Original Research

Effect of luteolin on lipid peroxidation and antioxidants in acute and chronic periods of isoproterenol induced myocardial infarction in rats

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Abstract

Aim: The present study was designed to investigate the preventive effect of luteolin on lipid peroxidation, antioxidants and histopathological findings in acute and chronic periods after isoproterenol (ISO)-induced myocardial-infarction (MI) in male Wistar rats.

Methods: Luteolin supplemented by intragastric intubation at a daily dose of 0.3 mg/kg body weight in acute and chronic periods following MI. In acute MI model, luteolin had been administered once per day to rat groups during 30 days. On days 29 and 30th the rats of the acute MI control groups were administered 85mg/kg body weight, isoproterenol, intraperitoneally intravel of 24h. In chronic MI model luteolin administered of rat group during 30 days, and on the 1st and 2nd days, the rats of the chronic MI control and luteolin treatment groups were administered ISO by the same way.

Results: The ISO-induced rats both in acute and chronic models showed significant increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides in the heart and erythrocyte, and significant decrease in the activities of heart and erythrocytes superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione. Oral treatment with luteolin at a daily dose of (0.3 mg/kg b.wt) in both acute and chronic models showed significant decrease in the levels of heart and erythrocyte lipid peroxidation by products and significant increase in the levels of antioxidant system. The protective role of luteolin (0.3 mg/kg b.wt) on ISO-induced myocardial infarction was further confirmed by histopathological examination.

Conclusion: Thus, the experiment clearly showed that luteolin ameliorates cardiac damage in ISO-induced myocardial infarction by prevented the accumulation of lipids due to the anti-lipid peroxidative effect.

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Keywords: Isoproterenol; Luteolin; Lipid peroxidation; Antioxidants

1. Introduction

Myocardial infarction (MI) continues to be a major public health problem, not only in Western countries but also increasingly in developing countries and makes a significant contribution to the mortality statistics.1 In India, the number of patients being hospitalized for myocardial infarction, commonly known as heart attack, is increasing over the past 35 years and male patients have shown a more striking increase.2 MI is the acute condition of necrosis of the myocardium that occurs as a result of critical imbalance between coronary blood supply and myocardial demand.3 Isoproterenol, a synthetic catecholamine and β-adrenergic agonist is a well known inducer of myocardial hypertrophy has been reported to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscles.4 It is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor in irreversible damage to the myocardial membrane.5 By studying the biochemical alterations that take place in an animal model, it
is possible to gain more insight into the mechanisms leading to the altered metabolic process in human myocardial infarction. The flavonoids appear to have played a major role in the successful medical treatments and their use has persevered up to now. Flavonoids are widely distributed polyphenol compounds in the plant kingdom and constitute an important source of natural products which plays an important role in the development of various human diseases including cardiovascular diseases.

Luteolin, 3', 4', 5, 7-tetrahydroxyflavone (Fig. 1), is a polyphenolic compound is widely distributed in fruits, herbs and green vegetables such as artichoke, celery, peppermint, broccoli, cauliflower, green pepper, perilla leaf, camomile tea, cabbage, thyme, honeysuckle and spinach. Several epidemiological studies have shown that high consumption of foods containing luteolin is associated with a reduced risk of developing chronic diseases. Numerous experimental data have revealed that luteolin possesses a wide range of pharmacological effects, including antioxidant, anti-neoplastic, anti-hepatotoxic, anti-allergic, anti-osteoporotic, anti-diabetic, anti-inflammatory, anti-platelet and vasodilatory activity. But till now there are no studies related to the effect of luteolin against isoproterenol induced myocardial infarction in rats. So we made an attempt to study the protective role of luteolin in ISO-induced cardiac damage with reference to lipid peroxidation and antioxidants that play an important role in cardiac damage.

2. Materials and methods

2.1. Chemicals

Isoproterenol, luteolin were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and reagents used were of analytical grade.

2.2. Formulation and administration of luteolin

Luteolin powder was suspended in 0.5% carboxymethyl cellulose (CMC) and each animal belonging to three different groups received 1.0 ml of luteolin suspension at a dose of 0.3 mg/kg body weight everyday respectively by intragastric intubation.

