Morin protects heart from beta adrenergic stimulated myocardial infarction; an electrocardiographic, biochemical and histological study in rats

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Abstract

Introduction: In recent years, polyphenols have attracted considerable attention as agents that protect cells or molecules from oxidative myocardial injury. This study was aimed to prove the cardioprotective benefits of the flavonoid morin in isoproterenol induced myocardial infarcted rats.

Materials and methods: Male Wistar rats were treated orally with morin (10 and 20 mg/kg) daily for a period of 21 days. After 21 days of pretreatment, isoproterenol (100 mg/kg) was injected subcutaneously to rats at an interval of 24 h for 2 days to induce myocardial infarction. Electrocardiographical abnormalities and bio markers were measured in normal and experimental rats.

Results: Isoproterenol-induced myocardial infarcted rats showed significant ($p<0.05$) increase in the levels of cardiac markers. Pretreatment with morin regulated the abnormalities in electrocardiograph and bio markers. The lipid peroxidation products were increased and indicated the increased lipid peroxidation in isoproterenol-induced myocardial infarcted rats. The rats pretreated with morin significantly reduced lipid peroxidation. The altered lipid metabolism was observed in isoproterenol-induced myocardial infarcted rats and pretreatment with morin regulated lipid metabolism. Histopathological study evidenced that the pretreatment with morin inhibited myocardial damage.

Conclusion: The results of this study proved the protective effect of morin as pretreatment and are rational to understand the beneficial effects of morin on cardio protection against myocardial injury. Based on the results the cardioprotective ability of morin on human being can be studied in future.

Key words: Morin, Isoproterenol, Electrocardiogram, Lipid peroxidation, Lipid profile, Histopathology

1. Introduction

Coronary artery diseases (CAD) are the most prevalent cause of death and disability worldwide. CAD is a condition in which the vascular supply to the heart is impeded and cause ischaemia. A prolonged ischemia may cause the death of cardiomyocytes i.e., myocardial infarction [MI]. It means necrosis of a region of myocardium caused by an interruption in the supply of blood to the heart usually as a result of occlusion of a coronary artery. [7] Hyperlipidemia is a major cause of CAD [19] due to increase intake of high fat diet in daily life [40]. There are some treatment protocols available to control the cholesterol level in CAD patients that includes statins, fibrates and nicotinic acid therapy and are not used routinely in all patients. The regular cardioprotective therapies are used after the diagnosis of CAD. But still, cardiovascular disease is estimated to be the leading cause of morbidity and mortality. Currently there are numbers of studies focused to identify new therapeutic strategies to prevent or reverse CAD. Alternative treatment strategies with lipid regulating nutrients are becoming increasingly popular as these therapies have no or minimal side effects and cost effective. [13].

In recent years, polyphenols have attracted considerable attention as agents that protect cells or molecules from oxidative myocardial injury. Polyphenols are excellent cardioprotectants [21]. Morin is one such polyphenolic phytonutrient found in wide varieties of Maclura pomifera (Osage orange), Maclura tinctoria (old fustic) and from leaves of Psidium guajava (common guava) and it has received fastidious consideration because of its extensive assortment of biological properties. Previous studies indicated that morin exhibits a variety of pharmacological activities such as free radical scavenging action [27], anti-inflammatory activity [11, 13], antioxidant activity [26, 48] and an anti-tumor promotion effect [17, 28] and there are no reports on its toxicity in any of the preclinical and clinical studies. Hence, we hypothesized that morin can be screened for its cardioprotective effect.

Isoproterenol (ISO), a catecholamine and β-adrenergic agonist, is used to study the protective effect of various drugs on cardiac functions. MI induced by ISO, has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human MI [30]. It induces myocardial necrosis by a multiple step mechanism [36]. Chagoya de sanchez et al. [5] have reported that ISO causes an increase in the levels of circulatory and myocardial lipids. Further, ISO promotes lipolysis in the myocardium [44].

Electrocardiographic changes are the gold standard markers of cardiovascular disorders. Therefore, we included ECG analysis along with the measurement of lipid profile and cardiac markers to evaluate the cardioprotective potential of morin in experimentally induced MI in rats. The objective of the present study was to examine the cardioprotective potential of the morin as a pretreatment against experimentally induced MI in male Wistar rats.

This study attempted to provide the evidence of the cardioprotective effect of morin and explain the possible underlying mechanism of action. The results of this study would be an ideal indicator for further research.

2. Methods

2.1. Drug and chemicals

Morin and Isoproterenol were purchased from Sigma Chemical Co., St. Louis, MO, USA. Xylenol orange, dithionitro bis benzoic acid, nitroblue tetrazolium, 2, 3, 5–triphenyl tetrazolium chloride, sodium sulphite, dimethyl sulfoxide, phenazine methosulphate and oxidized glutathione were obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals used in this study were of analytical grade.

2.2. Animals

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 and approved by the Institutional Animal ethical committee of Jayamukhi College of Pharmacy (approval number 23/IAEC/JCP/2011, Dated 22-02-2011). All the experiments were carried out with male albino Wistar rats weighing 180-200 g, purchased from Mahaveer Enterprises, Hyderabad, India. They were housed in polypropylene cages (47 cm × 34 cm × 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. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Pune, Maharashtra, India) once a day.

2.3. Induction of experimental myocardial infarction

ISO (100 mg/kg) was dissolved in saline and injected subcutaneously to rats at intervals of 24 h for 2 days [35]. Animals were sacrificed 48 h after the first dose of ISO.

2.4. Study design

The rats were divided into six groups of eight rats each. Two rats from each group were used for the

histological study. Group I: normal control rats were given 2 ml of saline orally by gastric intubation daily for a period of 21 days; Group II: normal rats were treated with morin (10 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 21 days; Group III: normal rats were treated with morin (20 mg/kg) in 2 ml of

saline orally by gastric intubation daily for a period of 21 days, Group IV: rats were subcutaneously injected with ISO (100 mg/kg) in 2 ml of saline once a day for 2 days (on the 22nd and 23rd days); Group V: rats were pretreated with morin (10 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 21 days and then subcutaneously injected with ISO (100 mg/kg) once a day for 2 days (on the 22nd and 23rd days); Group VI: rats were pretreated with morin (20 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 21 days and then subcutaneously injected with ISO (100 mg/kg) once a day for 2 days (on the 22nd and 23rd days).

2.5. Electrocardiogram and heart rate

Twenty-four hours after the second dose of ISO, the rats of all the groups were anesthetized with ketamine hydrochloride (100 mg/kg body weight) intraperitoneally and the electrocardiograph patternswere recorded by a 16 channel polygraph (Biopac systems Inc., USA). The type of alterations (P wave, QRS complex, ST-segment elevation, PR interval and RR interval) in the normal and experimental rats was recorded. The heart rate of all groups of animals was recorded by the pulse analyzer.

2.6. Assessment of left ventricular hypertrophy

The weights of the whole heart and left ventricle were measured. The ratio of left ventricle weight to heart weight was calculated to assess left ventricular hypertrophy [34].

2.7. Biochemical analysis

After recording the electrocardiogram, the animals were sacrificed by cervical decapitation and blood was collected in two tubes, i.e., one with anticoagulant (ethylene diamine 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 tissues were excised immediately and rinsed in ice-chilled saline. Internal coagulated blood was removed, and the heart was weighed to the nearest 0.1g. known weights of the tissues were weighed and homogenized in 5ml of chilled 0.1M 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.

2.7.1. Assay of Cardiac markers

The activity of serum creatine kinase (CK), CK-MB, LDH were assayed by a standard diagnostic kit (Infinite CK-

MB) purchased from Accurex Diagnostics Private Limited, Mumbai, India, and Troponin I was estimated in the serum by the standard diagnostic kit (Vitros Immunodiagnostic Products), purchased from Ortho Clinical diagnostics, New York, USA.

