Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: Biochemical and histopathological evidences

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Received 28 July 2006; received in revised form 15 September 2006; accepted 18 September 2006
Available online 24 September 2006

Abstract

This study was designed to evaluate the cardioprotective potential of naringin on lipid peroxides, enzymatic and nonenzymatic antioxidants and histopathological findings in isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Subcutaneous injection of ISO (85 mg/kg) to male Wistar rats showed a significant increase in the levels of thiobarbituric acid reactive substances and lipid hydroperoxides in plasma and the heart and a significant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the heart and the levels of reduced glutathione, vitamin C and vitamin E in plasma and heart and ceruloplasmin in plasma. Oral administration of naringin (10, 20 and 40 mg/kg, respectively) to ISO-induced rats daily for a period of 56 days showed a significant decrease in the levels of lipid peroxidative products and improved the antioxidant status by increasing the activities of antioxidant enzymes and nonenzymatic antioxidants. Histopathological findings of the myocardial tissue showed the protective role of naringin in ISO-induced rats. The effect at a dose of 40 mg/kg of naringin was more pronounced than that of the other two doses, 10 and 20 mg/kg. The results of our study show that naringin possess anti-lipoperoxidative and antioxidant activity in experimentally induced cardiac toxicity.

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Keywords: Naringin; Isoproterenol; Myocardial infarction; Lipid peroxidation; Antioxidants

1. Introduction

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in the Western world, and according to the World Health Organization, it will be the major cause of death in the world by the year 2020 (Lopez and Murrau, 1998). IHD is a condition in which an imbalance between myocardial oxygen supply and demand results in myocardial hypoxia and accumulation of waste metabolites most often due to atherosclerotic disease of the coronary arteries (Hegstad et al., 1994). It is well known that ischemic tissue generates oxygen-derived free radicals, and often leading to chain reactions which contribute to cell death (Cai et al., 1997).

Isoproterenol (ISO), a synthetic catecholamine and a β-adrenergic agonist causes severe oxidative stress in the myocardium, resulting in infarct like necrosis of the heart muscles (Wexler, 1978). Catecholamines rapidly undergo autooxidation and has been suggested that the oxidative products of catecholamines are responsible for the changes in the myocardium (Yates and Dhala,
Exposure of the heart to high concentrations of catecholamines had been reported to result in the development of necrotic lesions in the myocardium of experimental animals (Knufman et al., 1987).

Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They are widely distributed in foods of plant origin such as vegetables, fruits, tea and wine. Scavenging of free radicals seems to play a considerable part in the antioxidant activity of flavonoid compounds. Regular ingestion of flavonoid-containing foods may protect against death from coronary artery disease in elderly men (Hertog et al., 1993). Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and non-toxic nature. Novel antioxidants may offer an effective and safe means of counteracting some of the problems and bolstering the body’s defense against free radicals and cardiovascular diseases (CVD).

Grapefruit is a part of the diet in most countries, where it is usually consumed as juice. The chemical responsible for the characteristic sour flavor of the fruit is naringin a flavonone, which is rapidly transformed into naringenin by the action of the enzymes such as α-rhamnosidase and β-glucosidase (Kim et al., 1998). Naringin (4′,5,7-trihydroxy flavonone 7-rhamnoglucoside) (Fig. 1) is the predominant flavonone found in grape fruit Citrus paradisi, Citrus sinensis, Citrus unishu, Citrus nobilis, Citrus tachibana, Citrus junos, Artemisia selengensis, Artemisia stolonifera, roots of Cudrania cochinchinensis var. gerontatgea, aerial parts of Thymus barona, fruits of Poncirus species, Mabea fistulifera, Swartiza polyphylla and related citrus species (Jagetia and Reddy, 2002). The role of naringin had recently received considerable attention as dietary antioxidant. Naringin exhibits various pharmacological and therapeutic properties: antimicrobial, antimutagenic, anticancer, anti-inflammatory, cholesterol lowering, free radical scavenging and antioxidant effects (Jeon et al., 2004).

In our laboratory, we previously reported the preventive effect of naringin on lipids, lipoproteins and enzymes associated with lipid metabolism in ISO-induced male Wistar rats (Rajadurai and Stanely Mainzen Prince, 2006). In the present study, we evaluated the preventive effect of naringin on lipid peroxides, enzymatic, nonenzymatic antioxidants and histopathological findings in normal and ISO-induced myocardial infarction (MI) in male albino Wistar rats.