2.3. Induction of myocardial infarction

2.3.1. Acute myocardial infarction (AMI)

The acute myocardial infarction was induced by intra-peritoneal (i.p.) injection of isoproterenol hydrochloride (85 mg/kg body weight, dissolved in physiological saline, for 29th and 30th days.

2.3.2. Chronic myocardial infarction (CMI)

The Chronic myocardial infarction was induced by intra-peritoneal (i.p.) injection of isoproterenol hydrochloride (85 mg/kg body weight, dissolved in physiological saline, for 1st and 2nd days.

2.4. Animal housing and diets

Male Wistar albino rats aged 6 weeks and weighing about 150 g were obtained from Sri Venkateshwara enterprises Bangalore, India. After one week of acclimatization all animals were housed six per polypropylene plastic cage covered with metal grids and a hygienic bed of husk in a specific-pathogen free animal room under controlled conditions of a 12 h light/12 hour dark cycle, and provided with standard food pellets (diet composition, wheat broken-moisture 9.0%, crude protein, 11.5% crude fat, 1.9% crude fibre 4% ash 0.2%, nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India) and tap water ad libitum. The study was conducted after obtaining a clearance from the institutional animal ethical committee (IAEC) (Reg .no P.Col/52/2010/IAEC/VMCP) of Vinayaka Mission College of Pharmacy, Salem, Tamil Nadu.

2.5. Study design and treatment schedule

In the experiment, the total of 36 rats (24 myocardial rats, 12 normal rats) were used. The rats were randomly assigned into six groups of 6 animals each. Total duration of the experiment was 30 days.

The rats in group I received 1.0 ml of 0.5% CMC every day via intragastric intubation and served as the untreated control. The rats in group II received luteolin via intragastric intubation at a daily dose of (0.3 mg/kg body weight) respectively for a period of 30 days. Group III rats received isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24 h on the 29th and 30th days (Acute myocardial infarction). Group IV rats received isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24 h on the 1st and 2nd days (Chronic myocardial infarction). Group V rats received luteolin as in group II for 30 days and at the last of the experimental period on 29th and 30th days rats received isoproterenol (85 mg/kg body weight) injections intraperitoneally twice at an intravel of 24 h (Acute myocardial infarction + Luteolin). Group VI rats received isoproterenol as in group IV for 2 days and at the of the experimental period rats received luteolin (0.3 mg/kg body weight) (Chronic myocardial infarction + Luteolin).

Fig. 1. Structure of luteolin and its molecular formula C15H10O6, molecular weight 286.924.
At the end of the experimental period, rats were sacrificed by cervical decapitation. The blood was collected and serum obtained after centrifugation were used for various biochemical estimations. Heart were removed, cleared of blood and immediately transferred to ice cold containers containing 0.9% sodium chloride. Samples of tissues were homogenized in appropriate buffer and used for the determination of the following parameters.

2.6. Biochemical estimations

Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances (TBARS) in tissues via the method of Niehaus and Samuelson\(^\text{17}\) and plasma or erythrocyte via the method of Yagi.\(^\text{18}\) The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, was measured at 532 nm. The values are expressed as nmols/100 g tissue or nmols/mg Hb. Reduced glutathione (GSH) content was determined via the method of Moron et al.\(^\text{19}\) GSH determination is based on the development of yellow colour when 5, 5'-dithio 2-nitro benzoic acid (DTNB) is added to compounds containing sulfhydryl groups. The values are expressed as nmols g\(^{-1}\) wet tissue. Glutathione peroxidase (GPx EC.1.11.1.9) activity was assayed via the method of Rotruck\(^\text{20}\) with a modification: a known amount of enzyme preparation was incubated with H\(_2\)O\(_2\) in the presence of GSH for a specified time period. The amount of H\(_2\)O\(_2\) utilized was determined via the method of Habig et al.\(^\text{21}\) The values are expressed as µmols of GSH utilized/min/mg Hb or protein. Superoxide dismutase (SOD EC.1.15.1.1) was assayed using the method of Marklund and Marklund\(^\text{22}\) based on the 50% inhibition of the formation of NADH-phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. One unit of the enzymes is taken as the amount of enzyme for 50% inhibition of NBT reduction/min/mg protein. The activity of catalase (CAT EC. 1.11.16) was determined via the method of Sinha\(^\text{23}\): dichromate in acetic acid was reduced to chromic acid when heated in the presence of hydrogen peroxide (H\(_2\)O\(_2\)), with the formation of perchromic acid as an unstable CAT intermediate. The chromic acid formed was measured at 590 nm. Catalase was allowed to split H\(_2\)O\(_2\) for different periods of time. The reaction was stopped at different time intervals via the addition of a dichromate acetic acid mixture, and heating the reaction mixture and measuring chromic acid colorimetrically and determined the remaining H\(_2\)O\(_2\).

