Ellagic acid inhibits cardiac arrhythmiasis and hypertrophy via regulating lipid metabolism during myocardial infarction in rats

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Abstract

Objective: To evaluate the protective effect of pretreatment with ellagic acid against cardiac arrhythmiasis and hypertrophy during myocardial infarction in rats and its association with the lipid lowering ability of ellagic acid to prevent ventricular tachycardia.

Methods: We used isoproterenol to induce myocardial infarction in male Wistar rats. Rats were treated with ellagic acid (7.5 and 15 mg/kg) orally for a period of 10 days. After 10 days of pretreatment, isoproterenol (100mg/kg) was injected subcutaneously at an interval of 24 h for 2 days to induce myocardial infarction. On the 12^{th} day, the cardiac rhythm was observed. Ventricular hypertrophy and myocardial necrotic score were analysed. Changes in the lipid profile were also measured.

Results: Isoproterenol-induced rats showed significant changes in the cardiac rhythm, increased levels of myoglobin and creatine kinase-MB when compared to the normal control rats. The ventricular hypertrophy and increased myocardial necrotic score were observed in isoproterenol-induced rats. Additionally, increased lipid peroxidation and altered lipid metabolism were observed. The oral pretreatment with ellagic acid inhibited the ventricular tachycardia and cardiac hypertrophy. Pretreatment with ellagic acid decreased the lipid peroxidation and regulated the lipid metabolism in isoproterenol-induced myocardial infarcted rats.

Conclusions: Oral pretreatment with ellagic acid showed cardio protection against arrhythmiasis, hypertrophy and myocardial necrosis. Anti-hyperlipidemic activity was an added advantage of this drug. *In vitro* studies on ellagic acid suggested that the free radical scavenging activity may be the mechanism of action for the protective effect.

Key words: isoproterenol, ventricular tachycardia, lipid peroxidation, antioxidant

List of abbreviations

MI: myocardial infarction; ISO: isoproterenol; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PVC: premature ventricular contractions; VF: ventricular fibrillation; VF: ventricular fibrillation; CK-MB: creatine kinase-MB; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: very low density lipoprotein; HMG CoA reductase: 3-hydroxy-3 methyl glutryl CoA reductase; FFA: free fatty acid.

1. Introduction

Coronary artery disease is the leading cause of death worldwide. Of all death due to coronary artery disease, more than 60 % occur within the first few hours as a consequence of VT and/or VF [1]. Abnormal impulse formation and reentrant arrhythmia in the setting of myocardial scar tissue are the mechanisms that lead to sudden death. Hypertrophied myocardium has been shown to generate arrhythmias more readily than normal tissue and it is one of the main ways in which cardiomyocyte respond to mechanical and neurohormonal stimuli. A study by Liu et al. [2] stated that, dyslipidemia is an independent predictor of VT /VF in the acute phase of myocardial infarction (MI). Therefore it is of great interest to focus arrhythmias, hypertrophy and its association with the lipid lowering therapy which could help to prevent VT and fatal death due to coronary events.

Many synthetic drugs are used for the treatment of coronary artery disease, arrhythmiasis and MI. However, they cannot meet the demands due to some potential adverse effects and great drug dependence [3]. Thus, a number of studies have focused to identify new therapeutic strategies to prevent or reverse cardiac failure. Alternative therapies using phyto nutrients are becoming increasingly popular as these preparations have no or minimal

side effects and cost effective. Polyphenols are excellent cadioprotectants. Ellagic acid, (4,4',5,5',6,6'-hexahydroxydiphenic acid 2,6,2',6'-dilactone) is a phenolic acid found naturally in strawberries, raspberries, grapes and pomegranate [4]. It has shown anti-inflammatory, hepatoprotective and chemopreventive activities [5-7]. A recent study from our laboratory showed ellagic acid exhibit cardioprotective activity against experimentally induced MI [8].

Isoproterenol (ISO), a β-adrenergic receptor agonist, has been reported to have deleterious effects on the heart, when administered at higher doses by inducing necrosis and oxidative damage [9]. ISO-induced arrhythmiasis, hypertrophy and alterations in lipid metabolism have been extensively discussed [10,11]. The model is characterized by an extraordinary technical simplicity, an excellent reproducibility as well as an acceptable low mortality [12].

The present study was designed to investigate the potential therapeutic effect of pretreatment with ellagic acid against ISO-induced arrhythmiasis, hypertrophy, lipid peroxidation, dyslipidemia and myocardial necrosis in connection with its anti lipid peroxidation and anti hyperlipidemic activity. Our study also reports the possible underlying mechanism of action of ellagic acid by its superoxide radical scavenging and hydroxyl radical scavenging effect *in vitro*.

2. Materials and Methods

2.1. Experimental animals

All the experiments were carried out with male albino Wistar rats (*Rattus norvegicus*) weighing 180-200 g, purchased from Mahaveer Enterprises, Hyderabad, India. They were housed in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under

a 12 h light/dark cycle at around 22 °C with 50 % humidity. The rats had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Pune, Maharashtra, India). The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Institutional Animal Ethical Committee of Jayamukhi College of Pharmacy (Approval No. 022; 28.2.2010).

2.2. Drugs and Chemicals

Ellagic acid and isoproterenol were purchased from Sigma Chemical Co., St. Louis, MO, USA. Sodium sulphite, dimethyl sulfoxide, potassium tetraborate, deoxyribose, nitroblue tetrazolium reagent and hydroxylamine hydrochloride were purchased from Himedia, Mumbai, India. All other chemicals used in the study were of the highest analytical grade.

2.3. Induction of experimental myocardial infarction

ISO (100 mg/kg body weight) was dissolved in saline and injected subcutaneously to rats at an interval of 24 h for 2 days [13].

2.4. Experimental design

The rats were divided into six groups of six rats each. Group I: normal control rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group II: normal rats were treated with ellagic acid (7.5 mg/kg) dissolved in 2 ml of saline orally by

gastric intubation daily for a period of 10 days; Group III: normal rats were treated with ellagic acid (15 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group IV: normal control rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg) in 2 ml of saline at an interval of 24 h for 2 days (on 11^{th} and 12^{th} day); Group V: rats were pretreated with ellagic acid (7.5 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg) at an interval of 24 h for 2 days (on 11^{th} and 12^{th} day); Group VI: rats were pretreated with ellagic acid (15 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg) once a day for 2 days (on 11^{th} and 12^{th} day).

2.5. Determination of arrhythmia score

Electrocardiographic transducers were fixed and signals from the heart were obtained continuously. The Electro cardiographic tracings were analyzed off-line using AD Instruments Lab Tutor software. All arrhythmic events were classified by the observer according to the guidelines provided by "the Lambeth conventions" [14]. VT was defined as more than 4 consecutive ventricular premature beats. VF was defined as a signal that changed from beat to beat in rate and configuration or where individual QRS-deflections could not easily be distinguished from one another [15]. Arrhythmias were quantitated via a modified scoring system explained by Fryer et al. [16]. Arrhythmia scores were assigned as follows: 0=<10 PVCs/3-min period; 1=<10 to 50 PVCs/3-min period; 2= >50 PVCs/3-min period; 3 = 1 episode of VF /3-min period; 4 = 2 to 4 episodes of VF/3-min period; and 5 = > 4 episodes of VF/3-min period [17, 18].