2. Materials and methods

2.1. Experimental animals

All the experiments were carried out with male albino Wistar rats weighing 140–160 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk, renewed every 24 h under a 12:12 h light dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.25% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen-free extract (carbohydrates). The diet provided metabolisable energy of 3000 kcal. 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 Animal Ethical Committee of Annamalai University (Approval no. 276; dated 1/7/2005).

2.2. Drugs and chemicals

Isoproterenol hydrochloride, naringin, butylated hydroxy toluene, nitroblue tetrazolium, phenazine methosulphate and glutathione were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other biochemicals and chemicals used in the study were of analytical grade.

2.3. Induction of experimental myocardial infarction

Isoproterenol (85 mg/kg) dissolved in normal saline was injected subcutaneously to rats at an interval of 24 h for two days to induce experimental MI (Rajadurai and Stanely Mainzen Prince, 2006).

2.4. Experimental design

The animals were grouped as eight rats in each group—Group 1: normal control rats; Groups 2, 3 and 4: normal rats treated with naringin (10, 20 and 40 mg/kg, respec-
developed was read at 412 nm. GPx activity was assayed by the added to compounds containing sulfhydryl groups. The colour development of yellow colour, when dithionitro benzoic acid is by the method of Ellman (1959). This method is based on the dichromate in acetic acid is converted to perchromic acid was assayed by the method of Sinha (1972). In this method, ide radicals react with nitroblue tetrazolium in the presence of superoxide radicals and the formation of chromophore, which absorbance was measured at 520 nm.

2.5. Biochemical assays

Plasma thiobarbituric acid reactive substances (TBARS) were estimated by the method of Yagi (1987). TBARS were quantitated by their reactivity with thiobarbituric acid (TBA) in acidic conditions to generate a pink coloured chromophore, which was read at 530 nm. TBARS in the heart was estimated by the method of Fraga et al. (1988). In this method, malondialdehyde and other TBARS were measured by their reactivity with TBA in acidic conditions to generate a pink coloured chromophore, which was read at 535 nm. Estimation of plasma and cardiac tissue lipid hydroperoxides (HP) was done by the method of Jiang et al. (1992). In this method, oxidation of ferric ion (Fe$^{2+}$) under acidic conditions in the presence of xylene orange led to the formation of a chromophore, which was read at 560 nm.

Superoxide dismutase (SOD) activity in the myocardium was assayed by the method of Kakkar et al. (1984). Superoxide radicals react with nitroblue tetrazolium in the presence of reduced nicotinamide adenine dinucleotide and produce formazin blue. SOD removes the superoxide radicals and inhibits the formation of feormazin blue. The intensity of the colour is inversely proportional to the activity of the enzyme and read at 560 nm. The activity of catalase in myocardium was assayed by the method of Sinha (1972). In this method, dichromate in acetic acid is converted to perchromic acid and then to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate formed was measured at 620 nm.

Estimation of GSH in plasma and the heart tissue was done by the method of Ellman (1959). This method is based on the development of yellow colour, when dithionitro benzoic acid is added to compounds containing sulfhydryl groups. The colour developed was read at 412 nm. GSH for a specified time period. The GSH content remain-

2.6. Histopathological examination

The heart tissue obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissue was processed embedding in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin (H&E) and examined under high power microscope (320×) and photomicrographs were taken.

2.7. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± S.D. from eight rats in each group. $P$ values <0.05 were considered as significant.

3. Results

Table 1 shows the levels of TBARS and HP in plasma and the heart of normal and experimental rats. Rats induced with ISO, showed a significant ($P < 0.05$) increase in the levels of TBARS and HP in plasma and the heart when compared to normal control rats. Oral pretreatment with naringin (10, 20 and 40 mg/kg, respectively) to ISO-induced rats daily for a period of 56 days significantly ($P < 0.05$) decreased the levels of TBARS and HP in plasma and the heart when compared with ISO-alone induced rats.