2.7. Preparation of hemolysate

Blood was collected in heparinized tubes and plasma was separated by centrifugation at 2000×g for 10 min. After the separation of plasma, the buffy coat was removed and packed cells (RBCs) were washed thrice with cold physiological saline. To determine the activity of RBC antioxidant enzymes, RBC lysate was prepared by lysing a known volume of RBCs with hypotonic phosphate buffer, pH 7.4 and centrifuged at 3000×g for 10 min at 2°C to separate the hemolysate.

2.8. Preparation of tissue homogenate

Heart tissue was removed immediately and washed with ice-cold saline and homogenized in the appropriate buffer in a tissue homogenizer.

2.9. Histopathological studies

At the end of the study, all the rats were sacrificed by cervical decapitation and the hearts were dissected out, washed in ice cold saline. Then myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under a light microscope and photographs were taken.

2.9.1. Statistical analysis

The results presented here are the means ± SD of 6 rats in each group. The results were analyzed using one-way analysis of variance [ANOVA] and the group means were compared using Duncan’s multiple range test [DMRT] using SPSS version 12 for Windows. The findings were considered as statistically significant if \(P < 0.05\).\(^\text{24}\)

3. Results

3.1. Histopathological observations

Normal architecture of the cardiac cells was observed with no evidence of microscopic changes in the control and luteolin treated groups (Fig. 2). In isoproterenol (85 mg/kg body weight) treated rats’ heart showing focal confluent necrosis of muscle fiber with inflammatory cell infiltration, edema with fibroblastic proliferation and phagocytosis were seen. In luteolin (0.3 mg/kg body weight) and isoproterenol (85 mg/kg body weight) treated rats, showing mild degree of necrosis and less infiltration of inflammatory cells.

3.2. Effect of luteolin on lipid peroxidation in the myocardial infarction rats

Table 1 depicts the effect of luteolin on circulatory and tissue levels of TBARS in the control and experimental rats. The levels of plasma, erythrocyte and tissue TBARS was significantly (\(P < 0.05\)) increased in the acute and chronic periods of ISO-induced rats (groups 3 and 4) as compared to the control rats (group 1). However, pre-treatment with luteolin reduced TBARS levels to normal levels. Luteolin administration to isoproterenol treated rats (groups 5 and 6) significantly (\(P < 0.05\)) decreased in the levels of TBARS tissue erythrocyte and serum as compared with acute and chronic periods of ISO-induced rats (groups 3 and 4).
Fig. 2. Histopathology changes in the myocardial infarction of control and experimental rats. Effect of luteolin on isoproterenol induced myocardial infarction in rats. A) Normal control heart showing normal cardiac muscle fibers. B) Group II (luteolin) heart showed no changes. C) Group III (AMI) ISO (85 mg/kg twice with a duration of 24 h) control heart showing focal confluent necrosis of muscle fiber with inflammatory cell infiltration, edema with fibroblastic proliferation and phagocytosis along extravasation of red blood cells. D) Group IV (CMI) ISO (85 mg/kg twice with a duration of 24 h) control heart showing cardiac muscle fibers with muscle separation and inflammatory collections, cardiac necrosis and splitting of muscle bundles. E) Group V (AMI + luteolin) rats showing mild degree of necrosis and less infiltration of inflammatory cells; F) Group VI (CMI + luteolin) showing some cardiac muscle cell denaturation alone with infiltration of a small amount of inflammatory cells could be observed, necrosis was not obvious.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Luteolin (0.3 mg/kg b.wt)</th>
<th>Acute MI (85 mg/kg b.wt)</th>
<th>Chronic MI (85 mg/kg b.wt)</th>
<th>Luteolin + Acute MI</th>
<th>Luteolin + Chronic MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma thiobarbituric acid reactive substances (nM/ml)</td>
<td>3.01 ± 0.16</td>
<td>1.9 ± 0.26</td>
<td>3.4 ± 0.20</td>
<td>4.0 ± 0.19</td>
<td>3.3 ± 0.16</td>
<td>3.6 ± 0.21</td>
</tr>
<tr>
<td>Heart tissue thiobarbituric acid reactive substances (nM/100 g wet tissue)</td>
<td>37.74 ± 0.05</td>
<td>33.16 ± 0.35</td>
<td>110 ± 0.35</td>
<td>114 ± 0.27</td>
<td>85.7 ± 0.32</td>
<td>90.12 ± 0.32</td>
</tr>
<tr>
<td>Erythrocyte thiobarbituric acid reactive substances (nmoles MDA released min-1 mg-1 protein)</td>
<td>1.7 ± 0.14</td>
<td>1.2 ± 0.24</td>
<td>3.1 ± 0.19</td>
<td>3.5 ± 0.19</td>
<td>2.3 ± 0.26</td>
<td>2.9 ± 0.26</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD of six rats in each group.

