Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats

S. Sharmila Queenthy, a, b, Babu John, c, * a Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India
b Department of Biotechnology, Waljat College of Applied Sciences, Muscat, Oman
c PG and Research Department of Chemistry, Government Arts College, C.Mutlur, Chidambaram, Tamilnadu, India

A R T I C L E I N F O
Article history:
Received 16 February 2013
Received in revised form
15 August 2013
Accepted 30 August 2013
Available online 12 September 2013

Keywords:
Diosmin
Electrocardiogram
Isoproterenol
Myocardial infarction
Lipids
Lipoproteins

A B S T R A C T
The aim of the present study was to evaluate the protective effects of diosmin on experimentally induced myocardial infarcted rats. Diosmin (5 and 10 mg/kg body weight) was administered orally as pretreatment daily for a period of 10 days. Then isoproterenol (100 mg/kg) was injected subcutaneously into rats at an interval of 24 h for 2 days (on 11th and 12th day). Isoproterenol-induced myocardial infarcted rats showed significant changes in electrocardiogram and an increase in the levels of cardiac markers, compared with normal rats. Additionally, increased plasma lipid peroxidation products and altered lipid metabolism in the plasma were observed in the isoproterenol-induced myocardial infarcted rats. Pretreatment with diosmin (5 and 10 mg/kg body weight) minimized the electrocardiographic changes, decreased the levels of serum cardiac marker enzymes reduced plasma lipid peroxidation and minimized the alterations in the lipid metabolism of isoproterenol-induced myocardial infarcted rats. Also, diosmin inhibited the enhanced activity of liver HMG CoA reductase. The in vitro study revealed the free radical scavenging activity of diosmin. The free radical scavenging and anti-hyperlipidaemic effects are the reasons for the cardioprotective effects of diosmin.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction
Myocardial infarction is a disease that occurs when the coronary arteries reduce the blood supply to a part of the myocardium that leads to hypoxia (Sangeetha and Darlin Quine, 2008). Cardiovascular risk factors have long been studied in order to gain insight into the pathophysiology, epidemiology and therapy of acute myocardial infarction (López Messa et al., 2004). As data from the original Framingham cohort were analyzed, newer cohorts were added over the years, such as life style and diet, smoking, high blood pressure, altered lipid profile and obesity as important risk factors for myocardial infarction (O’Donnell and Eloisa, 2008). Therapeutic doses of isoproterenol (β-β-(3, 4-dihydroxyphenyl)-a-isopropyl aminoethanol hydrochloride), a β-adrenergic receptor agonist are regulating heart function. But in excessive dose, isoproterenol depletes the energy reserve of cardiomyocytes and thus results in biochemical and structural changes, which are responsible for the development of irreversible damage (Aman Upaganlawar et al., 2011). Isoproterenol also increases the intracellular levels of calcium, thereby causing myocardial infarction. It serves as a well accepted and standardized model to study the cardio protective effects of new drugs in preclinical trials.

The existing pharmacotherapy is effective in managing the myocardial infarction but with multiple adverse effects and drug–drug interactions (Wiffen et al., 2002). These disadvantages have enhanced the need for alternative phytoconstituents, which may be used to prevent the cardiovascular diseases. Flavonoids are present in fruits and various plants, which are pharmacologically active and can be used for the treatment of degenerative diseases. They have multiple biological activities including vasodilator (Duarte et al., 1993), anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiallergic, and antiviral effects (Brown, 1980; Middleton and Kandaswami, 1992). In general, antioxidant activity of flavonoids is closely related to preventive effects on various diseases including arteriosclerosis, liver injury, and carcinogenesis (Ho et al., 1994). Diosmin (3′, 5,7-Trihydroxy-4′-methoxyflavone 7-rutinoside) is an unsaturated flavone that can be found mainly in Hyssop and Rosemary (Del Baño et al., 2004; Camarda et al., 2007). It exhibits anti-inflammatory, antioxidant and anti-mutagenic properties (Kuntz et al., 1999). Literature survey revealed that there are no scientific reports available on the effects of diosmin in myocardial infarction. The aim of the study is to understand the protective effect of diosmin in isoproterenol induced myocardial infarcted rats.
2. Materials and methods

2.1. Experimental animals

All the experiments were carried out with healthy 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 cm³) (3 rats per cage) 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, and Maharashtra, India). The experiments were 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. 02; 10/06/2011).