2.7.2. Assay of lipid peroxidation products.

The level of plasma thiobarbituric acid reactive substances was estimated by the method of Yagi et al [47]. Lipid hydroperoxides in the plasma was estimated by the method of Jiang et al [20].

2.7.3. Lipid profile

The cholesterol content and triglyceride was estimated by a reagent kit (Qualigens Diagnostics, Product no. 72181/72 191, Mumbai, India). Free fatty acids were estimated by the method of Falholt et al [10]. Phospholipid content was estimated by the method of Zilversmit and Davis [53].

2.7.4. Estimation of lipoproteins.

High density lipoprotein (HDL) was estimated by a reagent kit 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. Histopathology

After the sacrifice of the normal and experimental rats, the heart was rapidly dissected and washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissue was processed by embedding it in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope for histopathological changes and photomicrographs were taken.

2.9. Myocardial necrosis score

Histological examination of the hearts was undertaken to study the severity of infarction. Necrosis produced by ischemia reperfusion injury was graded as described by Vogel [46]. Tissues were fixed in 4 % paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin for histological evaluation of tissue

damage. To have a quantitative estimation of the histological cardiac damage, sections (n = 6 for each experimental group) were scored by 2 independent observers blinded to the experimental protocol. The following morphological criteria were considered: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling and necrosis; score 3 (severe), necrosis with presence of contraction bands and neutrophil infiltrate; score 4 (highly severe), widespread necrosis with presence of contraction bands, neutrophil infiltrate, and hemorrhage.

2.10. In vitro free radical scavenging activity

The free radical scavenging activities of the morin were measured in vitro by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) assay [3]. The ABTS assay was conducted as described by Re et al [37].

2.11. Statistical analysis

Results are expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was carried out, and Statistical comparisons among the groups were performed with Duncan's multiple range test (DMRT) using a software, Statistical Package for the Social Science software package version 16.00 (SPSS, Chicago, IL). Throughout the report wherever we 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

Table 1 shows that the total heart weight and left ventricle weight of the normal and experimental rats. The ratio between total heart weight and left ventricle weight were measured in all the animals. We analysed the effect of morin on left ventricular hypertrophy. We observed an increase in left ventricle/ total heart weight ratio in ISO induced rats as compared to the normal control rats. Pretreatment with morin significantly reduced left ventricle/total heart weight ratio when compared with non-treated ISO-induced myocardial infarcted rats (Table 1). We observed a significant increase in the heart rate of the isoproterenol-induced rats. The oral pretreatment of morin reduced the heart rate dose dependently and 20 mg/kg produced a better effect than 10mg/kg. Table 1 also indicates the myocardial necrosis score on normal and experimental animals. Normal control rats and the rats received morin alone (5 and 10 mg/kg) were shown no necrosis. The ISO-induced myocardial infarcted rats showed higher values of necrosis score when compared with normal control rats. Oral pretreatment with morin in

ISO-induced rats (10 and 20 mg/kg) showed a significant decrease in necrosis score when compared with ISO-induced rats.

Electrocardiogram pattern of normal and experimental animals is shown in Fig. 1 and changes in the duration of each event are mentioned in Fig 2. Normal control and morin treated rats showed normal pattern, whereas ISO-induced rats showed a significant increase in ST-segment, QT interval along with a significant decrease in the P wave, QRS complex, PR interval and RR interval as compared to the normal control group. Oral pretreatment with morin in ISO-induced rats (10 and 20 mg/kg) showed a significant decrease in ST segment, QT interval along with a significant increase in P wave, QRS complex, PR interval and RR interval, when compared to ISO-induced rats.

Fig.3. shows the effect of morin on heart rate of the normal and experimental rats. We observed a significant increase in the heart rate of the ISO-induced rats. Pretreatment with morin (10mg and 20mg/kg) daily for a period of 21 days significantly reduced the heart rate dose dependently and 20mg/kg produced better effect than 10mg/kg.

In the present study there was a significant increase in the levels of cardiac markers creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH) and troponin-I in the serum of ISO-induced rats, when compared to that of the normal control rats. Pretreatment with morin (10mg and 20mg/kg) daily for a period of 21 days significantly decreased the levels of cardiac marker enzymes in the serum, when compared to rats induced with ISO alone-induced rats (Fig 4 and 5).

The endogenous defense against the peroxidation of membrane lipids remain an area of continuous interest. The ISO-induced myocardial infarcted rats showed a significant increase in the levels lipid peroxidation products of thiobarbituric acid reactive substances and lipid hydroperoxides, when compared with the normal control rats. Peroxidation of endogenous lipid might be a major factor involved in the cytotoxic nature of excessive dose of ISO. Pretreatment with morin (10mg and 20mg/kg) significant 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 morin (Fig 6).

Fig 7 showed the alteration in the lipid profile. The levels of total cholesterol, LDL and VLDL were increased and HDL was decreased in the ISO-induced rats, when compared to the normal control rats.

Pretreatment with morin (10mg and 20mg/kg) daily for a period of 21 days regulated the altered lipid profile levels significantly by decreasing the levels of total cholesterol, LDL, VLDL and increased the levels of HDL.

The levels of triglycerides, free fatty acids and phospholipids in the serum of ISO-induced rats increased

significantly. We also analyzed the levels of total cholesterol, triglycerides, free fatty acids and phospholipids in the heart tissue of the normal and experimental rats. The ISO-induced rats showed significantly increased the levels of triglycerides and free fatty acids and decreased levels of phospholipids in the heart tissue homogenate, when compared to the normal control rats. Pretreatment with morin (10mg and 20mg/kg) daily for a period of 21 days regulated the altered lipid profile levels significantly by decreasing the levels of total cholesterol, triglycerides and free fatty acids and increasing the levels of phospholipids in the heart tissue homogenate, when compared to rats induced with ISO alone-induced rats (Fig 8).

Rats treated with morin (10 mg/kg and 20 mg/kg) daily for a period of 21 days did not show any significant toxic effect and no impact with the biochemical parameters in the normal rats that indicating that these doses appear safe.

Histopathological examination of the myocardium of normal control animals showed clear integrity of myocardial cell membrane (Fig. 9A). No inflammatory cell infiltration was observed. Morin (10mg/kg, 20mg/kg) treated rats did not show any significant effect on the integrity of myocardial membrane and the architecture is normal (Fig. 9 B, C). Heart tissues from rats induced with ISO (100mg/kg) showed widespread myocardial structure disorder and subendocardial necrosis with moderate infiltration of lymphocytes and macrophages (Fig. 9D). Pretreatment with morin 10 mg/kg, exhibited decreased degree of necrosis (mild) and less infiltration of inflammatory cells (Fig. 9 E). The percentage of infarction area (infarct ratio) is 64.3 ± 6.6% in ISO -alone induced group. However, pretreatment with morin (10 and 20 mg/kg,) decreased the infarct size ratio (33.5 ± 8.0%, 12.6 ± 2.3% respectively). Fig 10 shows the percentage scavenging effect of morin on DPPH and ABTS free radicals. Morin scavenges DPPH and ABTS free radicals in vitro in a dose-dependent manner (10-20mg/ml). The percentage scavenging effect of morin increased with increase in concentration.