2.6. Sample preparation

After measuring the arrhythmic score, the rats were sacrificed by cervical decapitation and blood was collected in two tubes, i.e., one with anticoagulant (ethylenediamine tetra acetic acid) for plasma separation, and another without anticoagulant for serum separation. Both the plasma and serum were separated from each sample and used for the biochemical analysis. The heart and liver tissues were excised immediately and rinsed in ice-chilled saline. Internal coagulated blood was removed, and the whole heart was weighed to the nearest 0.1 g. Left ventricle potion was removed and weighed. The heart tissue was homogenized in 5 ml of chilled 0.1 M tris-hydrochloric acid buffer (pH: 7.4). The homogenate was centrifuged (3,000 ×g for 5 min) and the supernatant was used for the estimation of various biochemical parameters.

2.7. Assessment of left ventricular hypertrophy

Left ventricular hypertrophy was calculated as the ratio of left ventricular weight /whole heart [19].

2.8. Biochemical analysis

2.8.1. Assay of cardiac damage biomarkers

The activity of serum CK-MB was assayed by a standard diagnostic kit (Infinite-CK-MB) purchased from Accruex Diagnostics Private Limited, Mumbai, India, and myoglobin

was estimated in the serum by the standard diagnostic kit (Vitros Immunodiagnostic Products Myoglobin Assay), purchased from Ortho Clinical diagnostics, New York, USA.

2.8.2. Assay of lipid peroxidation products

The concentration of plasma thiobarbituric acid reactive substances was estimated by the method of Yagi et al. [20]. To 0.5 ml of plasma, 4 ml of 0.083 N sulphuric acid was added. To this mixture, 0.5 ml of 10 % phosphotungstic acid was added and mixed thoroughly. After allowing the tubes to stand at room temperature for 5 min, the mixture was centrifuged at 3,000 ×g for 10 min. The supernatant was discarded and the sediment was mixed with 2 ml of sulphuric acid and 0.3 ml of 10 % phosphotungstic acid. The mixture was shaken well and centrifuged at 3,000 ×g for 10 min. The sediment was suspended in 4 ml of double distilled water and 1 ml of thiobarbituric acid reagent was added. The reaction mixture was heated at 95 ºC for 60 min. After cooling, 5 ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at 3,000 ×g for 15 min. The colour extracted in n- butanol layer was measured at 530 nm in a UV-Spectrophotometer. Lipid hydroperoxides in the plasma was estimated by the method of Jiang et al. [21]. 1.8 ml of the Fox reagent was mixed with 0.2 ml of plasma homogenate and incubated for 30 min at room temperature and the absorbance was measured at 560 nm in a UV-Spectrophotometer.

2.8.3. Assay of lipid profile

Lipid profile was measured in the plasma and heart tissue homogenates. Lipids were extracted from the heart tissue by the method of Folch et al. [22]. A known weight (200 mg) of the heart tissue was homogenized in 7 ml of methanol using a Potter-Elvejhem Teflon

homogenizer. The contents were filtered. The residue was homogenized with 14 ml of chloroform-methanol (2:1 v/v) mixture. This was again filtered in to the side arm flask and the residue was successively homogenized in chloroform-methanol (2:1 v/v) mixture and each time this extract was filtered. The collective filtrates in the flask were adjusted to a final volume using chloroform-methanol (2:1 v/v) and evaporated to dryness to a constant weight. This preparation was used for further analysis. The cholesterol content and the triglyceride contents were estimated by reagent kits (Qualigens Diagnostics, Mumbai, India). The levels of FFA were estimated by the method of Falholt et al. [23]. 0.1 ml of lipid extract was evaporated to dryness. To this, 1 ml of phosphate buffer, 6 ml of extraction solvent and 2.5 ml of copper reagent were added. The tubes were shaken vigorously for 90 sec and kept aside for 15 min. Then, the tubes were centrifuged and 3 ml of the upper copper layer was transferred to another tube containing 0.5 ml of diphenyl carbazide solution and mixed carefully. The absorbance was measured at 550 nm immediately in a UV-Spectrophotometer. Phospholipid content was estimated by the method of Zilversmit and Davis [24]. 0.1 ml of lipid extract was evaporated to dryness. To this, 1 ml of concentrated sulphuric acid and 1 ml of concentrated nitric acid were added and digested to a colorless solution. 1 ml of the supernatant was taken and made up to 4.3 ml with double distilled water and 1ml of ammonium molybdate was added and incubated at room temperature for 10 min. To this, 0.4 ml of 1-amino-2-napthol-4-sulphonic acid reagent was added. The colour developed was measured at 640 nm in a UV-Spectrophotometer after 20 min.

High density lipoprotein (HDL) was estimated by a reagent kit (Auto Zyme: HDL-Cholesterol) from Accurex Diagnostics Private Limited, Mumbai, India. Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) were calculated as follows: LDL = Total cholesterol–(HDL+VLDL); VLDL= Triglycerides/5.

2.8.4. Assay of 3-hydroxy-3 methyl glutryl CoA reductase (HMG CoA reductase)

The activity of HMG-CoA reductase was assayed by the method of Rao and Ramakrishnan [25]. Equal volumes of fresh 10 % tissue homogenate and diluted perchloric acid were mixed, kept for 5 min and centrifuged at 2,000 ×g for 10 min. To this, 1 ml of filtrate, 0.5 ml of freshly prepared hyroxylamine reagent (alkaline hydroxylamine in the case of HMG-CoA) was added, mixed and after 5 min 1.5 ml of ferric chloride was added and shaken well. Readings were taken after 10 min at 540 nm in a UV- Spectrophotometer against a similarly treated saline arsenate blank. The ratio of HMG-CoA to mevalonate was calculated. Lower ratio indicates higher enzyme activity and vice-versa.

2.9. Myocardial necrosis score

Histological examination of the hearts was undertaken to study the severity of infarction [26]. Tissues were fixed in 4 % paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin for histological evaluation of tissue damage. Transverse sections of the ventricular myocardium were graded for the severity of necrosis and mononuclear cell infiltration by the 2 independent pathologist blinded to the experimental protocol, using the following scale: grade 1, lesions involving < 25 % of the ventricular myocardium; grade 2, lesions involving 25 to 50 % of the myocardium; grade 3, lesions involving 50 to 75 % of the myocardium; and grade 4, lesions involving > 75 % of the myocardium.

2.10. In vitro free radical scavenging analysis

2.10.1. Determination of superoxide radical scavenging activity (in vitro)

The superoxide radical scavenging activity was measured by the method of Sabu and Kuttan [27]. Ellagic acid (10-50 µg/ml) was taken in a test tube. To this, reaction mixture consisting of 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) nitro blue tetrazolium

chloride and 0.2 ml of 0.1 mM EDTA solutions were added and reading was taken at 560 nm immediately. About 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction then reaction mixture was incubated at 25 °C for 15 min and reduction of nitro blue tetrazolium chloride was measured at 560 nm. Ascorbic acid was used as the reference compound. Decreased absorbance of the reaction mixture indicates
increased superoxide anion scavenging activity. All the samples were treated in the similar manner. Absorbance was recorded and the percentage of inhibition was calculated.