The activities of SOD and catalase in the heart of normal and experimental rats are shown in Table 2. Rats induced with ISO, exhibited a significant ($P < 0.05$)
Table 1
Effect of naringin on the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in plasma and the heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma TBARS (nM/ml)</th>
<th>Plasma HP (values × 10⁻⁵ mM/dL)</th>
<th>Heart TBARS (mM/100 g wet tissue)</th>
<th>Heart HP (mM/100 g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.08 ± 0.19a</td>
<td>11.21 ± 0.70a</td>
<td>0.76 ± 0.05a</td>
<td>22.43 ± 1.39a</td>
</tr>
<tr>
<td>Normal + naringin (10 mg/kg)</td>
<td>3.13 ± 0.18a</td>
<td>11.23 ± 0.66a</td>
<td>0.75 ± 0.04a</td>
<td>22.37 ± 1.32a</td>
</tr>
<tr>
<td>Normal + naringin (20 mg/kg)</td>
<td>3.00 ± 0.22a</td>
<td>11.17 ± 0.82a</td>
<td>0.76 ± 0.06a</td>
<td>22.30 ± 1.63a</td>
</tr>
<tr>
<td>Normal + naringin (40 mg/kg)</td>
<td>3.02 ± 0.19a</td>
<td>11.07 ± 0.70a</td>
<td>0.73 ± 0.05a</td>
<td>22.08 ± 1.39a</td>
</tr>
<tr>
<td>ISO (85 mg/kg) control</td>
<td>6.20 ± 0.39b</td>
<td>18.34 ± 1.14b</td>
<td>1.16 ± 0.07b</td>
<td>48.59 ± 3.02b</td>
</tr>
<tr>
<td>Naringin (10 mg/kg) + ISO</td>
<td>5.25 ± 0.31c</td>
<td>17.21 ± 1.02c</td>
<td>1.01 ± 0.06c</td>
<td>40.14 ± 2.37c</td>
</tr>
<tr>
<td>Naringin (20 mg/kg) + ISO</td>
<td>4.63 ± 0.34d</td>
<td>14.21 ± 1.04d</td>
<td>0.92 ± 0.07d</td>
<td>32.25 ± 2.35d</td>
</tr>
<tr>
<td>Naringin (40 mg/kg) + ISO</td>
<td>3.77 ± 0.28e</td>
<td>12.13 ± 0.89f</td>
<td>0.83 ± 0.06f</td>
<td>25.37 ± 1.19e</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for eight rats in each group. Values not sharing a common superscripts (a, b, c, d, and e) differ significantly at P < 0.05 (DMRT).

Table 2
Effect of naringin on the activities of superoxide dismutase (SOD) and catalase in the heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (μmoles of H₂O₂ consumed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>12.51 ± 0.77a</td>
<td>7.56 ± 0.47a</td>
</tr>
<tr>
<td>Normal + naringin (10 mg/kg)</td>
<td>12.69 ± 0.75a</td>
<td>7.58 ± 0.45a</td>
</tr>
<tr>
<td>Normal + naringin (20 mg/kg)</td>
<td>12.61 ± 0.92a</td>
<td>7.61 ± 0.56a</td>
</tr>
<tr>
<td>Normal + naringin (40 mg/kg)</td>
<td>12.67 ± 0.80a</td>
<td>7.66 ± 0.48a</td>
</tr>
<tr>
<td>ISO (85 mg/kg) control</td>
<td>6.50 ± 0.40b</td>
<td>3.05 ± 0.18b</td>
</tr>
<tr>
<td>Naringin (10 mg/kg) + ISO</td>
<td>8.28 ± 0.49c</td>
<td>3.98 ± 0.23c</td>
</tr>
<tr>
<td>Naringin (20 mg/kg) + ISO</td>
<td>9.14 ± 0.67d</td>
<td>5.15 ± 0.37d</td>
</tr>
<tr>
<td>Naringin (40 mg/kg) + ISO</td>
<td>10.72 ± 0.78e</td>
<td>6.21 ± 0.45e</td>
</tr>
</tbody>
</table>

SOD units: 1 U is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min. Each value is mean ± S.D. for eight rats in each group. Values not sharing a common superscripts (a, b, c, d, and e) differ significantly at P < 0.05 (DMRT).

decrease in the activities of these antioxidant enzymes in the heart on comparison with normal control rats. Pretreatment with naringin (10, 20 and 40 mg/kg, respectively) to ISO-induced rats significantly (P < 0.05) increased the activities of these enzymes when compared with ISO-alone induced rats.