Discussion

Supramaximal doses of ISO induce subendocardial myocardial ischemia, hypoxia and necrosis as seen in human myocardial infarction [22]. Our results are also in consisting with the previous reports. In the electrocardiogram of rats, we observed a decrease in the QRS complex, decrease in P wave, decrease in PR interval and RR interval, an elevated ST-segment and a hyper acute T wave with an asymmetric configuration in ISO-induced rats. This may be due to the reduced capacity of ventricular contraction. The ST-segment elevation is the most sensitive

marker for myocardial infarction and it reflects myocardial necrosis and the consequent loss of cell membrane in an injured myocardium [25]. The T wave represents the repolarization time, and a prolonged T wave may be due to a delay in recovery and the depleted energy level in the ischemic tissue [21]. PR segment depression in ISO-induced rats indicates concomitant atrial ischaemia or infarction. The oral pretreatment with morin significantly reduced the pathological alterations including decrease ST-segment elevation, decrease pathological Q wave, increase P wave and increase PR interval and RR interval. Normalization of the ST-segment by morin indicates sufficient perfusion all the way through the myocardial microvasculature. These results showed the cardio protective effect of morin against ISO-induced myocardial infarction.

We observed an increased heart rate and it may be due to the positive inotrophic effect of ISO on myocardium. Stimulation of β-adrenergic receptors by ISO was the reason for increased heart rate. As β-adrenergic receptors are one of the susceptible receptors to phenolic binding [52], morin might have bind with them and controlled increased heart rate in the ISO-induced rats.

We observed an increase in the activities of cardiac marker enzymes in the serum of ISO-induced myocardial infarcted rats. Of all the macromolecules that leak from damaged tissue, enzymes because of their tissue specificity and catalytic activity are the best markers of tissue damage [17]. The release of cellular enzymes reflects the alterations in plasma membrane integrity and/or permeability as a response to β-adrenergic stimulation [9]. This might be due to the damage caused to the sarcolemma by the β-agonist that has rendered it leaky. ISO-induction produces free radicals via β-adrenoceptor mechanism, affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis [43]. When myocardial cells are damaged or destroyed due to deficient oxygen supply, the cell membrane becomes permeable or may rupture, which results in the leakage of these enzymes. This accounts for the increased activities of CK, CK-MB, LDH and troponin-I in the serum of ISO-induced rats. Our results showed significant elevation of serum levels of CK, CK-MB, LDH and troponin-I in ISO-induced rats, which was in line with the previous reports [32, 38]. Oral pretreatment with morin (10 mg and 20 mg/kg) restored the levels of these enzymes to near normal in the serum of ISO-induced rats. This could be due to the membrane stabilizing effect of morin on the myocardium. Previous reports on morin indicated the cytoprotective activity against oxygen radical damage [51] which supports our findings.

In order to provide better evidence on membrane stabilizing effect, we studies anti lipid peroxidation activity of morin. Lipid peroxidation is also an important pathogenic event in myocardial infarction [13]. Karthikeyan et al explained that it is an indication of the severity of necrotic damage of the heart, and has been linked with severity

of the damage [23]. The increased levels of TBARS and LOOH were measured in the plasma of ISO-induced rats. Free radical mediated membrane damage may be the reason for this activity. An enormous amount of superoxide radicals formed by ISO can stimulate the Haberweiss reaction for further generation of reactive oxygen species initiating lipid peroxidation [21, 2]. Oral pretreatment with morin (10 mg and 20 mg/kg), decreased the levels of TBARS and LOOH in ISO-induced rats. These results suggest that morin scavenges the lipid peroxidation caused by ISO. In this context, previous studies also reported the scavenging action of morin on free radicals [27, 48]. Flavonoids have been shown to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages [23]. The free radical inhibitory activity of morin, may effectively scavenge the reactive oxygen species and decreases lipid peroxidation end products.

An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death that follows coronary artery occlusion [24]. Increased levels of blood cholesterol and their accumulation in the heart are well associated with myocardial damage [39]. The cardiac muscle generally utilizes fatty acid as the major source of energy of the total oxygen consumption; 60–90% is utilized to oxidize fatty acid under aerobic condition. Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these long chain fatty acyl coA derivatives [47].

ISO-induced rats showed increased levels of total cholesterol, triglycerides, phospholipids, and free fatty acids in serum of rats. An increase in the serum LDL and VLDL fractions, along with a decrease in HDL cholesterol, were also observed in ISO-induced rats. These changes could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate [33]. High levels of LDL cholesterol and low levels of HDL cholesterol show a positive correlation with myocardial infarction [4]. Anandan et al. have reported that the increase in the myocardial cholesterol content in ISO induced rats is due to increased uptake of LDL from blood by myocardial membranes [1]. Pretreatment with morin restores the levels of LDL and HDL by decreasing the LDL cholesterol and increasing the HDL cholesterol in myocardial infarcted rats.

The mechanism of observed increase in TGs 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 morin decreased

the levels of TG, VLDL in myocardial infarcted rats. Decreased triglycerides might be the result of decrease atherogenic lipoproteins such as VLDL on the other hand it might by due to increase HDL-cholesterol.

Under severe ischemic conditions, oxidation of carbohydrates and FFAs will cease [16]. Thus, circulating FFAs

may be increased in myocardial infarcted rats. There is a report showing that increased levels of blood FFAs may depress cardiac function, promote arrhythmias and further increase the extent of myocardial damage [18]. Crass et al. [8] also reported that after addition of catecholamine to heart homogenate resulted in the release of FFAs. The heart derives a significant portion of its fatty acid substrates as FFAs derived by lipolysis from adipose tissue. Although lipid availability is important for the heart, excess levels of fatty acids in myocytes can be deleterious [45]. Pretreatment with morin lowered the levels of FFAs in myocardial infarcted rats.

Membrane PLs are essential factors in maintaining cell function and degradation of PL by phospholipase A2 and other phospholipases have been implicated as a vital factor in the genesis of ischemic cellular injury [6]. In this context, Franson et al. [12] have reported that ISO increases the activity of phospholipase A2. Thus, the observed decreased content of PL in ISO-induced myocardium is due to accelerated membrane degradation of PLs by phospholipases. Pretreatment with morin showed a stabilizing effect on myocardial phospholipids. This effect is due to the ability of morin to prevent peroxidation of membrane phospolipids.

The histopathological results showed a better insight of cardioprotective effect of morin. The normal heart showed no change in the structural integrity of myocardial tissue. The rats treated with morin also showed the similar architecture as normal heart. The histopathological findings of the ISO-induced myocardium showed infarcted zone with edema, necrosis and separation of cardiac muscle fibers. The neutrophisl characteristically invade the myocardial tissue during ischemia. The ISO-induced rats showed the evidence of myocardial necrosis, tissue damage, infiltration of inflammatory cells and hemorrhage. The rats pretreated with morin (10mg/kg) showed decreased necrosis, less tissue damage and reduced infiltration of inflammatory cells. The rats pretreated with morin (20mg/kg) further reduced the pathological changes and brings back the cellular architecture almost similar to the normal.

The pathological changes including increased cardiac markers, increased lipid peroxidation, altered lipid metabolism and histological changes were due to the excessive free radicals produced by ISO. It was reported that phenolic compounds are effective hydrogen donors, making them very good antioxidant [50]. For the quantitative investigation of hydrogen-radical donation, stable radicals have the advantage that their concentrations are readily and directly measurable. Among them, a stable free radical, DPPH was investigated as a reactive hydrogen acceptor. Since, then DPPH has been mainly used to examine radical scavenging activity of antioxidative vitamins and polyhydroxy aromatic compounds [31]. As a result, their capability to donate hydrogen to the free radical was more pronounced [29]. The effect of antioxidants on DPPH radical scavenging is generally due to their hydrogendonating ability [41]. The antioxidant activity of morin increased with increasing concentration. The

high radical scavenging activity of morin was probably attributed to the higher degree of hydroxylation in their structure.

The improved technique for the generation of ABTS described herein involves the direct production of the blue/green ABTS chromophore through the reaction between ABTS and potassium persulfate. As seen in Fig 10, the ABTS radical scavenging activity of morin was concentration dependent (10–20 µg ml-1). There was a significant decrease in the concentration of ABTS due to the scavenging capacity of morin and standards.