2.10.2. Determination of hydroxyl radical scavenging activity (in vitro)

The hydroxyl radical scavenging capacity was measured using modified method as described by Halliwell et al. [28]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1 ml of ellagic acid (10-50 μg/ml) each dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. About 1 ml portion of the incubated mixture was mixed with 1 ml of (10 %) trichloro acetic acid and 1 ml of (0.5 %) thiobarbituric acid (in 0.025 M NaOH containing butylated hydroxy anisole) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the ellagic acid was reported as the percentage of inhibition of deoxyribose degradation

2.11. Statistical analysis

Results are expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed

with Duncan's multiple range test (DMRT) using a software, statistical package for the social science (SPSS) version 16.00. Throughout the report, wherever we have used the word "significant" to describe results, we mean "statistically significant at an alpha level of 0.05 (p<0.05) for the two-sided alternative hypothesis.

3. Results

Examples of the patterns of cardiac rhythm of normal and experimental rats are shown in (Fig. 1). Regular cardiac rhythm was observed in normal and ellagic acid treated rats. The ISO-induced rats showed increased arrhythmiasis (VT and VF) with higher arrhythmic score (4.55 ± 0.52) when compared to normal rats. The rats received ellagic acid (7.5 and 15 mg/kg) as pretreatment showed significant decrease in VT without VF and the arrhythmic scores were significantly reduced (3.11 ± 0.52 and 1.08 ± 0.31 respectively) when compare to non-treated ISO-induced myocardial infarcted rats.

The mean body weight of rats in all experimental groups had no significant change. Interestingly, and left ventricle weight/heart weight ratio were significantly increased in ISO-induced rats as compared to the controls. Pretreatment with ellagic acid significantly reduced the heart weight and reduced left ventricular weight. ISO-induced rats showed a significant increase in the left ventricle to body weight ratio. Pretreatment with ellagic acid (7.5 and 15 mg/kg) showed significantly reduced left ventricle to body weight ratio in comparison with non-treated ISO-induced myocardial infarcted rats. (Fig. 2).

In the present study there was a significant increase in the levels of CK-MB and myoglobin in the serum of ISO-induced rats, when compared to that of the normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) daily for a period of 10 days

significantly decreased the activities of cardiac marker enzymes in the serum, when compared to non-treated ISO-induced myocardial infarcted rats (Fig. 3).

The ISO-induced myocardial infarcted rats showed a significant increase in the levels of thiobarbituric acid reactive substances and lipid hydroperoxides, when compared with the normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) significantly reduced the levels of thiobarbituric acid reactive substances and lipid hydroperoxides, when compared to rats induced with ISO alone that reflecting the anti lipid-peroxidative effect of ellagic acid (Fig. 4).

Fig. 5 showed the alterations in the lipid profile in normal and experimental rats. The levels of total cholesterol, LDL and VLDL in the serum were significantly increased and HDL was decreased in the ISO-induced rats, when compared to the normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) regulated the altered lipid profile levels by decreasing the levels of total cholesterol, LDL, VLDL and increasing the levels of HDL (Fig. 5).

ISO-induced rats showed a significant increase in triglycerides, FFA and phospholipids in the serum and increased levels of total cholesterol, triglycerides and FFA and decreased level of phospholipids in the heart tissue homogenate when compared with normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) significantly decreased the levels of triglycerides, FFA and phospholipids in the serum, decreased total cholesterol, triglycerides, FFA and increasing the levels of phospholipids in the heart tissue homogenate, when compared to rats induced with ISO alone (Fig.6-8).

The significant increase in the activity of HMG-CoA reductase in the liver of ISO-induced rats was indicated by the lower ratio of HMG-CoA/mevalonate. Pretreatment with ellagic acid (7.5 and 15 mg/kg) significantly decreased the activity of HMG-CoA reductase,

when compared to ISO-induced rats and it is indicated by higher ratio of HMG-CoA/mevalonate (Fig. 9).

For all the biochemical parameters studied, treatment with ellagic acid (7.5mg/kg and 15 mg/kg) daily for a period of 10 days to the normal control rats did not show any significant effect.

Ellagic acid showed superoxide and hydroxyl free radicals scavenging effect *in vitro*. The percentage scavenging effects of ellagic acid on super oxide free radical at different concentrations (10, 20, 30, 40 and 50 μM) were found to be 18.54. 29.53. 48.62, 68.66 and 93.55 % respectively. The percentage scavenging effects of ellagic acid on hydroxyl free radical at different concentrations (10, 20, 30, 40 and 50 μM) were found to be 24.65, 48.43, 69.76, 74.34**,** and 88.43 % respectively. These results indicated the stronger ability of the ellagic acid to act against superoxide and hydroxyl free radicals at minimum doses.

Though our previous report indicated histological analysis of heart tissue [8], a detailed analysis of necrosis such as the degree of damage, percentage of inflammation and the area at risk of infarction were observed. Histology of ellagic acid (7.5 and 15 mg/kg) pretreated rats showed improvement in the cardiac muscle structure when compare to ISO-induced rats. Mild alterations in the architecture were observed with interstitial edema and localized necrotic areas. The damage score was significantly reduced in ellagic acid pretreated rats, when compared with ISO-induced rats (Table 1).

4. Discussion

It is well accepted that cardiac contractile function and energy metabolism are actively downregulated during myocardial ischemia and infarction. VT arising from myocardial ischemia and infarction is a leading cause of death. We observed VT in ISO-

induced myocardial infarcted rats. The primary disturbances of ISO-induced MI lead to the lypolysis in the myocardium [29]. Increased lipolysis during acute ischemia would result in increased circulating FFA, which would, in turn, contribute to arrhythmogenesis. There have been interests in the theory that oxygen free radicals play a role in the pathogenesis of myocardial ischemia and infarction. Therefore, treatment with antioxidants might be beneficial [30]. Our results showed that the oral pretreatment with ellagic acid is beneficial to VT and VF. The current observation was in line with our previous report on the effect of ellagic acid against increased heart rate in ISO-induced rats [8].

The left ventricular hypertrophy in the ISO-induced rat hearts was observed. Structural remodeling of components of the ventricular walls due to its adaptive response to physiological and pathological alterations during ISO-induced damage may be the reason for this condition. The NF-κB signalling pathway was reported o play a pivotal role in myocardial hypertrophy [31, 32]. Pretreatment with ellagic acid decreased left ventricular hypertrophy significantly in the ISO-induced rats. Polyphenolic compounds have been known for its ability to block NF-**κ**B activation by inhibiting I kappa B kinase activity [33]. Ellagic acid, be a polyphenolic acid may underwent the same mechanism to block the NF-**κ**B activation and reduced hypertrophy.

Previous study from our laboratory demonstrated that the pretreatment with ellagic acid showed beneficial effects on cardiac markers such as troponin-I, creatinine kinase, lactate dehydrogenase and plasma homocystein. In clinical diagnosis, the detection of myoglobin and CK-MB isoforms in the serum will be helpful in the accurate and specific conclusion of MI [8]. Myoglobin may represent an intracellular fatty acid transporter [34]. The presence of myoglobin in the serum plays an important role in the prediction of infarct size, either alone or in combination with other cardiac markers. CK-MB isoforms have been found to be effective for rapid early diagnosis of MI [35], and there are a few reports in

which myoglobin and CK-MB isoforms have been compared for early diagnosis of MI [36, 37]. We observed a significant increase in the level of myoglobin and CK-MB in the ISO-induced myocardial infarcted rats. These elevated levels of cardiac markers exposed the changes in membrane integrity and disturbed the permeability of the cardiomyocytes. ISO-induced membrane damage was reported previously [8, 9]. The oral pretreatment with ellagic acid decreased the levels of myoglobin and CK-MB in ISO-induced rats. Based on its structure, ellagic acid can interact with cell membranes, altering the permeability of the cells and can activate or deactivate proteins and transcription factors by key metabolioc pathways.