Table 3 illustrates the effect of naringin on the activities of myocardial GPx and GST and the levels of GSH in plasma and the heart in normal and ISO-induced rats. Rats induced with ISO, showed a significant (P < 0.05) decrease in the activities of these antioxidant enzymes and the levels of GSH on comparison with normal control rats.

Table 3
Effect of naringin on the activities of myocardial glutathione peroxidase (GPx), glutathione-S-transferase (GST) and the levels of reduced glutathione (GSH) in plasma and the heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart GPx (μg of GSH consumed/min/mg protein)</th>
<th>Heart GST (μmoles of CDNB conjugated/min/mg protein)</th>
<th>Plasma GSH (mg/dL)</th>
<th>Heart GSH (mM/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.28 ± 0.27a</td>
<td>830.23 ± 52.1a</td>
<td>23.75 ± 1.48a</td>
<td>8.27 ± 0.51a</td>
</tr>
<tr>
<td>Normal + naringin (10 mg/kg)</td>
<td>4.33 ± 0.26a</td>
<td>837.27 ± 49.5a</td>
<td>23.95 ± 1.41a</td>
<td>8.46 ± 0.49a</td>
</tr>
<tr>
<td>Normal + naringin (20 mg/kg)</td>
<td>4.40 ± 0.32a</td>
<td>840.79 ± 61.7a</td>
<td>24.17 ± 1.74a</td>
<td>8.63 ± 0.62a</td>
</tr>
<tr>
<td>Normal + naringin (40 mg/kg)</td>
<td>4.31 ± 0.27a</td>
<td>845.46 ± 53.2a</td>
<td>24.22 ± 1.51a</td>
<td>9.06 ± 0.55b</td>
</tr>
<tr>
<td>ISO (85 mg/kg) control</td>
<td>1.97 ± 0.12b</td>
<td>557.07 ± 34.5a</td>
<td>12.95 ± 0.80b</td>
<td>4.45 ± 0.28b</td>
</tr>
<tr>
<td>Naringin (10 mg/kg) + ISO</td>
<td>2.51 ± 0.15c</td>
<td>620.41 ± 37.6a</td>
<td>16.65 ± 0.98b</td>
<td>5.27 ± 0.31d</td>
</tr>
<tr>
<td>Naringin (20 mg/kg) + ISO</td>
<td>3.15 ± 0.23d</td>
<td>678.07 ± 49.3a</td>
<td>19.27 ± 1.41d</td>
<td>6.55 ± 0.45e</td>
</tr>
<tr>
<td>Naringin (40 mg/kg) + ISO</td>
<td>3.94 ± 0.29c</td>
<td>750.22 ± 54.3a</td>
<td>22.14 ± 1.58b</td>
<td>7.97 ± 0.58e</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for eight rats in each group. Values not sharing a common superscripts (a, b, c, d, and e) differ significantly at P < 0.05 (DMRT).
 Oral administration of naringin (10, 20 and 40 mg/kg, respectively) to ISO-induced rats significantly \((P < 0.05)\) increased the activities of these antioxidant enzymes and the levels of GSH when compared with ISO-alone induced rats. Naringin 40 mg/kg to normal rats showed a significant effect on myocardial GSH.

**Table 4** shows the effect of naringin on the levels of plasma and heart vitamin C and E and plasma ceruloplasmin in normal and ISO-induced rats. Rats induced with ISO, exhibited a significant \((P < 0.05)\) decrease in the levels of vitamin C and E in plasma and heart and ceruloplasmin in plasma when compared with normal control rats. Oral administration of naringin (10, 20 and 40 mg/kg, respectively) to ISO-induced rats significantly \((P < 0.05)\) increased the levels of vitamin C and E in plasma and the heart and ceruloplasmin in plasma when compared with ISO-alone induced rats.