The attempt has been made to find out the mechanism of cardioprotective action of morin. Based on the in vitro studies, the free radical scavenging effect of morin may be one of the important mechanisms of its cardioprotective activity. A previous scientific report has also shown that rutin exhibits protective effects on lipid peroxidation, antioxidant system and histology of heart in ISO-induced myocardial infarction [42].

In conclusion, the present study provides experimental evidence that the oral pretreatment with morin (10 mg and 20 mg/kg) was non toxic, safe and highly effective in cardio protection, in addition improved cardiac dysfunction during ISO-induced cardiotoxicity. These findings are rational to understand the beneficial effects of morin on cardio protection against myocardial injury, in which oxidative stress was known to contribute to the pathogenesis. The beneficial effects of morin on cardiotoxic rats indicating its possible candidature for the treatment of myocardial infarction. Further research with the aim of determining the effects of morin on oxidative stress-induced myocardial infarction in human beings should include in clinical trails.

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Figure captions

Fig. 1: Effect of morin on electrocardiographic pattern in normal and experimental rats. (A). Electrocardiogram pattern of normal control group showing normal cardiograph. (B). Electrocardiogram pattern of morin (10 mg and 20 mg/kg) pretreated group rats showing normal cardiograph. (C). Electrocrdiogram pattern of morin (10 mg and 20 mg/kg) treated group rats showing normal cardiograph. (D). Electrocardiogram pattern of isoproterenol-induced group rats showing pathological changes such as ST-segment elevation. (E). Electrocardiogarm pattern of morin (10 mg and 20 mg/kg) treated isoproterenol-induced group rats showing minimized ST-segment elevation. (F). Electrocardiogram pattern of morin(10 mg and 20 mg/kg) pretreated isoproterenol-induced group rats showing almost normal cardiograph without any elevation in ST-segment.

Fig. 2: Changes in the cardiac events. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test);Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg). Units*: the electrocardiographic events such as P wave, QRS complex, QT and PR intervals (value X10), RR intervals: seconds (s). ST-segment elevation: milli volt (mv).

Fig. 3: Effect of morin on heart rate. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig 4: Effect of morin on the levels of CK, CK-MB and LDH in the serum of normal and experimental rats. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol

(100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig 5: Effect of morin on the levels of troponin I in the serum of normal and experimental rats. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig 6: Effect of morin on the levels of lipid peroxidation products in the plasma of normal and experimental rats. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig. 7: Effect of morin on levels of lipoproteins and lipids in the plasma of normal and experimental rats. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig. 8: Levels of TG, FFA and PL in the serum of normal and experimental rats. Each coloumn is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,d) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

Fig. 9: (A-F) Histopathological study of the heart tissue of normal and experimental rats. (A): Section of heart tissue of normal control rat showing normal architecture.

(B) : Section of heart tissue of morin (10mg/kg) treated rat showing no significant change from the normal architecture.

(C) : Section of heart tissue of morin (20mg/kg) treated rats also showing nearly normal architecture.

(D) : Section of heart tissue of ISO-induced rats showed necrotic damage, infiltration of neutrophils and altered structure of myofiber.

(E) : Section of heart tissue of morin (10mg/kg) pretreated and ISO-induced rat showing mild focal degenerative changes in the cardiac muscle fiber

(F) : Section of heart tissue of morin (20mg/kg) pretreated and ISO-induced rat showing almost normal architecture.

Fig 10: Percentage scavenging effect of morin on DPPH and ABTS free radicals.

Table 1. Effects of morin on total heart weight, left ventricle weight, cardiac hypertrophy, heart rate and muocardial necrosis score in normal and experimental rats

Group	I	II	III	IV	V	VI
Total Heart Weight (THW) (g)	1.07 ± 0.07 [a]	1.05 ±0.09[a]	1.06 ± 0.07 [a]	1.37 ± 0.07[b]	1.23 ± 0.05 [c]	1.10 ± 0.06 [a]
Left Ventricle Weight (LVW)(g)	0.61 ± 0.04 [a]	0.58 ±0.06[a]	0.60 ± 0.07 [a]	0.81 ± 0.05[b]	0.72 ± 0.05 [c]	0.62 ± 0.06 [a]
Left ventricle hypertrophy Ratio of LVW/THW	0.57 ± 0.03 [a]	0.55 ±0.04[a]	0.57 ± 0.04[a]	0.60 ± 0.05[b]	0.59 ± 0.04 [c]	0.57 ± 0.04 [a]
Heart rate (bpm)	381 ± 13 [a]	382 ± 11 [a]	381 ± 12[a]	441 ± 13 [b]	412 ± 14 [c]	387 ± 13 [a]
Myocardial necrosis score	0.0 [a]	0.0 [a]	0.0 [a]	3.72 ± 0.06[b]	2.5 ± 0.08 [c]	1.7 ± 0.07 [a]

Each value is mean ± standard deviation for six rats in each group. Columns not sharing a common letter (a,b,c,) differ significantly with each other, Duncan's multiple rang test); Group I: Normal control rats. Group II: Rats treated with morin (10mg/kg) Group III: Rats treated with morin (20mg/kg). Group IV: Rats induced with isoproterenol (100mg/kg). Group V: Rats pretreated with morin (10mg/kg) and induced with isoproterenol (100mg/kg). Group VI: Rats pretreated with morin (20mg/kg) and isoproterenol (100mg/kg).