The increase in lipid peroxidation observed in ISO-induced rats may be a consequence of higher levels of free radicals, which are produced in significant amounts in response to the oxidized metabolic products of ISO metabolites. The increased levels of thiobarbituric acid reactive substances and lipid hydroperoxides confirmed increased lipid peroxidation in ISO-induced rats. Pretreatment of ISO-induced rats with ellagic acid significantly decreased the levels of thiobarbituric acid reactive substances and lipid hydroperoxides in the systemic circulation. Ellagic acid inhibits the generation of free radicals through its metal chelating property, thus providing protection against lipid peroxidation [38]. This same activity may possibly account for the protective effect of ellagic acid against lipid peroxidation in ISO-induced MI.

The supramaximal dose of ISO promotes lipolysis in the myocardium, and increases the accumulation of lipids in the heart. The increased myocardial cholesterol content in ISO-induced rats is due to the increased uptake of LDL-cholesterol from the blood by myocardial membranes [39]. Enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate is another reason for increased cholesterol levels [40]. Oral pretreatment with ellagic acid significantly decreased the levels of cholesterol in ISO-induced myocardial infarcted rats. Ellagic acid has a great affinity for the lipid membrane of myocardium and also reduces the

uptake of LDL from the circulatory system, which may be one of the reasons for its action against hyperlipidemia. Our results suggest that ellagic acid may be a candidate agent for further development in the anti-hyperlipidemic agent.

We observed increased levels of LDL and VLDL and a decreased level of HDL in the serum of ISO-induced rats. A defect in the LDL receptor, either through failure in its production or function, and increased uptake of LDL from the blood by myocardial membranes, may be reasons for the increased LDL level in the ISO-induced rats [41]. Inhibition of the HDL level makes the heart vulnerable to oxidized LDL and produces MI. Elevated flux of fatty acids and impaired removal of VLDL from the serum during hypoxia induced oxidative stress may be the reason for the enhanced level of VLDL in the ISO-induced rats [42]. Pretreatment with ellagic acid regulated the altered levels of lipoproteins in ISO-induced rats. Devipriya et al. (2008) reported that ellagic acid had protective effects against LDL oxidation and that ellagic acid supplementation effectively regulated the lipid and lipoprotein levels in alcohol induced toxicity [43]. Our reports on the effect of ellagic acid on lipid profile confirmed the protective effect of ellagic acid on lipid metabolism in ISO-induced MI.

The observed increase in the level of triglycerides in ISO-induced rats might have been be due to a decrease in the activity of lipoprotein lipase, resulting a decreased uptake of triglycerides from the circulation and enhancement of lypolysis [44]. The accumulation of acyl coA and augmented production of glycerol by increased glycolytic flux may be an additional reason for hyper-triglyceridemia. Pretreatment with ellagic acid significantly reduced the levels of triglycerides in ISO-induced rats. Polyphenolic compounds are known to regulate lipoprotein production and secretion through the inhibition of various enzymes, such as phospholipase A_2 [45]. Ellagic acid, a phenolic acid may also possess the same action and reduced the levels of increased triglycerides.

We observed an increase in the level of free fatty acids in ISO-induced rats and these results are in relation with the increased arrhythmiasis. Hypoxia, produced by a supramaximal dose of ISO, leads to oxidation of carbohydrates and produces free fatty acids. An increased level of phospholipids in the serum and decreased level in the heart tissue were also observed in the ISO-induced rats. Increased lipid peroxidation by ISO may cause damage to the phospholipid rich myocardial membrane and lead to a leakage of phospholipids from the myocardium. Pretreatment with ellagic acid significantly reduced the level of phospholipids in the serum and increased in the heart tissue homogenate. Anti lipid peroxidation of ellagic acid may helped to protect the phospholipids in myocardium. The metal chelating capacity of ellagic acid can effectively chelates calcium in the extracellular matrix, and suppresses the carbohydrate metabolism. This decreased carbohydrate metabolism may contributed for reduced FFA levels.

Though the exact mechanism by which ellagic acid regulated lipid profile is not clear, it can be speculated that ellagic acid decreases the activity of HMG-CoA reductase, a rate limiting enzyme in cholesterol biosynthesis; this may enhance the rate of the lipid degradative process and decrease the levels of other lipids [46]. To confirm this phenomenon, we studied the activity of ellagic acid in the liver of normal and experimental rats. Interestingly, we observed increased activity of HMG-CoA reductase in the liver of ISO-induced myocardial infarcted rats. Pretreatment with ellagic acid significantly decreased the activity of this enzyme in the liver of myocardial infarcted rats. The decreased activity of this enzyme in the ellagic acid pretreated rats showed that ellagic acid can act as HMG-Co-reductase inhibitor. A detailed study is necessary to explain the mechanism.

In our previous report, we discussed the free radical scavenging activity and reducing power of ellagic acid [8]. Further we decided to explore the radical scavenging activities against superoxide and hydroxyl free radicals. The highest percentages of the scavenging

effect of ellagic acid on superoxide and hydroxyl free radicals was found at 50 μg/ml concentration and were 93.55 % and 88.43 % respectively. Ellagic acid is partially hydrophobic and can be a perfect candidate for activating the signalling pathways responsible for stimulation of the pentose phosphate pathway, an important pathway for regulating the antioxidant stress response and it may be closely linked to stimulation of cellular antioxidant response pathways [47, 48].

In conclusion, our study provides the link between arrhythmiasis, hypertrophy and the involvement of hyperlipidemia in MI. Our reports showed that the exposure to excessive ISO cause VT and VF, which could be prevented by pretreatment with ellagic acid. Ellagic acid could be a very useful phyto-nutrient to prevent MI by both inhibiting lipid peroxidation and improving the lipid profile. Ellagic acid showed its beneficial effects on myocardial necrosis by improving the necrotic score of ISO-induced myocardial infarcted rats. The *in vitro* studies on superoxide and hydroxyl free radical scavenging effects of ellagic acid suggest that the free radical scavenging ability of ellagic acid may be the reason for its protective effects.

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Conflict of interest declaration

The authors of this article do not have any conflict of interest to disclose.

Authors' contributions

Both the authors (DQ and MK) were designed, organized and carried out the experimental works and wrote the manuscript. Both of the authors read and approved the final manuscript.

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Table 1. Effect of ellagic acid on myocardial necrosis.