**Table 5** shows the effect of naringin on the degree of histological changes in myocardial tissues in normal and ISO-induced rats. Histopathological findings of the ISO-alone induced myocardium (Fig. 6) showed infarcted zone with oedema and inflammatory cells and the myocardium also showed separation of muscles fibres. Pretreatment with naringin at a dose of 10 mg/kg (Fig. 7) showed focal area of infarction with coagulative necrosis and inflammatory cells with mild oedema. Pretreatment with naringin at a dose of...
Table 4
Effect of naringin on the levels of vitamin C and E in plasma and the heart and ceruloplasmin in plasma in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma vitamin C (mg/dL)</th>
<th>Plasma vitamin E (mg/dL)</th>
<th>Heart vitamin C (μmol/mg protein)</th>
<th>Heart vitamin E (μmol/mg protein)</th>
<th>Plasma ceruloplasmin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.07 ± 0.13a</td>
<td>1.72 ± 0.11a</td>
<td>0.97 ± 0.07a</td>
<td>0.72 ± 0.05a</td>
<td>31.82 ± 1.98a</td>
</tr>
<tr>
<td>Normal + naringin (10 mg/kg)</td>
<td>2.10 ± 0.12a</td>
<td>1.74 ± 0.10a</td>
<td>0.95 ± 0.08a</td>
<td>0.71 ± 0.06a</td>
<td>31.87 ± 1.88a</td>
</tr>
<tr>
<td>Normal + naringin (20 mg/kg)</td>
<td>2.12 ± 0.13a</td>
<td>1.72 ± 0.13a</td>
<td>0.96 ± 0.07a</td>
<td>0.73 ± 0.05a</td>
<td>31.95 ± 2.33a</td>
</tr>
<tr>
<td>Normal + naringin (40 mg/kg)</td>
<td>2.06 ± 0.13a</td>
<td>1.76 ± 0.11a</td>
<td>0.98 ± 0.06a</td>
<td>0.74 ± 0.05a</td>
<td>31.84 ± 2.01a</td>
</tr>
<tr>
<td>ISO (85 mg/kg) control</td>
<td>0.86 ± 0.05b</td>
<td>0.96 ± 0.06b</td>
<td>0.51 ± 0.04b</td>
<td>0.35 ± 0.03b</td>
<td>20.67 ± 1.28b</td>
</tr>
<tr>
<td>Naringin (10 mg/kg) + ISO</td>
<td>1.14 ± 0.07c</td>
<td>1.20 ± 0.07c</td>
<td>0.65 ± 0.05c</td>
<td>0.42 ± 0.04c</td>
<td>23.35 ± 1.38c</td>
</tr>
<tr>
<td>Naringin (20 mg/kg) + ISO</td>
<td>1.66 ± 0.12d</td>
<td>1.36 ± 0.10d</td>
<td>0.73 ± 0.05d</td>
<td>0.54 ± 0.04d</td>
<td>26.70 ± 1.95d</td>
</tr>
<tr>
<td>Naringin (40 mg/kg) + ISO</td>
<td>1.82 ± 0.13e</td>
<td>1.59 ± 0.12e</td>
<td>0.89 ± 0.08e</td>
<td>0.65 ± 0.05e</td>
<td>28.88 ± 2.17e</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for eight rats in each group. Values not sharing a common superscripts (a, b, c, d, and e) differ significantly at $P<0.05$ (DMRT).

Table 5
Effect of naringin on the degree of histological changes in myocardial tissues in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Necrosis</th>
<th>Oedema</th>
<th>Inflammatory cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Normal + naringin (10 mg/kg)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Normal + naringin (20 mg/kg)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Normal + naringin (40 mg/kg)</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>ISO (85 mg/kg) control</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Naringin (10 mg/kg) + ISO</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Naringin (20 mg/kg) + ISO</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Naringin (40 mg/kg) + ISO</td>
<td>A</td>
<td>+</td>
<td>A</td>
</tr>
</tbody>
</table>

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no changes; (+) mild changes; (++) moderate changes; (+++) marked changes.

20 mg/kg (Fig. 8) showed mild oedema and necrosis without inflammatory cells. Naringin pretreatment at a dose of 40 mg/kg (Fig. 9) showed mild oedema and the myocardial fibres were within normal limits. Naringin
Fig. 9. Naringin 40 mg/kg + ISO treated heart tissue showing mild oedema but no infarction and inflammatory cells. The cardiac fibres are within the normal limits (H&E; 320×).