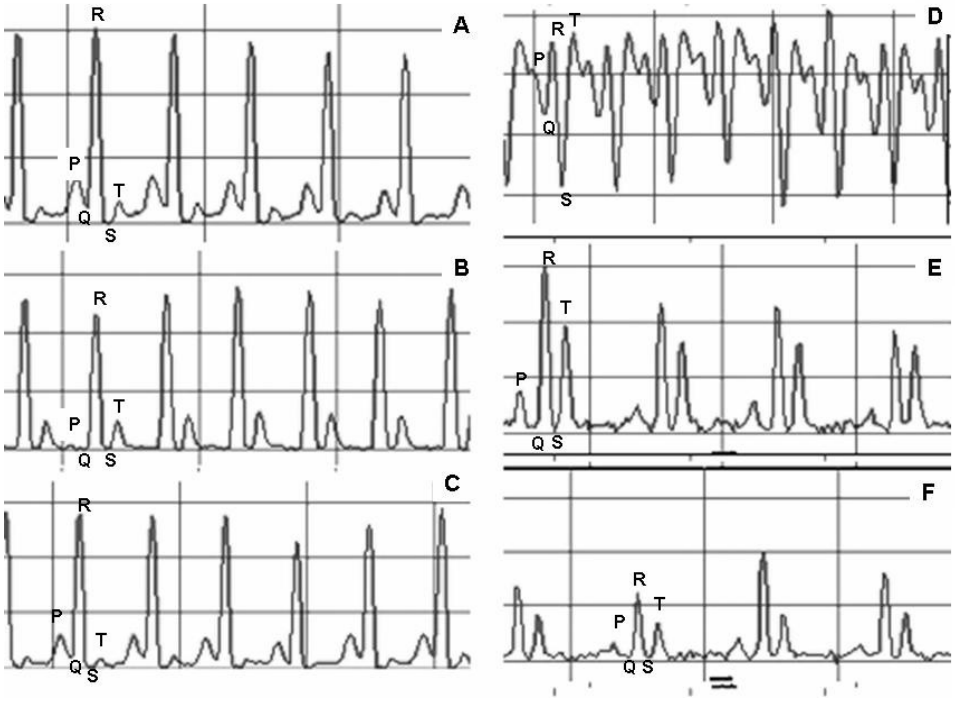

Figure 1

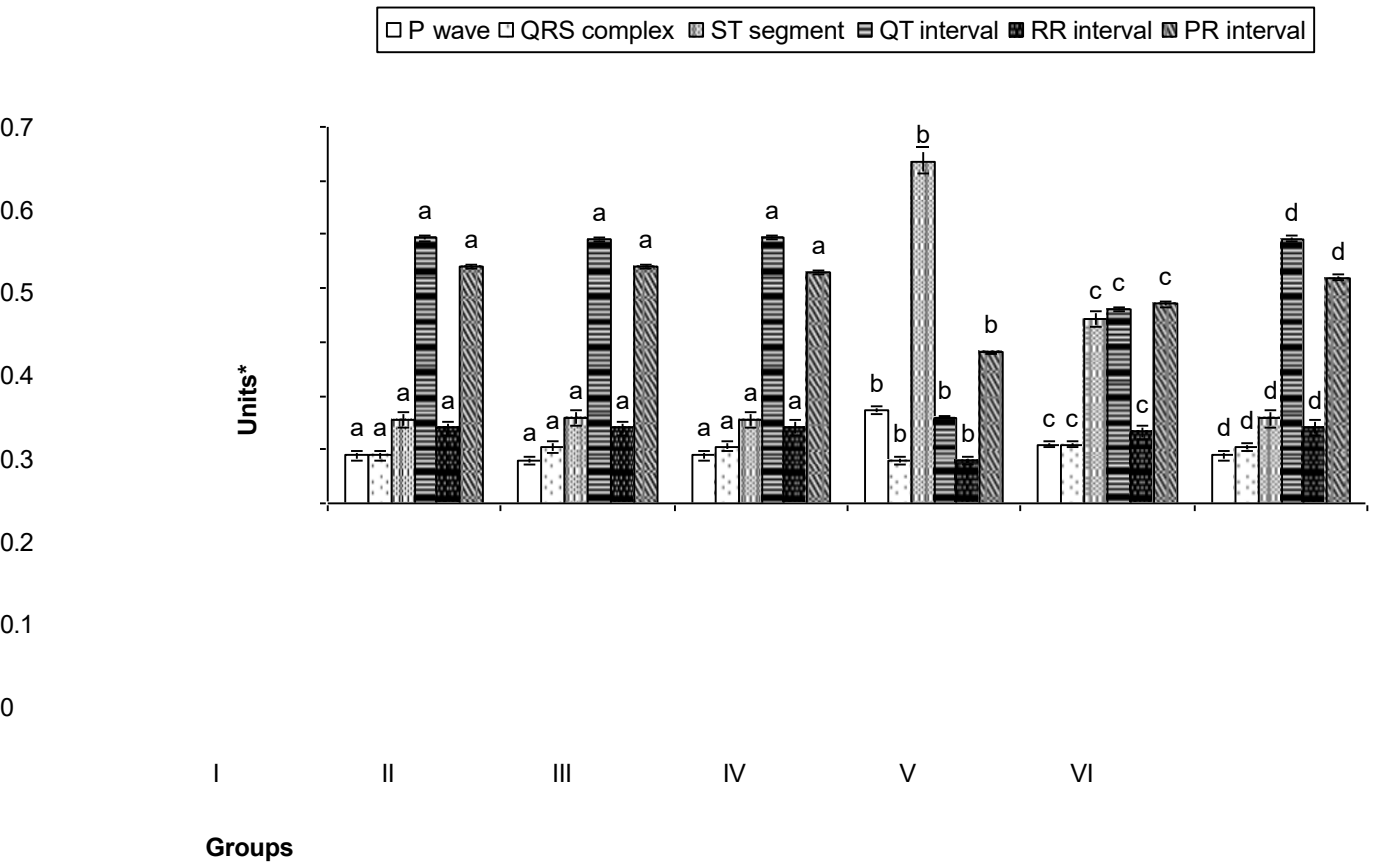
P wave
QRS complex
ST segment
QT interval
RR interval
PR interval
Units*
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
I
II
III
IV
V
VI
Groups

Figure 2

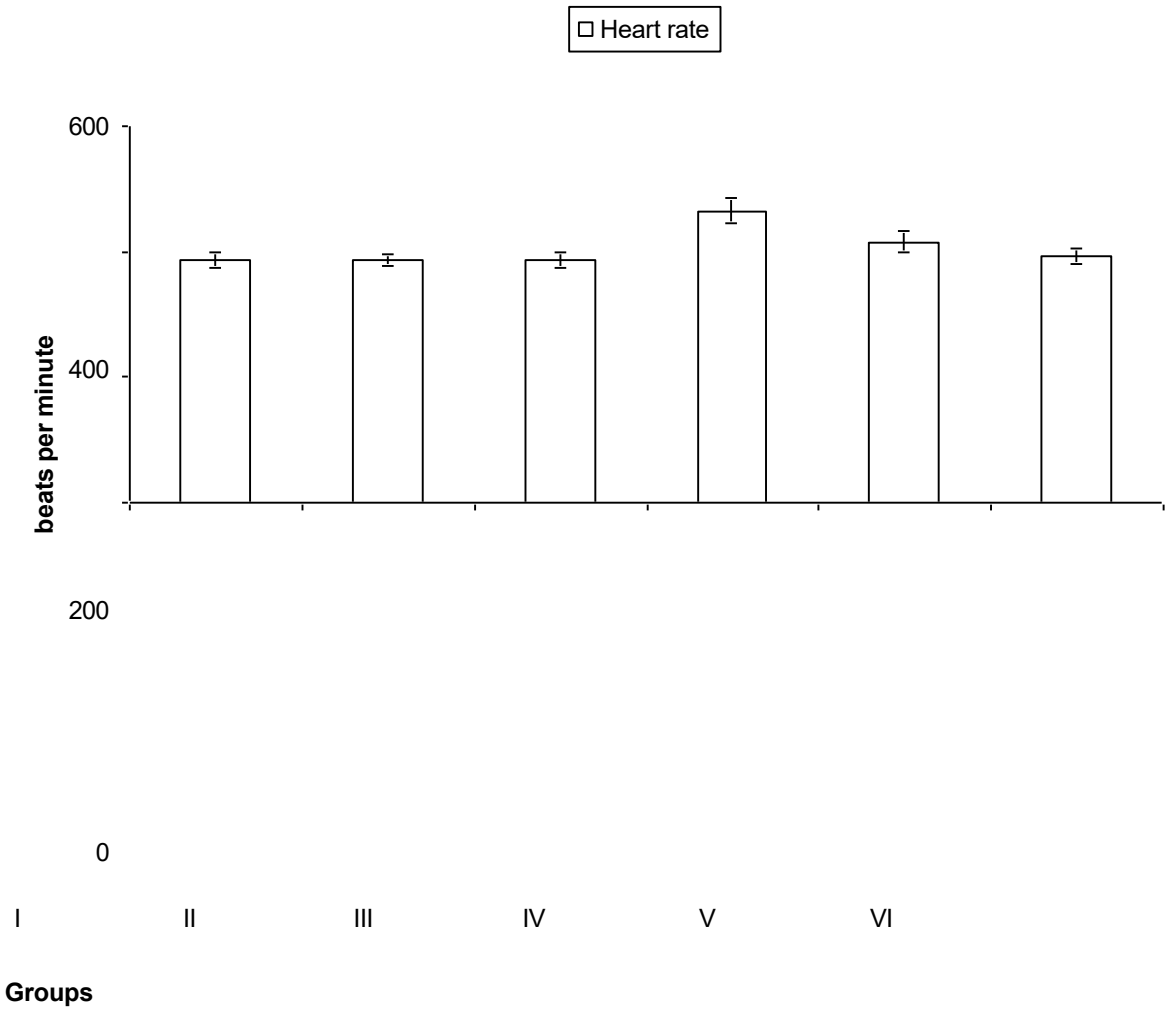
Heart rate
600
400
200
0
beats per minute
I
II
III
IV
V
VI
Groups