Normal control and ellagic acid (7.5 and 15 mg/kg) treated rats did not show any infarction/

Groups	% infarct size/ left ventricle	% infarct size/ area at risk	% area at risk/ left ventricle	Degree of damage
Isoproterenol-alone (100mg/kg)	37.34 ± 3.66*	34.55 ± 5.43*	53.52 ± 4.58*	3.45 ± 0.31*
Ellagic acid (7.5 mg/kg) + isoproterenol (100mg/kg)	24.23 ± 4.34†	18.84 ± 3.31†	39.63 ± 3.28†	2.67 ± 0.19†
Ellagic acid (15 mg/kg) + isoproterenol (100mg/kg)	16.64 ± 3.21‡	23.54 ± 3.62‡	27.44 ± 2.88‡	1.88 ± 0.21‡

necrosis. Each value is mean ± standard deviation for six rats in each group, values not sharing a common superscript (*,†, ‡) differ significantly with each other ($p < 0.05$, duncan's multiple range test).

Figure legends

Fig. 1(a-f): Effect of ellagic acid on ventricular tachycardia in normal and experimental rats.

Fig.1(a): Rhythmic pattern of normal control group.

Fig.1(b): Rhythmic pattern of ellagic acid (7.5mg/kg) treated group

Fig.1(c): Rhythmic pattern of ellagic acid (15mg/kg) treated group rats

Fig.1(d): Rhythmic pattern of isoproterenol(100mg/kg)-induced group rats showing ventricular tachycardia and ventricular fibrillation.

Fig.1(e): Rhythmic pattern of ellagic acid (7.5mg/kg) treated isoproterenol-induced rats showing minimized ventricular tachycardia.

Fig.1(f): Rhythmic pattern of ellagic acid (15mg/kg) pretreated isoproterenol-induced rats showing cardiac rhythm with mild arrhythmiasis.

Fig. 2: Effect of Ellagic Acid on left ventricular hypertrophy. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.3: Effect of ellagic acid on cardiac markers CK-MB and Myoglobin. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.4: Effect of ellagic acid on lipid peroxidation. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group

VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.5: Effect of ellagic acid on total cholesterol and lipoproteins. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.6: Effect of ellagic acid on triglycerides, free fatty acids, and phospholipids in the serum. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.7: Effect of ellagic acid on total cholesterol, triglycerides and phospholipids in the heart. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.8: Effect of ellagic acid on free fatty acid in the heart. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six

rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

Fig.9: Effect of ellagic acid on HMGCo-A enzyme activity. Group I: Normal control rats; Group II: Normal rats + ellagic acid (7.5 mg/kg); Group III: Normal rats + ellagic acid (15 mg/kg); Group IV: ISO-induced rats (100mg/kg); Group V: ellagic acid (7.5 mg/kg) + ISO; Group VI: ellagic acid (15 mg/kg) + ISO. Each column is mean ± standard deviation for six rats in each group. Columns not sharing a symbol (*, †, ‡, §,) differ significantly with each other.