2.2. Chemicals

Diosmin, a rhamnosogluco side (rutinoside), extracted from citrus species at 95% purity, and isoproterenol were purchased from Sigma Chemical Co., St. Louis, MO, USA. Sodium sulfite, dimethyl sulfoxide, potassium tetra borate, nitroblue tetrazolium and hydroxylamine hydrochloride were purchased from Himedia, Mumbai, India. All other chemicals and reagents used in the study were of analytical grade.

2.3. Induction of experimental myocardial infarction

Isoproterenol (100 mg/kg body weight) was dissolved in saline and injected subcutaneously into rats at an interval of 24 h for 2 days to induce myocardial infarction (Punithavathi and Stanely Mainzen Prince, 2010; Stanely Mainzen Prince, 2011).

2.4. Experimental design

The rats were divided into six groups of six rats each. Group I: normal rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group II: rats were treated with diosmin (5 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group III: rats were treated with diosmin (10 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group IV: rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) in 2 ml of saline at an interval of 24 h for 2 days (on 11th and 12th day); Group V: rats were pretreated with diosmin (5 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) at an interval of 24 h for 2 days (on 11th and 12th day); Group VI: rats were pretreated with diosmin (10 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) at an interval of 24 h for 2 days (on 11th and 12th day).

2.5. Electrocardiogram

Electrocardiographic patterns in the normal and experimental rats were recorded according to the method of Mari Kannan and Darlin Quine (2011). Twenty four hours after the second dose of isoproterenol, rats of all the groups were anesthetized with ketamine hydrochloride (100 mg/kg body weight) intraperitoneally and the electrocardiographic patterns were recorded by a 16 channel polygraph (Biopac systems Inc., USA). The types of alterations (P wave, QRS complex, ST-segment elevation, RR interval) in the normal and experimental rats were recorded.

2.6. Analysis of cardiac markers

The level of cardiac troponin-I in the serum was estimated by VITROS immunodiagnostic kit purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA. Creatine kinase-MB in the serum was estimated by a standard diagnostic kit obtained from Accurex Private Ltd, Mumbai, India.

2.7. Estimation of lipid peroxidation products

The levels of plasma thiobarbituric acid reactive substances were estimated by the method of Yagi et al. (1998). Four ml of 0.083 N sulfuric acid were added to 0.5 ml of plasma. 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 3000 × g for 10 min. The supernatant was discarded and the sediment was mixed with 2 ml of sulfuric acid and 0.3 ml of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3000 × 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 °C for 60 min. After cooling, 5 ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at 3000 × g for 15 min. The color extracted in the n-butanol layer was measured at 530 nm in a UV-Spectrophotometer.

Lipid hydroperoxides in the plasma were estimated by the method of Jiang et al. (1992). 1.8 ml of Fox reagent was added to 0.2 ml of plasma and incubated for 30 min at room temperature and the absorbance was measured at 560 nm in a UV-Spectrophotometer.

2.8. Estimation of lipid profile

Lipid profile was estimated in the plasma and heart tissue homogenates. Lipids were extracted from the heart tissue by the method of Folch et al. (1957). This lipid extract was used for further analysis. The total plasma cholesterol and triglyceride contents were estimated by reagent kits (Qualigens Diagnostics, Mumbai, India). Plasma high density lipoprotein (HDL) level was estimated by a reagent kit (Auto Zyme: HDL-Cholesterol) from Accurex Diagnostics Private Limited, Mumbai, India. Plasma low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated as follows: LDL-cholesterol = Total cholesterol− (HDL-cholesterol + VLDL-cholesterol); VLDL-cholesterol = Triglycerides/5 (Rajadurai and Stanely Mainzen Prince, 2006).

2.9. Assay of 3-hydroxy-3 methyl glutaryl CoA reductase (HMG CoA reductase)

The activity of HMG-CoA reductase was assayed in the liver by the method of Rao and Ramakrishnan (1975). The ratio of HMG CoA to mevalonate was taken as an index of enzyme activity which catalyzes the conversion of β-hydroxy-β-methyl glutaryl CoA to mevalonate. Lower ratio of β-hydroxy-β-methyl glutaryl CoA to mevalonate indicates higher enzyme activity and higher ratio of β-hydroxy-β-methyl glutaryl CoA to mevalonate indicates lower enzyme activity.