Figure 3

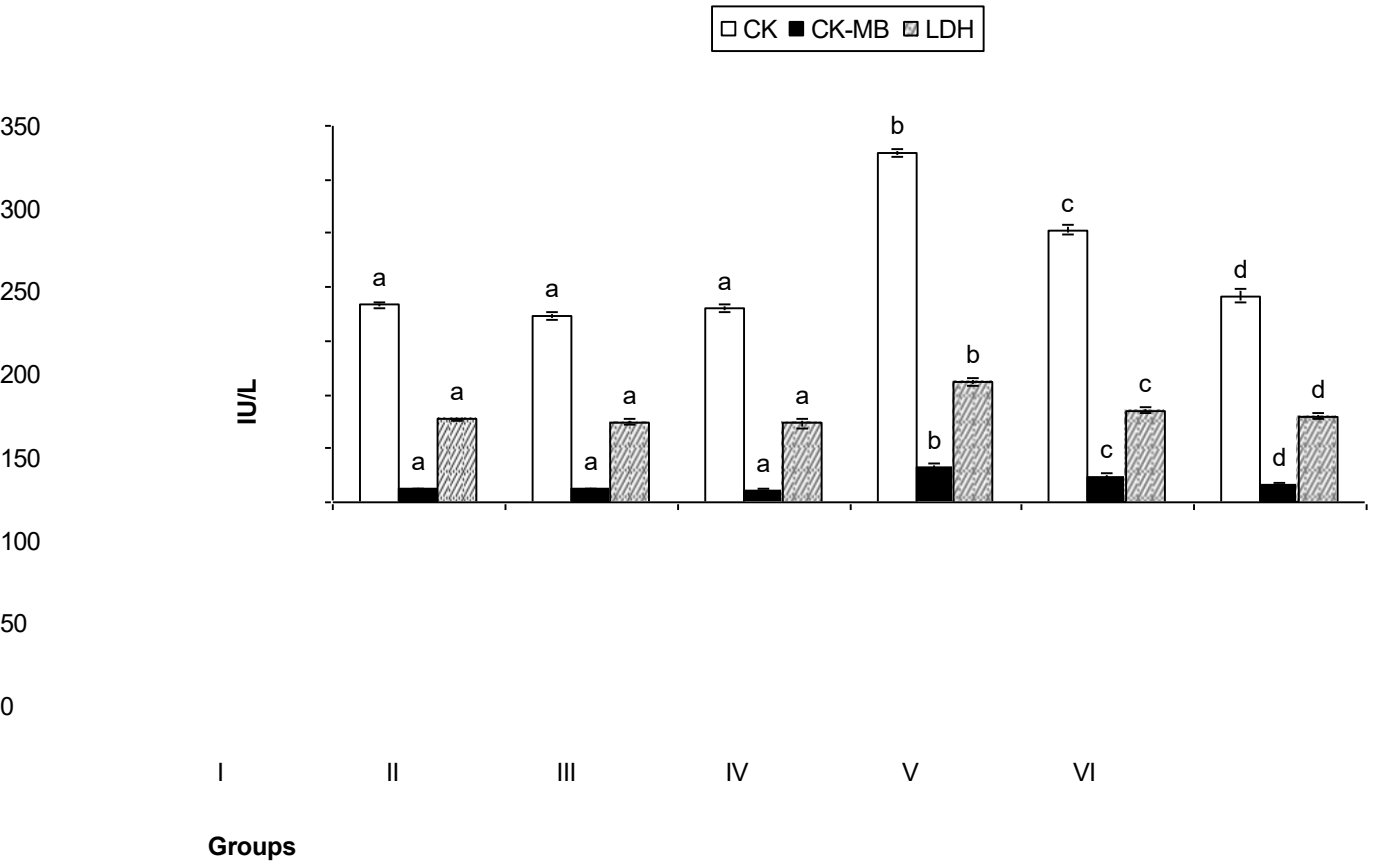
CK
CK-MB
LDH
IU/L
350
300
250
200
150
100
50
0
a
a
a
a
a
a
a
a
a
b
b
b
c
c
c
d
d
d
I
II
III
IV
V
VI
Groups

Figure 4

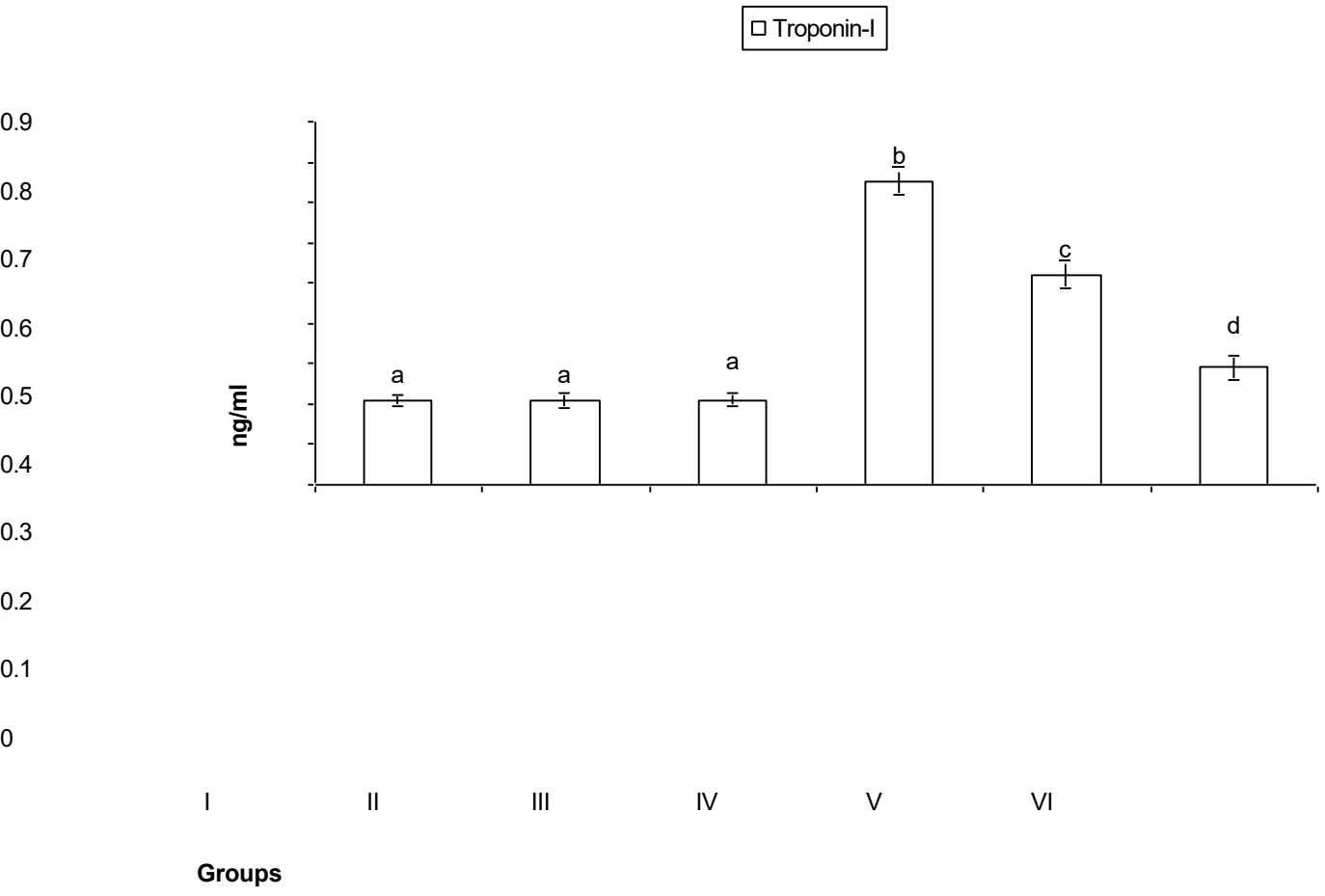
Troponin-I
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ng/ml
a
a
a
b
c
d
I
II
III
IV
V
VI
Groups