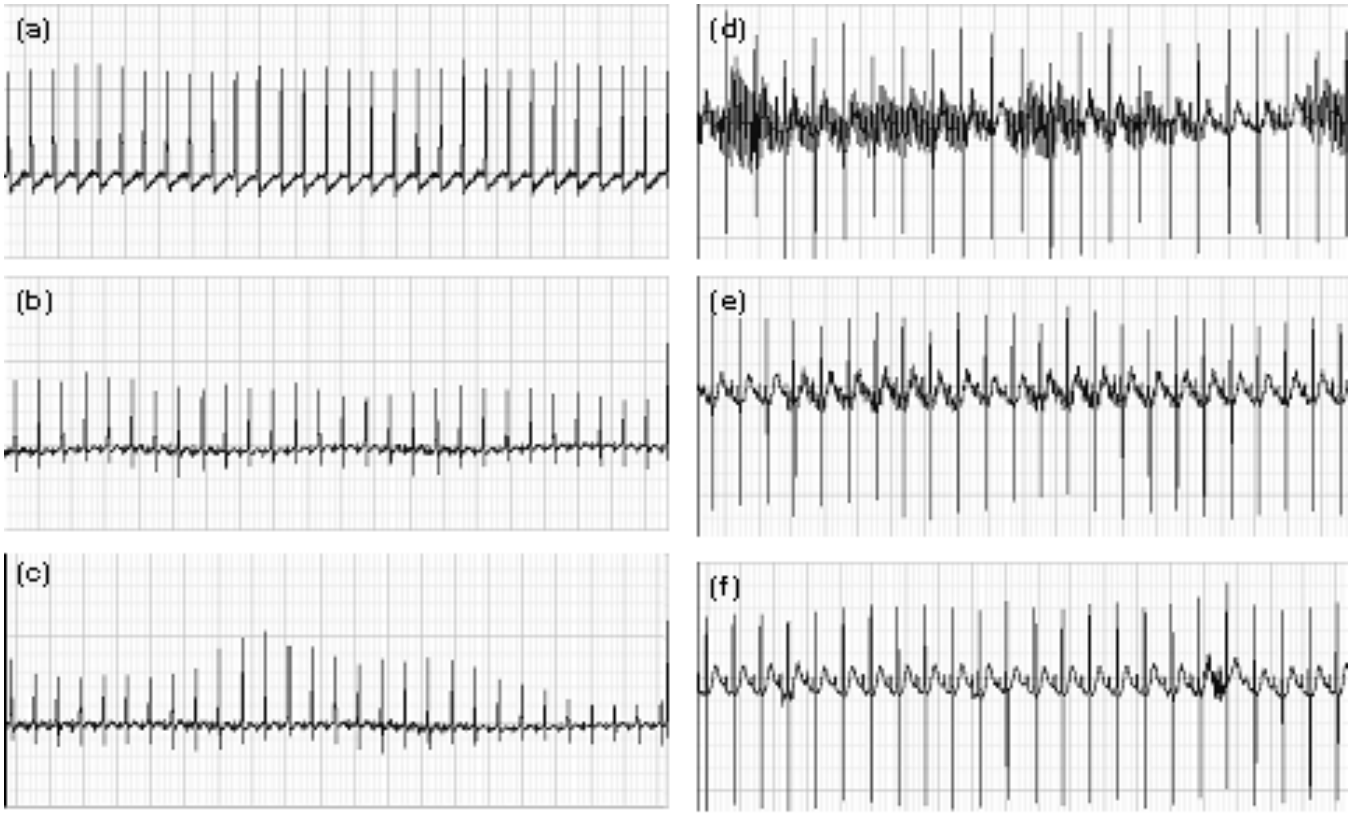

Figure 1

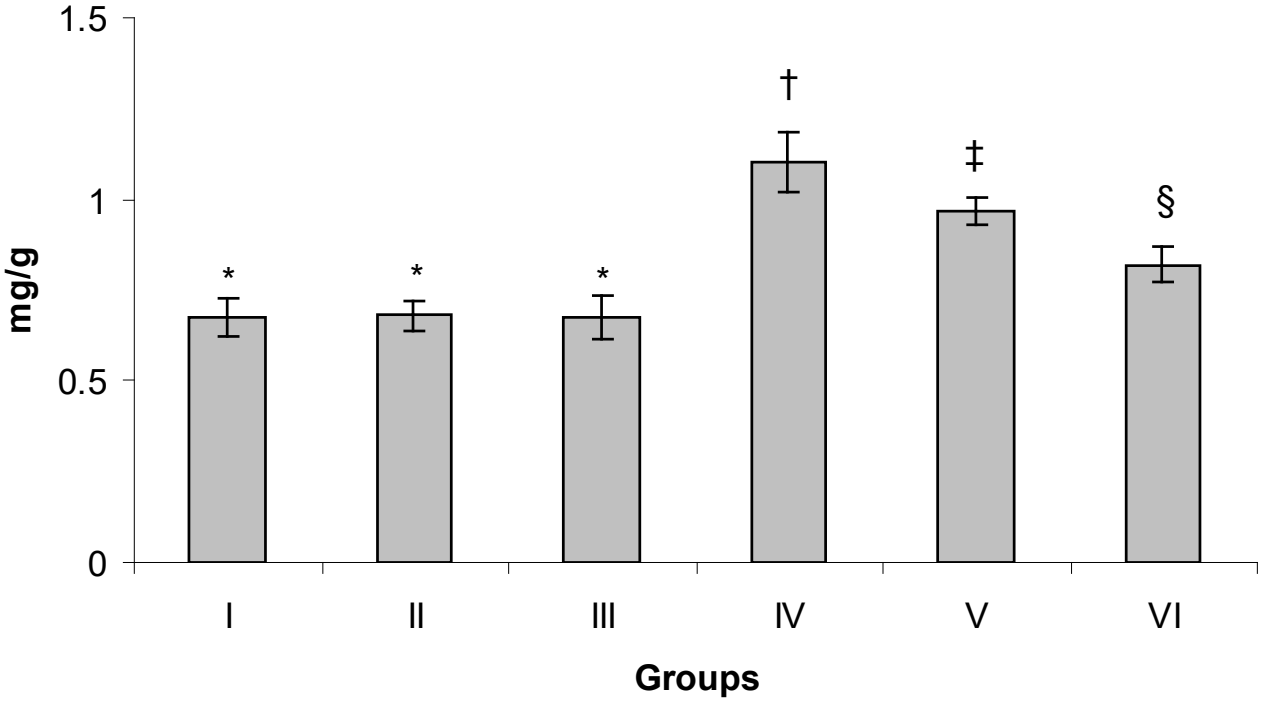

Figure 2

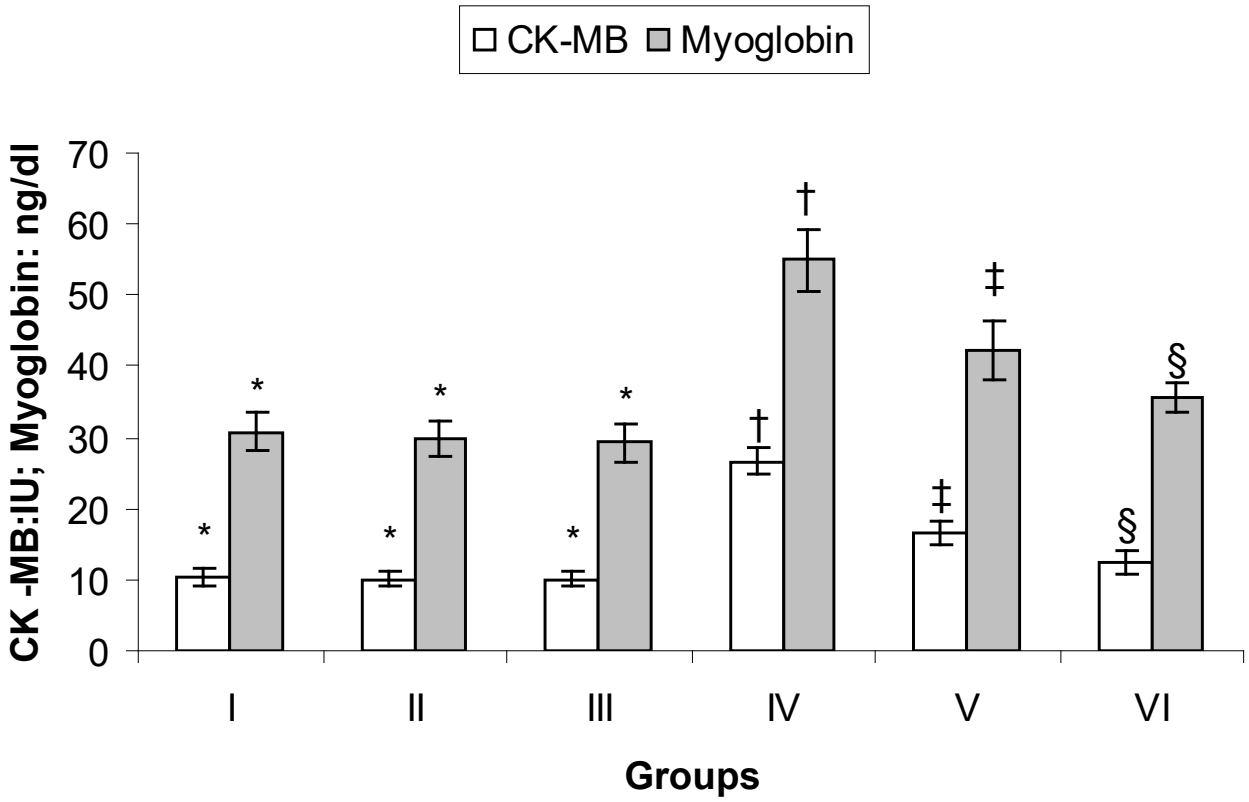
CK-MB
Myoglobin
CK -MB:IU; Myoglobin: ng/dl
70
60
50
40
30
20
10
0
I
II
III
IV
V
VI
Groups

Figure 3

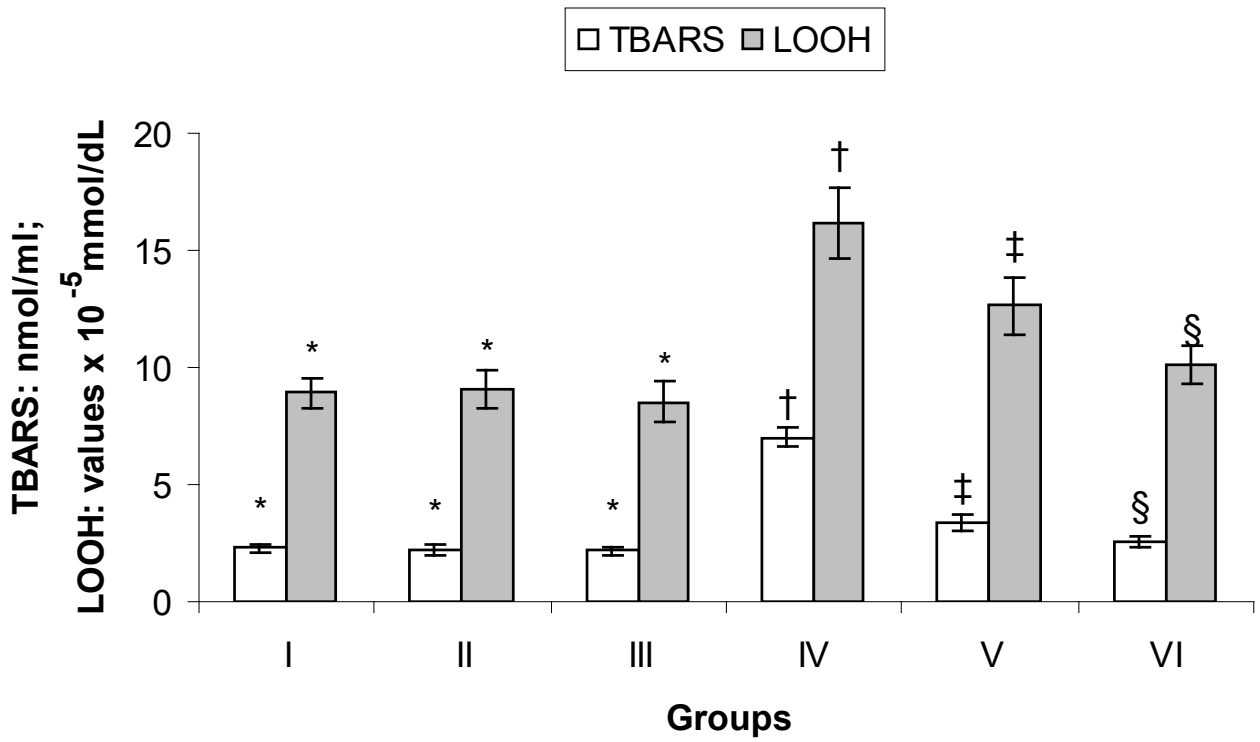
TBARS
LOOH
TBARS: nmol/ml;
LOOH: values x 10 -5 mmol/dL
20
15
10
5
0
*
†
‡
§
I
II
III
IV
V
VI
Groups

