation,

antioxidants and cardiac markers properties. These results support that the *Rosmarinus officinalis* may be used as cardioprotective drug.

Conflict of interest statement

We declare that we have no conflict of interest

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