2.10. Assessment of cardiac indices and left ventricular hypertrophy

The heart was then removed, cleaned from fat and fibrous tissue, and dried with filter paper before determining the weight of the whole heart and the left ventricle. Cardiac indices were
calculated as the ratio of whole heart weight to body weight, and
the ratio of left ventricle weight to body weight was calculated to
assess left ventricular hypertrophy (El-Bahai et al., 2009).

2.11. Determination of superoxide radical scavenging activity in vitro

The in vitro superoxide radical scavenging activity of diosmin
was determined by the method of Sabu and Kuttan (2002). One ml
of NBT (100 μM of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml
of NADH solution (14.68 μM of NADH in 100 mM phosphate
buffer) and varying volumes of diosmin (10–50 μg/ml) were mixed
well. The reaction was started by the addition of 100 μM of PMS in
(60 μM / 100 mM phosphate buffer, pH 7.4). The reaction mixture
was incubated at 30°C for 15 min. The absorbance was measured
at 560 nm in a spectrophotometer. Incubation without diosmin
was used as blank. Butylated hydroxytoluene was used as a
positive control for comparison. Decreased absorbance of the
reaction mixture was recorded 517 nm in a Systronics UV–visible
spectrophotometer. Decreased absorbance of the reaction mixture
indicates increased superoxide anion scavenging activity. All the
samples were treated in a similar manner. Absorbance was
recorded and the percentage of inhibition was calculated.

2.12. Determination of hydroxyl radical scavenging activity in vitro

The in vitro hydroxyl radical scavenging capacity of diosmin was
measured using modified method as described by Halliwell et al.
(1987). The incubation mixture in a total volume of 1 ml contained
0.1 ml of 100 mM of potassium dihydrogen phosphate-KOH buffer,
varying volumes of diosmin (10–50 μM), 0.2 ml of 500 mM of ferric
chloride, 0.1 ml of 1 mM of ascorbic acid, 0.1 ml of 1 mM of EDTA,
0.1 ml of 10 mM of H₂O₂ and 0.2 ml of deoxyribose. The contents
were mixed thoroughly and incubated at room temperature for
60 min. Then 1 ml of 1% TBA (1 g in 100 ml of 0.05 N NaOH) and 1 ml
of 28% TCA were added. All the tubes were kept in a boiling water
bath for 30 min. The absorbance was read in a spectrophotometer at
532 nm with reagent blank containing distilled water in place of
diosmin. The percentage scavenging activity was determined.
Decreased absorbance of the reaction mixture indicated increased
hydroxyl radical scavenging activity. The hydroxyl radical scavenging
activity of diosmin was reported as the percentage of inhibition of
deoxyribose degradation.

2.13. 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

We analyzed the function of cardiac conduction system by
recording electrocardiogram patterns of normal and experimental
rats (Fig. 1). We observed the changes in the various phases of each
cardiac cycle. Normal (Group-I) and diosmin treated rats (Group-
II) showed no change in the cardiac conduction activity with no
changes in depolarization waves and repolarization wave. The rats
induced with MI by isoproterenol (Group-III) showed significant
changes in ST-segment, QT interval, P wave, QRS complex and RR
interval compared to normal rats. These changes are almost
similar to the indications of MI. Oral pretreatment with diosmin
in isoproterenol-induced rats (5 and 10 mg/kg) (Group-IV and V)

![Fig. 1. (A–F): Effects of diosmin on electrocardiographic patterns in normal and experimental rats. (A). Electrocardiogram pattern of normal rats showing normal cardiograph. (B). Electrocardiogram pattern of diosmin (5 mg/kg) treated rats showing normal cardiograph. (C). Electrocardiogram pattern of diosmin (10 mg/kg) treated rats showing normal cardiograph. (D). Electrocardiogram pattern of isoproterenol (100 mg/kg)-induced rats showing pathological changes such as ST-segment elevation. (E). Electrocardiogram pattern of diosmin (5 mg/kg) treated isoproterenol-induced rats showing minimized ST-segment elevation. (F). Electrocardiogram pattern of diosmin (10 mg/kg) pretreated isoproterenol-induced rats showing almost normal cardiograph without any elevation in ST-segment.](image)
showed a significant improvement in the ECG parameters, compared to isoproterenol-induced rats (Group III).

In the isoproterenol-induced myocardial infarcted rats, significant ($P < 0.05$) increases in the levels of cardiac markers such as troponin-I and creatine kinase—MB fraction in the serum were observed. Pretreatment with diosmin significantly ($P < 0.05$) decreased the levels of these cardiac markers in the serum, compared to non-treated isoproterenol-induced myocardial infarcted rats (Table 1).