Figure 4

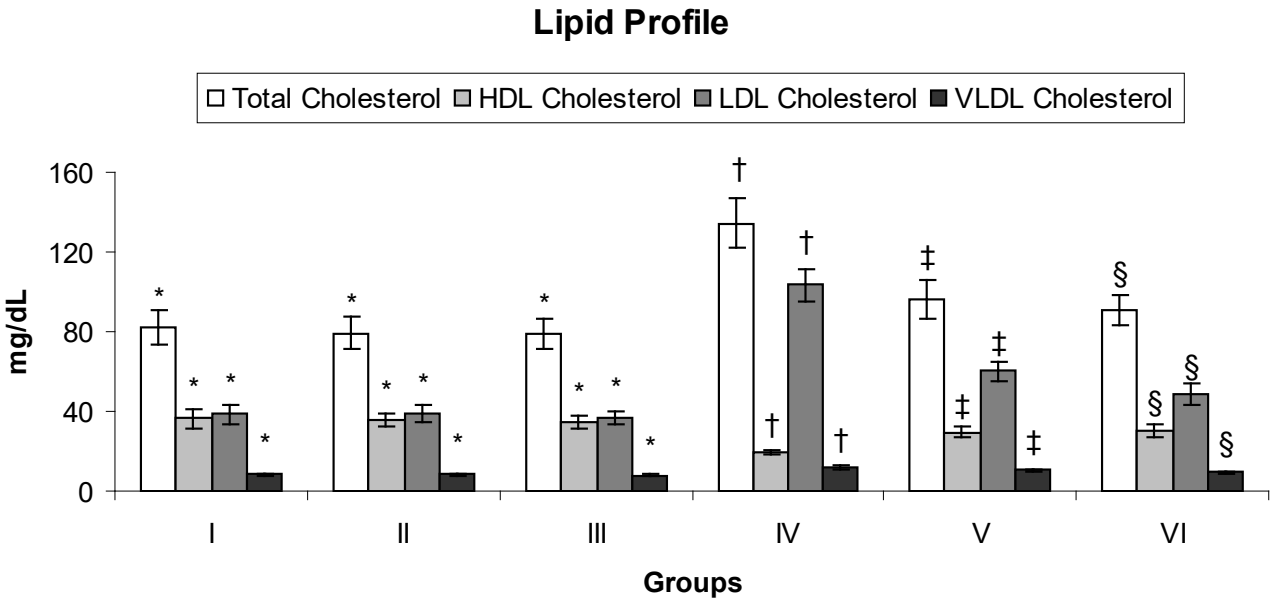
Lipid Profile
Total Cholesterol
HDL Cholesterol
LDL Cholesterol
VLDL Cholesterol
mg/dL
160
120
80
40
0
I
II
III
IV
V
VI
Groups

Figure 5

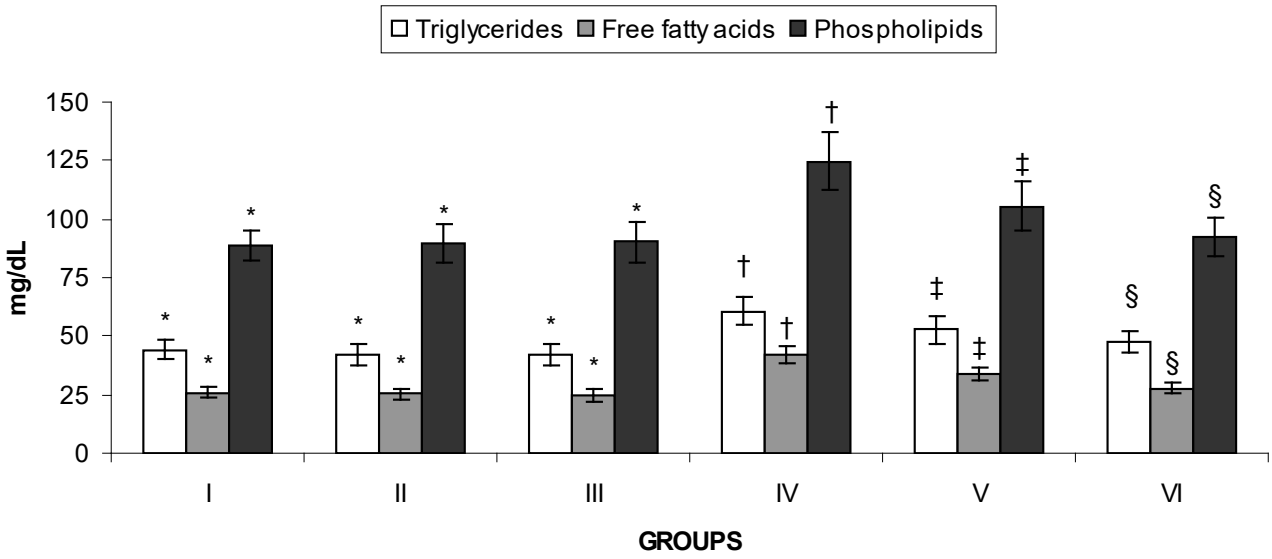
Triglycerides
Free fatty acids
Phospholipids
150
125
100
75
50
25
0
mg/dL
I
II
III
IV
V
VI
GROUPS