Figure 5

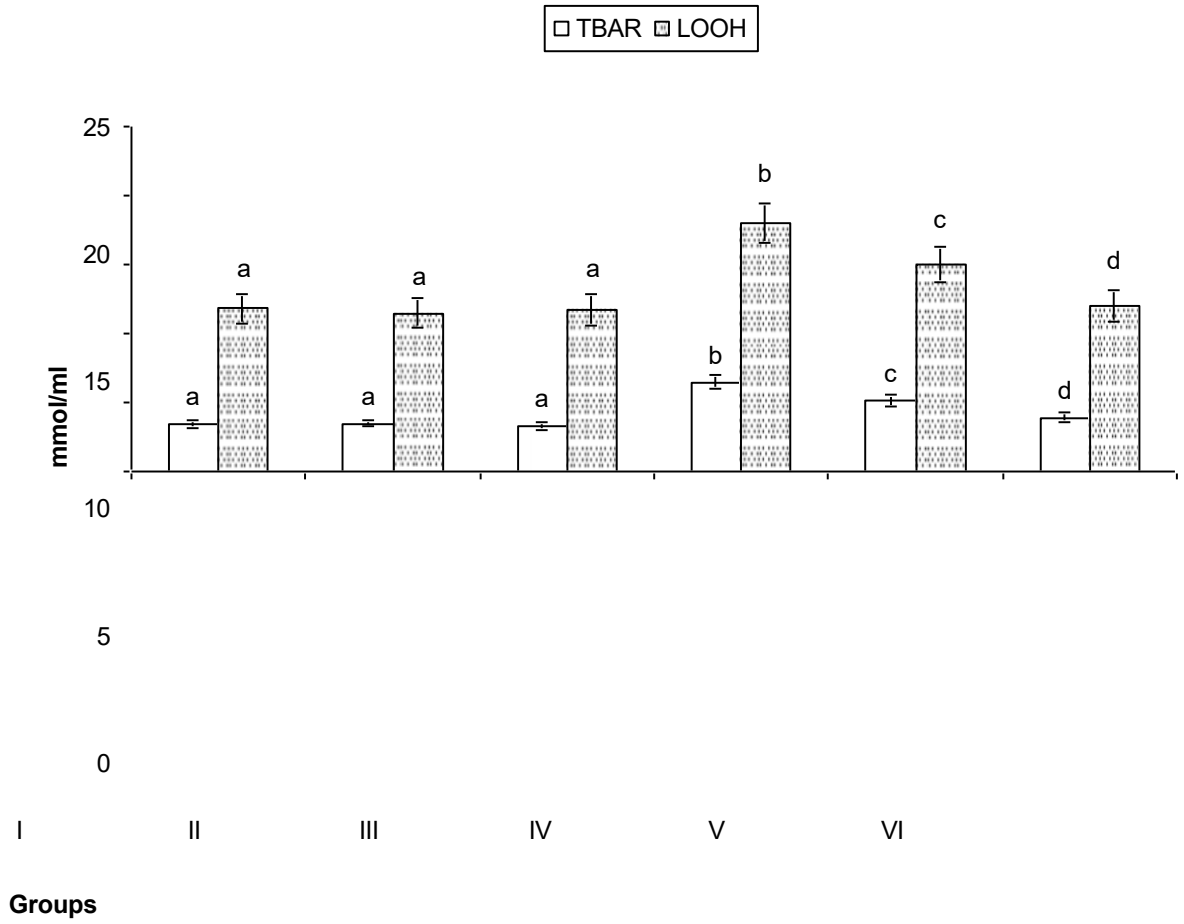
TBAR
LOOH
mmol/ml
25
20
15
10
5
0
a
a
a
a
a
a
b
b
c
c
d
d
I
II
III
IV
V
VI
Groups

Figure 6

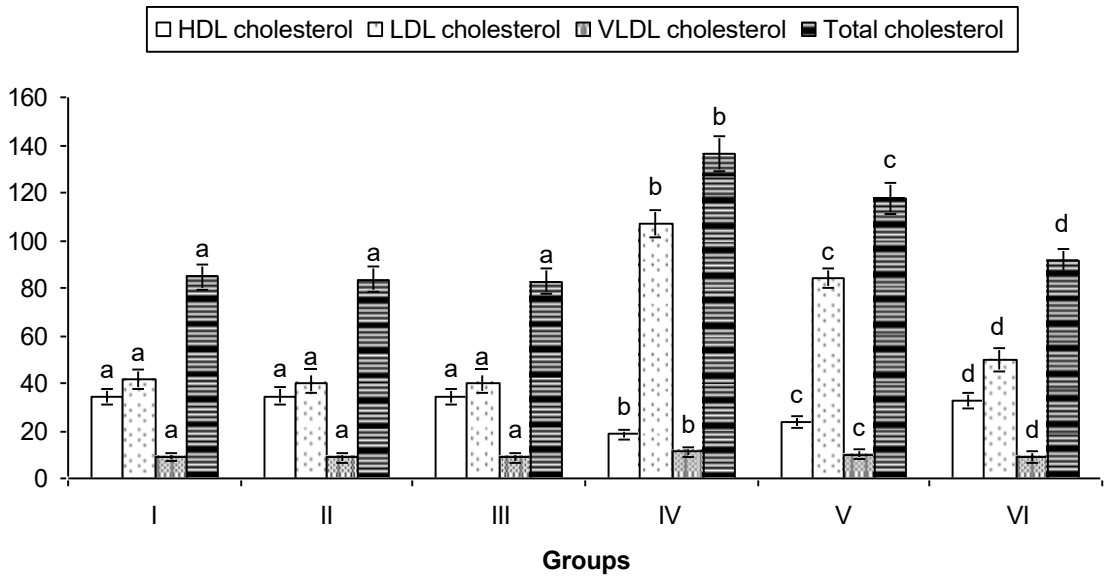
HDL cholesterol
LDL cholesterol
VLDL cholesterol
Total cholesterol
160
140
120
100
80
60
40
20
0
I
II
III
IV
V
VI
Groups