The isoproterenol-induced myocardial infarcted rats showed a significant ($P < 0.05$) increase in the levels of plasma thiobarbituric acid reactive substances and lipid hydroperoxides compared with the normal rats. Pretreatment with diosmin significantly ($P < 0.05$) reduced the levels of plasma thiobarbituric acid reactive substances and lipid hydroperoxides, compared to rats induced with isoproterenol alone that reflecting the anti lipid-peroxidative effect of diosmin (Fig. 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK-MB (IU/l)</th>
<th>Troponin-I (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.71 ± 0.59a</td>
<td>0.23 ± 0.01a</td>
</tr>
<tr>
<td>Diosmin (5 mg/kg)</td>
<td>7.93 ± 0.48a</td>
<td>0.24 ± 0.01a</td>
</tr>
<tr>
<td>Diosmin (10 mg/kg)</td>
<td>7.73 ± 0.57a</td>
<td>0.23 ± 0.01a</td>
</tr>
<tr>
<td>ISO (100 mg/kg)</td>
<td>17.92 ± 1.07b</td>
<td>0.98 ± 0.06b</td>
</tr>
<tr>
<td>Diosmin (5 mg/kg) + ISO</td>
<td>13.22 ± 0.84c</td>
<td>0.57 ± 0.05c</td>
</tr>
<tr>
<td>Diosmin (10 mg/kg) + ISO</td>
<td>9.37 ± 0.59d</td>
<td>0.36 ± 0.02d</td>
</tr>
</tbody>
</table>

ISO—isoproterenol, each value is mean ± standard deviation for six rats in each group, values not sharing a common superscript (a,b,c,d) differ significantly with each other ($P < 0.05$). Duncan’s multiple range test.

Isoproterenol-induced myocardial infarcted rats showed a significant ($P < 0.05$) increase in the levels of plasma total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and a significant decrease in the levels of plasma HDL-cholesterol compared with normal group. Pretreatment with diosmin significantly ($P < 0.05$) decreased the levels of plasma total cholesterol, LDL-cholesterol, VLDL-cholesterol and significantly ($P < 0.05$) increased the levels of plasma HDL-cholesterol (Table 2).

The significant ($P < 0.05$) increase in the activity of HMG-CoA reductase in the liver of isoproterenol-induced myocardial infarcted rats was indicated by the lower ratio of HMG-CoA/ mevalonate. Pretreatment with diosmin significantly ($P < 0.05$) decreased the activity of HMG-CoA reductase, compared to isoproterenol-induced myocardial infarcted rats and it is indicated by higher ratio of HMG-CoA/mevalonate (Fig. 3).

The mean body weight of rats in all experimental groups had no significant change (data not shown in the results). The ratio of left ventricle weight to body weight was significantly ($P < 0.05$) increased in isoproterenol-induced rats as compared to the normal rats (Fig. 4). Pretreatment with diosmin significantly ($P < 0.05$) reduced the ratio of left ventricle weight to body weight indicating the antihypertrophic effects of diosmin.

For all the biochemical parameters studied, treatment with diosmin (5 mg/kg and 10 mg/kg) (Groups III and IV) daily for a period of 10 days to the normal rats did not show any significant effect. Also, pretreatment with diosmin (10 mg/kg body weight) (Group VI) showed a higher effect than the lower dose (5 mg/kg body weight) (Group V).

Diosmin showed positive response with the scavenging of superoxide and hydroxyl free radicals in vitro. The percentage scavenging effects of diosmin on superoxide free radical at different concentrations (10, 20, 30, 40 and 50 μM) were found

Table 1
Effects of diosmin on serum cardiac markers.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK-MB (IU/l)</th>
<th>Troponin-I (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.71 ± 0.59a</td>
<td>0.23 ± 0.01a</td>
</tr>
<tr>
<td>Diosmin (5 mg/kg)</td>
<td>7.93 ± 0.48a</td>
<td>0.24 ± 0.01a</td>
</tr>
<tr>
<td>Diosmin (10 mg/kg)</td>
<td>7.73 ± 0.57a</td>
<td>0.23 ± 0.01a</td>
</tr>
<tr>
<td>ISO (100 mg/kg)</td>
<td>17.92 ± 1.07b</td>
<td>0.98 ± 0.06b</td>
</tr>
<tr>
<td>Diosmin (5 mg/kg) + ISO</td>
<td>13.22 ± 0.84c</td>
<td>0.57 ± 0.05c</td>
</tr>
<tr>
<td>Diosmin (10 mg/kg) + ISO</td>
<td>9.37 ± 0.59d</td>
<td>0.36 ± 0.02d</td>
</tr>
</tbody>
</table>

ISO—isoproterenol, each value is mean ± standard deviation for six rats in each group, values not sharing a common superscript (a,b,c,d) differ significantly with each other ($P < 0.05$). Duncan’s multiple range test.