For all the parameters studied, oral administration of naringin (10, 20 and 40 mg/kg, respectively) to normal rats for a period of 56 days showed a minor effect, but it was not statistically significant ($P < 0.05$) except myocardial GSH. Naringin at a dose of 40 mg/kg showed a better effect than the other two doses (10 and 20 mg/kg, respectively) in ISO-induced rats.

4. Discussion

The effects of ISO on heart are mediated through $\beta_1$ and $\beta_2$ adrenoceptors. Both $\beta_1$ and $\beta_2$ adrenoceptors mediate the positive inotropic and chronotropic effects to $\beta$ adrenoceptor agonists (Brodde, 1991). Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension (Yeager and Iams, 1981), and induce myocardial ischemia due to cytosolic Ca$^{2+}$ overload (Bloom and Davis, 1972). Additionally, ISO causes myocardial ischemia due to excessive production of free radicals resulting from oxidative metabolism of catecholamines (Singal et al., 1983). Grimm et al. (1998) have reported that a toxic dosage of ISO caused characteristic myocardial damage that subsequently resulted in heart failure. ISO administration causes ischemic necrosis in rats, which closely resembles histological damage seen in human MI.

We have observed increased levels of plasma and heart TBARS and HP in ISO-induced rats. Reactive oxygen species (ROS) may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids, which is the precursor of lipid peroxide formation (Gutteridge, 1982). Elevation of lipid peroxides in ISO-induced rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to the myocardial membranes. ROS are highly toxic byproducts of aerobic metabolism, react unfavorably with surrounding macromolecules resulting in severe cell and tissue damage. Increased levels of lipid peroxidation products injure blood vessels, causing increased adherence and aggregation of platelets to the injured sites. Our results are in agreement with previous reports (Sathish et al., 2003).

Pretreatment with naringin to ISO-induced rats significantly decreased the levels of TBARS and HP in both plasma and the heart. Flavonoids been have shown to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages. In this context, Jagetia and Reddy (2005) have reported that naringin treatment reduced the levels of TBARS in radiation-induced lipid peroxidation.

Oxidative stress in cells or tissues results in the enhanced generation of ROS and/or depletion of the antioxidants in the defense system, thereby causing an imbalance between the prooxidants and antioxidants. The ROS generation in tissues are efficiently scavenged by the enzymatic and nonenzymatic antioxidant systems. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of lipid peroxidation (Jagetia et al., 2003). Free radical scavenging enzymes such as SOD, catalase, GPx and GST are the primary defense system against oxidative stress.

In our study, ISO-induced rats exhibited decreased activities of SOD and catalase in the heart. SOD plays an important role in protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized by catalase to molecular oxygen and water. The decrease in the activities of these antioxidant enzymes might be due to myocardial cell damage. Superoxide radicals generated at the site of damage, modulates SOD and catalase resulting in the decreased activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium.

Pretreatment with naringin significantly increased the activities of SOD and catalase in the heart of ISO-induced rats. Recently, naringin has been demonstrated to play an important role in regulating antioxidative capacity by increasing SOD and catalase activities by upregulating their mRNA expression in high cholesterol fed rabbits (Jeon et al., 2002). Naringenin ($4',5,7$-trihydroxy flavonone), the major metabolite of naringin, is known to possess aorta dilation (Rojas et al., 1996), superoxide-scavenging and antioxidant activities (Kroyer, 1986).
In our study, we observed decreased concentration of GSH in plasma and the heart and decreased activities of glutathione dependent enzymes such as GPx and GST in the heart of ISO-induced rats. GSH is an abundant and ubiquitous antioxidant, a tripeptide and essential biofactor synthesized in all living cells. It functions mainly as an effective intracellular reductant (Rahman and MacNee, 1999). It protects the cells from free radical mediated damage caused by drugs and ionizing radiation. It forms an important substrate for GPx, GST and several other enzymes, which is involved in the free radical scavenging action (Jagetia et al., 2004). In the heart, GPx is a major enzymatic mechanism for the disposal of peroxides, a prolonged depression in the activity of this enzyme may lead to the intracellular peroxide accumulation. GST acts like peroxidase and removes the stable peroxides from the system, resulting in the reduction of peroxide-induced damage (Jagetia et al., 2004). Decreased GSH levels might be due to increased utilization in protecting ‘SH’ containing proteins from lipid peroxides. The unavailability of GSH may decrease the activities of GPx and GST in ISO-induced rats. Pretreatment with naringin significantly increased the concentration of GSH in plasma and the heart and the activities of GPx and GST in the heart of ISO-induced rats. Kanno et al. (2003) have reported that the antioxidant effect of naringin is similar to that of GSH, and furthermore, it inhibits the hydrogen peroxide-induced lipid peroxidation. There is a report showing that naringin treatment upregulated the gene expression of GPx in high cholesterol fed rabbits (Jeon et al., 2002).