Figure 7

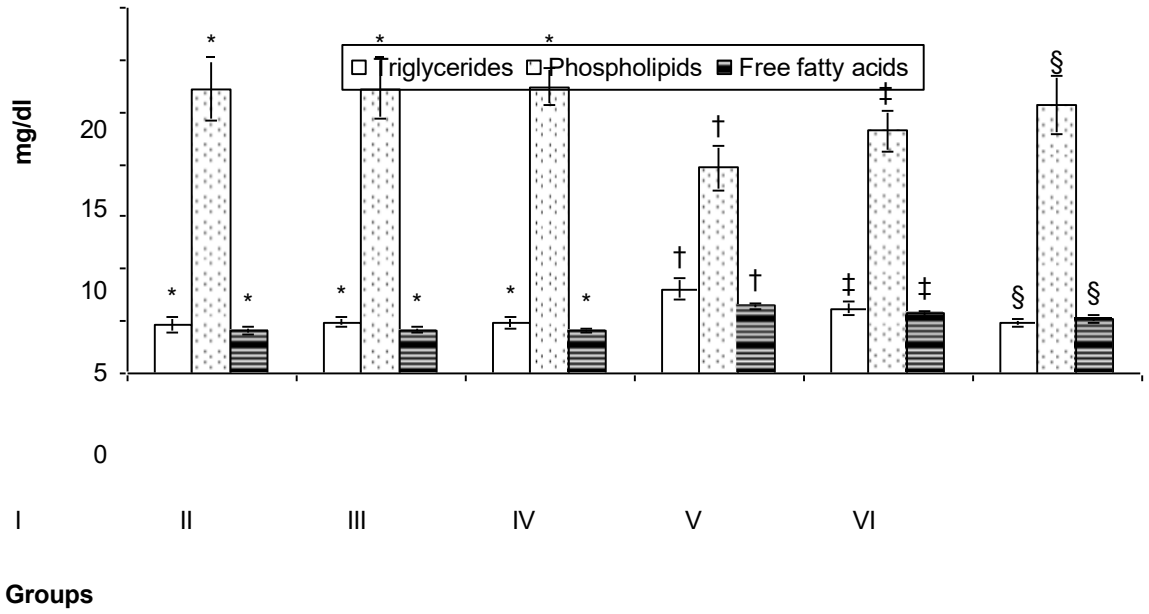
Triglycerides
Phospholipids
Free fatty acids
mg/dl
20
15
10
5
0
I
II
III
IV
V
VI
Groups

Figure 8

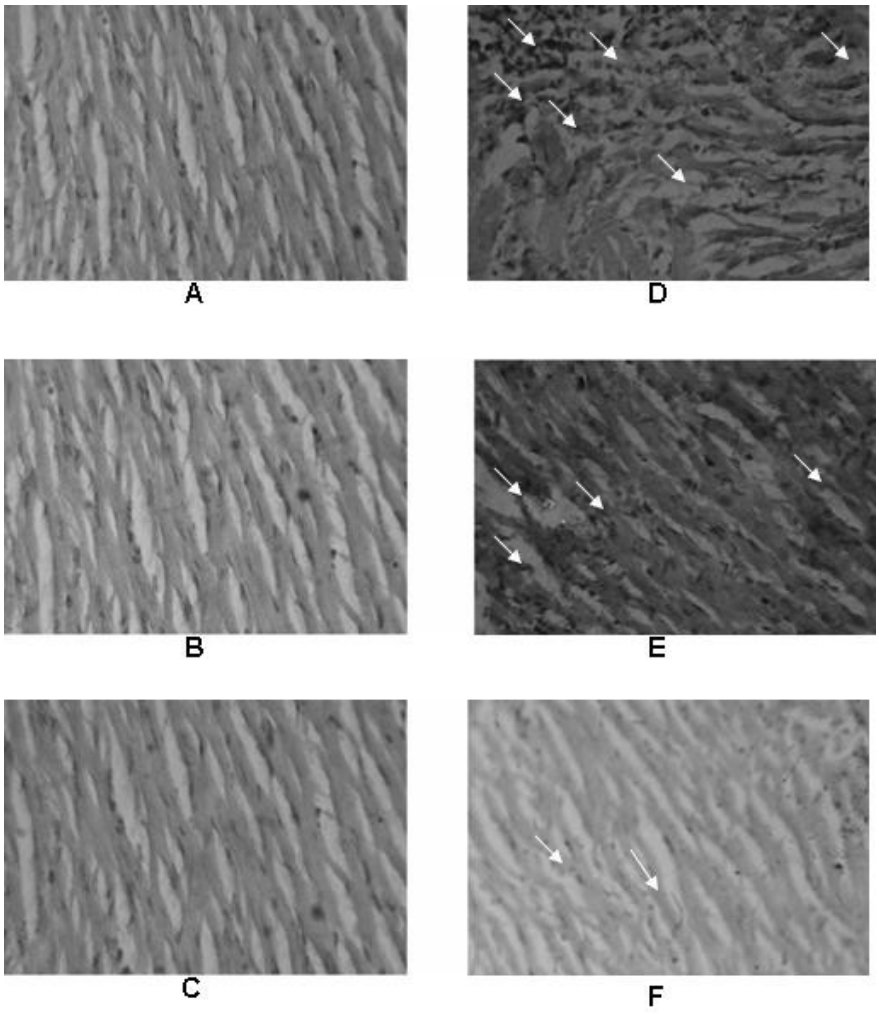

Figure 9

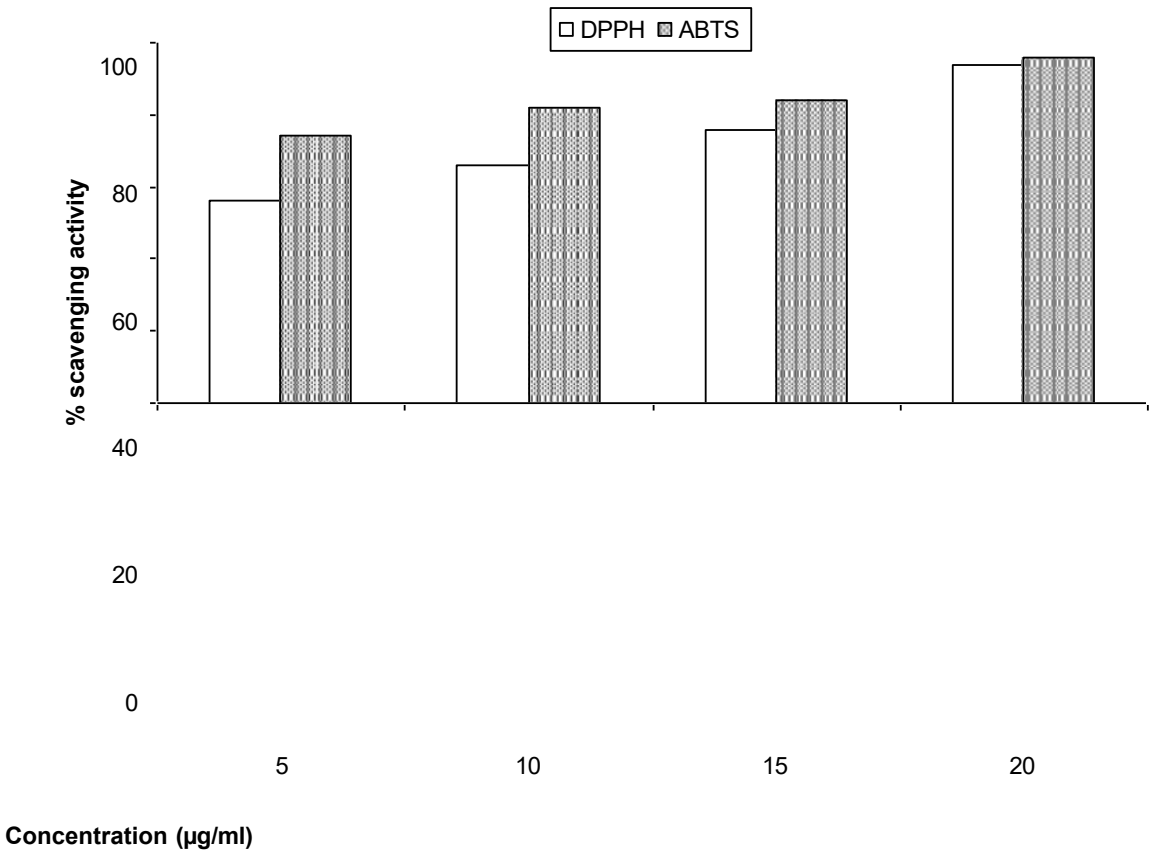
DPPH
ABTS
100
80
60
40
20
0
% scavenging activity
5
10
15
20
Concentration (µg/ml)

Figure 10