Table 2
Effects of diosmin on lipid profile in the plasma.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Diosmin (5 mg/kg)</th>
<th>Diosmin (10 mg/kg)</th>
<th>ISO (100 mg/kg)</th>
<th>Diosmin (5 mg/kg) + ISO (100 mg/kg)</th>
<th>Diosmin (10 mg/kg) + ISO (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>83.72 ± 3.42a</td>
<td>82.37 ± 5.90a</td>
<td>81.99 ± 5.63a</td>
<td>131.57 ± 9.28b</td>
<td>114.79 ± 6.06c</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>37.70 ± 2.21a</td>
<td>36.29 ± 2.24a</td>
<td>35.60 ± 2.44a</td>
<td>20.06 ± 1.83b</td>
<td>25.80 ± 1.97c</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>39.44 ± 2.82a</td>
<td>39.54 ± 5.83a</td>
<td>39.93 ± 6.60a</td>
<td>100.41 ± 7.68b</td>
<td>80.33 ± 5.86c</td>
</tr>
<tr>
<td>VLDL-Cholesterol (mg/dl)</td>
<td>6.58 ± 0.36a</td>
<td>6.55 ± 0.38a</td>
<td>6.46 ± 0.36a</td>
<td>11.10 ± 0.65b</td>
<td>8.66 ± 0.45c</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>32.57 ± 1.34a</td>
<td>32.75 ± 1.57a</td>
<td>32.30 ± 1.84a</td>
<td>55.95 ± 2.48b</td>
<td>42.57 ± 1.82c</td>
</tr>
</tbody>
</table>

ISO—isoproterenol, each value is mean ± standard deviation for six rats in each group, values not sharing a common superscript (a,b,c,d) differ significantly with each other ($P < 0.05$). Duncan’s multiple range test.
to be 20.24, 35.48, 52.37, 75.66 and 92.47% respectively. The percentage scavenging effects of diosmin on hydroxyl radical at different concentrations (10, 20, 30, 40 and 50 μM) were found to be 18.73, 33.89, 47.54, 72.56 and 87.65% respectively. These results clearly show the effects of diosmin to scavenge superoxide and hydroxyl free radicals even at lower doses.

4. Discussion

ECG is a good tool to analyze the function of heart. The anatomical difference of the atria and the ventricles, their sequential activation, depolarization and repolarization are the reasons to produce clearly differentiable deflections in electrical conductivity and these deflections are recorded using ECG. The decreased QRS complex may lead to inadequate contractile activity in the isoproterenol-induced myocardial infarcted rats.

Another strong finding was positive correlation between ST-segment elevation and a hyper acute T wave. Pharmacoepidemiological analysis clearly indicated that ST segment elevation remains one of the adverse predictors of myocardial infarction and it is also considered as a reliable biomarker in the clinical trials (Cannon et al., 1997). We also observed pathological Q wave in the isoproterenol-induced myocardial rats. These changes clearly indicate that there is some abnormality with respect to the conductivity and contractility of the heart. In this context, a previous study revealed that after 24 h of isoproterenol administration, decreased QRS complex, ST-segment elevation and a hyper acute T wave were observed in ECG of Wistar rats (Darlin Quine, 2013). Pretreatment with diosmin significantly reduced the pathological changes in the ECG of isoproterenol-induced rats.

The levels of troponins are the ideal biochemical markers, which are present at high concentration in the myocardium, absent in non-cardiac tissue and released rapidly following myocardial damage. The release of cardiac troponins has been correlated with the severity of infarction in experimental animal models (Bertinchant et al., 2000). The higher levels of troponin-I observed in the serum indicate that there is a myocardial damage in isoproterenol-induced myocardial infarcted rats. The increased activity of serum creatine kinase MB (CK-MB) in the isoproterenol-induced myocardial infarcted rats confirmed that there is myocardial cardiac damage. Pretreatment with diosmin (5 and 10 mg/kg) blocked the toxic effect of excessive dose of isoproterenol and inhibited cardiac damage, which was indicated by the decreased levels of cardiac markers in the diosmin pretreated rats. The results showed the cardioprotective effects of diosmin in the myocardium.