Decreased concentration of vitamin C and E in plasma and the heart and ceruloplasmin in plasma in ISO-induced rats were observed in our study. Vitamin C is a primary antioxidant, water-soluble vitamin that can directly scavenge singlet oxygen, superoxide and hydroxyl radicals. It has been suggested to reduce the risk of CVD by reducing blood pressure, blood cholesterol and the formation of oxidized low-density lipoprotein–cholesterol (Benidich et al., 1986). Vitamin E appears to be the most effective lipid soluble antioxidant in the biological system. It inhibits lipid peroxidation and regenerates reduced vitamin C and GSH. By protecting myocardial membranes and inhibiting the oxidation of lipoproteins, vitamin E inhibits membrane peroxidative damage and atherogenesis (Upston et al., 1999). Ceruloplasmin inhibits ferritin-dependent lipid peroxidation by catalyzing the oxidative reincorporation of released irons into ferritin (Samokyszyn et al., 1989).

Pretreatment with naringin to ISO-induced rats significantly increased the levels of vitamin C and E in both plasma and heart and ceruloplasmin levels in plasma. In this context, Jeon et al. (2002) have shown that naringin possess a strong antioxidant property and reduce the incidence of lipid peroxidation in high cholesterol fed rabbits.

Flavonoid antioxidants function as scavengers of free radicals by rapid donation of hydrogen atom to radicals (Amic et al., 2003). Naringin has many of the structural components that contribute to its antioxidant property. Naringin has carbonyl group at C-4 of the C ring, and hydroxyl groups at C-5 of A ring and at C-4’ of B ring. Moreover, flavonoids having both a C-4 carbonyl group and a C-3 or C-5 hydroxyl group such as naringin form chelates with iron ions and the ability of flavonoids to sequester metal ions may contribute to their antilipoperoxidative property by preventing the formation of free radicals in the Fenton system. Flavonoids retain their free radical scavenging activities after forming complexes with iron ions and thus formation of metal ion chelates is also one of the antioxidant mechanisms of flavonoids (Cook and Samman, 1996).

The histopathological findings of the ISO-induced myocardium showed infarcted zone with oedema and inflammatory cells and separation of cardiac muscle fibres. Naringin (10 mg/kg) pretreated myocardium showed focal area of infarction with coagulative necrosis and inflammatory cells with mild oedema and naringin (20 mg/kg) pretreated myocardium showed mild oedema and necrosis without inflammatory cells. There was mild oedema but no infarction and inflammatory cells and the cardiac fibres were within the normal limits at the dose of naringin (40 mg/kg) pretreated rat myocardium. Naringin (10, 20 and 40 mg/kg, respectively) treated normal rats myocardium showed normal cardiac fibres without any pathological changes. This indicates that naringin does not possess any adverse effects under normal conditions.

5. Conclusion

The biochemical and histopathological findings obtained from our study indicates that naringin offers protection to the myocardium against ISO-induced oxidative stress in rats. This could be due to prevention or inhibition of lipid peroxidative system by its antioxidant effect. Thus, naringin has been proved to possess cardioprotective effect in ISO-induced cardiac toxicity in rats.

Acknowledgement

We thank the Indian Council of Medical Research (ICMR), New Delhi, for funding support in the form
of Senior Research Fellowship (SRF) to M. Rajadurai. For histopathological studies, the assistance of Dr. S. Vairamuthu, Assistant Professor, Department of Veterinary Pathology, Tamilnadu Veterinary and Animal Sciences University, Chennai, Tamilnadu, is gratefully acknowledged.

References


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