Figure 6

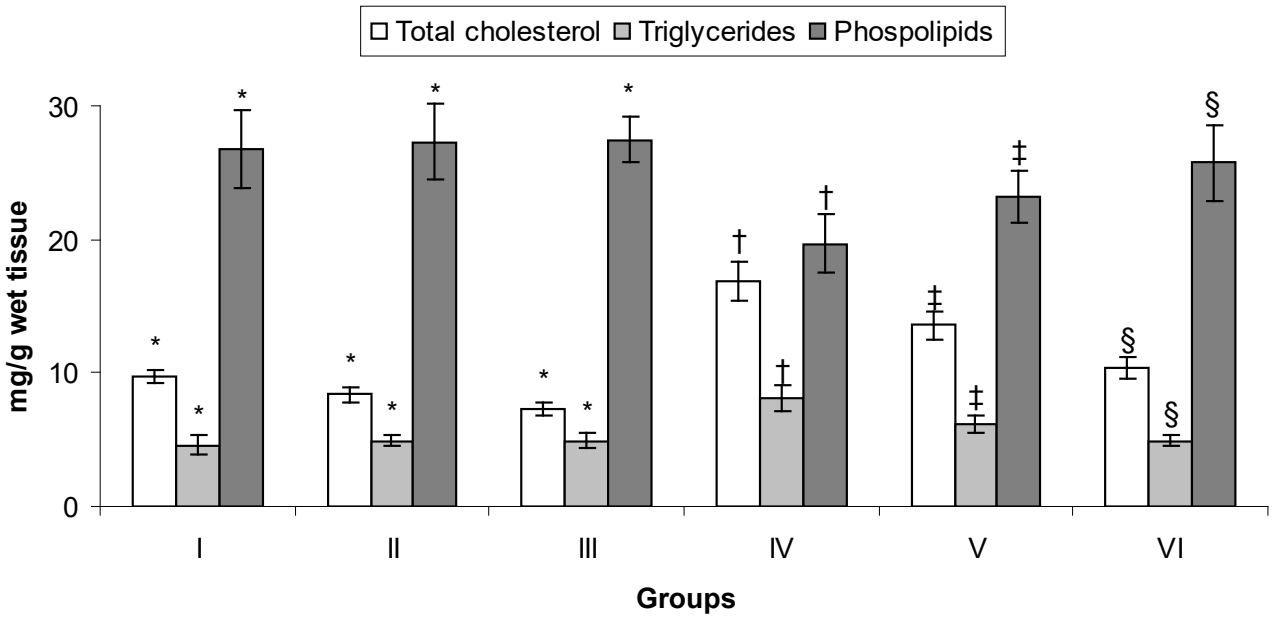
Total cholesterol
Triglycerides
Phospolipids
mg/g wet tissue
30
20
10
0
I
II
III
IV
V
VI
Groups

Figure 7

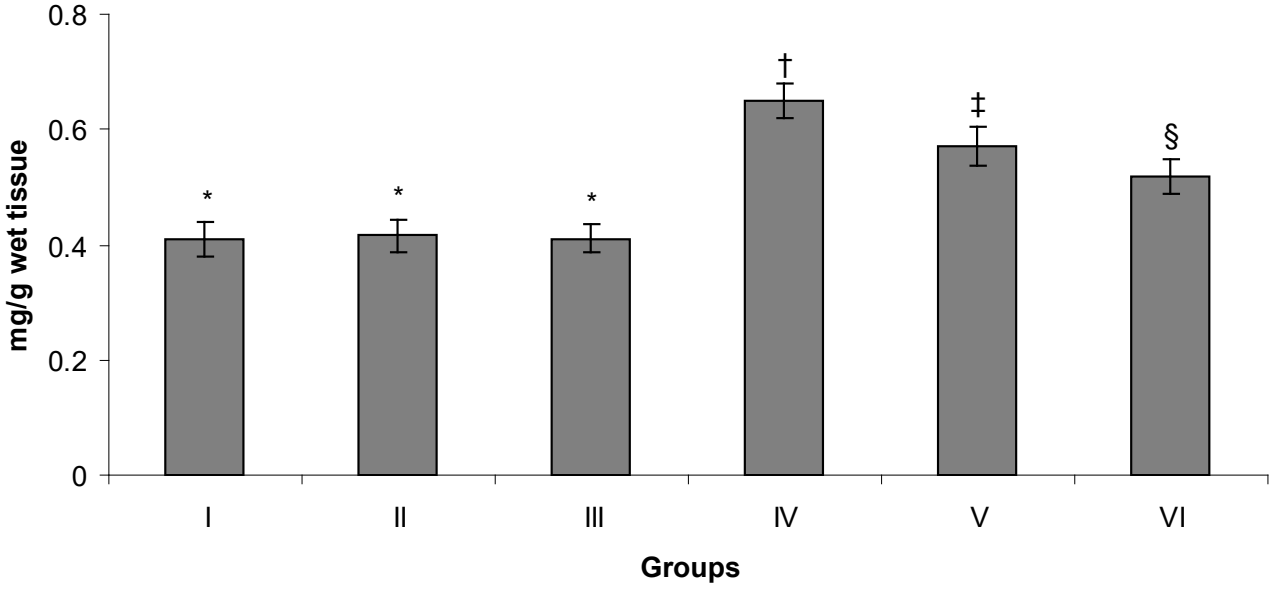

Figure 8

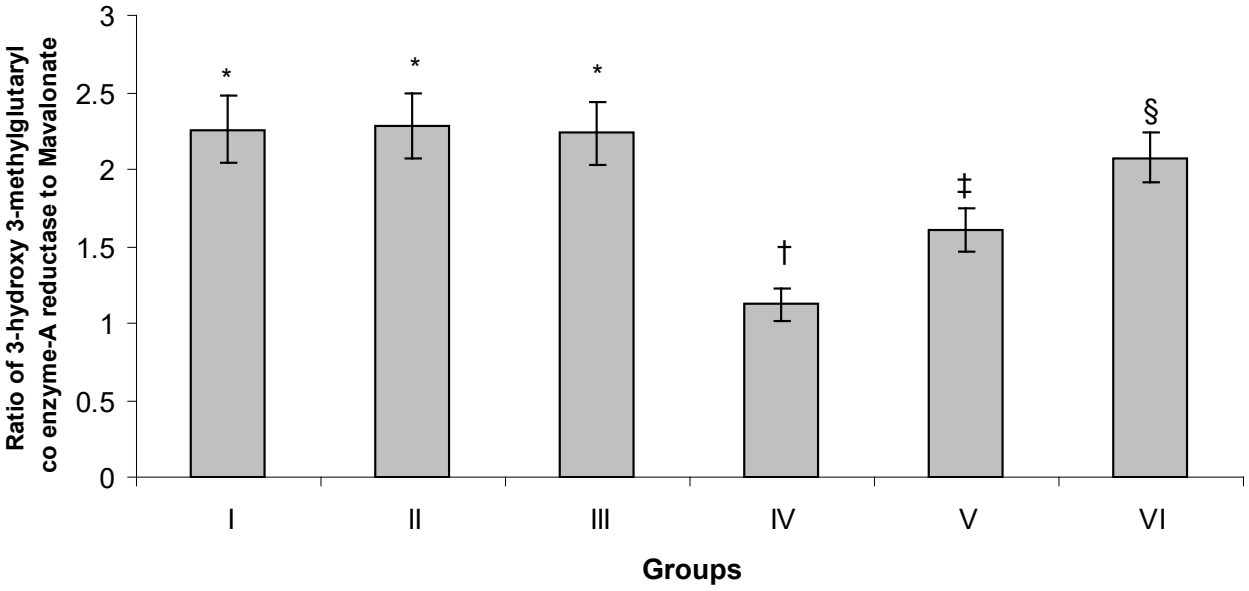
Ratio of 3-hydroxy 3-methylglutaryl co enzyme-A reductase to Mavalonate
3
2.5
2
1.5
1
0.5
0
*
*
*
†
‡
§
I
II
III
IV
V
VI
Groups

Figure 9