The excessive free radicals produced by isoproterenol, damages lipophilic cell membrane which is indicated by the elevated lipid peroxidation in terms of thiobarbituric acid reactive substances and lipid hydroperoxides. The observed increase in the plasma lipid peroxidation products in isoproterenol-induced myocardial infarcted rats indicating that there is an increased lipid peroxidation (Becker, 2004) reported that Haber–Weiss reaction stimulated by excessive amount of superoxide radicals formed by isoproterenol, and initiating lipid peroxidation. Rats pretreated with diosmin significantly reduced lipid peroxidation in the plasma of isoproterenol-induced myocardial infarcted rats by its antilipid peroxidation property.

The altered lipid profile is a matter of great concern given their role in MI. It has been suggested that hyperlipidaemia and hypertriglyceridaemia are directly related to coronary artery diseases. The obtained results of diosmin encouraged us to focus on its antihyperlipidaemic effect for better understanding of its mode of action. In the present study, an altered plasma lipid profile was observed in isoproterenol-induced myocardial infarcted rats. Cardiac cyclic adenosine monophosphate enhanced lipid biosynthesis during the hypoxic condition is the reason for the increased levels of lipids in isoproterenol-induced myocardial infarcted rats (Leiroth, 2007). Pretreatment with diosmin significantly decreased the levels of plasma total cholesterol in isoproterenol-induced myocardial infarcted rats by its antihypercholesterolemic effects. Paritha and Devi (1997) reported that decreased activity of lipoprotein lipase reduced the uptake of triglycerides from the circulation. This is the reason for the observed increased levels of plasma triglycerides in isoproterenol-induced myocardial infarcted rats. Pretreatment with diosmin significantly reduced the levels of plasma triglycerides in isoproterenol-induced myocardial infarcted rats.

Decreased number of LDL receptors in isoproterenol-induced myocardial infarcted rats is the reason for high levels of LDL-cholesterol. In addition, the decreased levels of HDL make the heart vulnerable to oxidized LDL and cause myocardial infarction. The increased lipolysis induced by isoproterenol is the reason for increased VLDL-cholesterol. Pretreatment with diosmin significantly minimized the alterations in lipoproteins and regulated lipid profile. Diosmin was reported for its protective effect on isolated human lipoproteins from in vitro peroxidation (Dumon et al., 1994). The same action may be responsible for the protective effects against lipoprotein changes in isoproterenol-induced myocardial infarcted rats.

Most of the drugs acting positively on lipid profile are acting through HMG-CoA reductase, a rate limiting enzyme in cholesterol synthesis. The beneficial effects of diosmin on lipid profile stimulated us to find out its effect on the activity of HMG-CoA reductase. The observed increased activity of HMG-CoA reductase in the liver of isoproterenol-induced myocardial infarcted rats resulted in increased levels of cholesterol. Fascinatingly, we observed that diosmin significantly decreased the activity of this enzyme in the liver of myocardial infarcted rats. The observed effect of diosmin on the HMG-CoA reductase is the reason for the decreased levels of lipids.

Free radicals play a deleterious role in the biological systems and act as a major cause for degenerative disorders. Generally, flavonoids inhibit oxygen free radical formation. As diosmin produced cardioprotective effects in this study, we are interested to find out the superoxide and hydroxide radical scavenging effects of diosmin in vitro. The in vitro studies on diosmin showed its free radical scavenging activity. The hydroxyl free radical scavenging and superoxide free radical scavenging activities of diosmin were evidenced by the in vitro studies. The dose dependent activity of diosmin confirmed that the small quantity of the
drug can also produce positive impact. This free radical scavenging mechanism of diosmin may be the reason for its protective effect against isoproterenol-induced cardiac toxicity.

In conclusion, pre-treatment with diosmin provides cardioprotection by preventing the accumulation of lipids by its free radical scavenging and antihyperlipidaemic effects. Also, diosmin pre-treatment appears to be instrumental in correcting myocardial rhythm by regulating electrical conductivity within the myocardium. The pre-treatment in status of lipid profile by HMG-CoA reductase inhibiting capacity of diosmin further contributes to its overall cardioprotective property.

References