a $P < 0.05$ when compared with normal.

b $P < 0.05$ when compared with isoproterenol.
### Table 2
Effect of luteolin on circulatory antioxidants of control and experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (Acute MI) (85 mg/kg b.wt)</th>
<th>Luteolin (Acute MI) (85 mg/kg b.wt)</th>
<th>Acute MI (Chronic MI)</th>
<th>Luteolin + Acute MI (Chronic MI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulatory antioxidants</td>
<td>7.8 ± 0.34 82.2 ± 0.25</td>
<td>3.3 ± 0.39a</td>
<td>3.1 ± 0.29a</td>
<td>6.05 ± 0.42b 6.3 ± 0.39b</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (IU/L)</td>
<td>21 ± 1.00</td>
<td>13 ± 0.29a</td>
<td>11 ± 0.544</td>
<td>14 ± 0.56b 17 ± 0.29b</td>
</tr>
<tr>
<td>Catalase (CAT) (IU/L)</td>
<td>0.33 ± 0.03 0.48 ± 0.03</td>
<td>0.20 ± 0.02e</td>
<td>0.16 ± 0.02e</td>
<td>0.28 ± 0.02b 0.29 ± 0.02b</td>
</tr>
<tr>
<td>Glutathione peroxidase (GPx) (IU/L)</td>
<td>1.8 ± 0.58 2.2 ± 0.15</td>
<td>1.3 ± 0.23d</td>
<td>1.1 ± 0.13d</td>
<td>1.5 ± 0.11b 1.65 ± 0.22b</td>
</tr>
<tr>
<td>Glutathione reductase (IU/L)</td>
<td>0.25 ± 3.3</td>
<td>0.15 ± 1.3</td>
<td>0.03 ± 0.20</td>
<td>1.02 ± 13</td>
</tr>
<tr>
<td>Glutathione-S-transferase (IU/L)</td>
<td>795.21 ± 58.8</td>
<td>822.6 ± 56.5</td>
<td>554.6 ± 36.1a</td>
<td>534.3 ± 35.2a 688.1 ± 49.4d 665.6 ± 53.1b</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD of ten rats in each group.

a $p < 0.05$ when compared with normal.

b $p < 0.05$ when compared with isoproterenol.

#### 3.3. Effect of luteolin on circulatory antioxidants in the myocardial infarction rats

Table 2 illustrates the effect of luteolin on circulatory antioxidants (SOD, CAT, GSH and GPx) in the control and experimental rats. The circulating levels of (SOD, CAT, GSH and GPx) were significantly ($p < 0.05$) decreased in the acute and chronic periods of ISO-induced rats (groups 3 and 4) as compared to the control rats (group 1). However, pre-treatment with luteolin (group 2) increased the circulatory antioxidants near to normal levels. Luteolin administration to acute and chronic periods of ISO-induced rats (groups 5 and 6) was significantly ($p < 0.05$) increased the levels of antioxidants (SOD, CAT, GSH and GPx) as compared to the unsupplemented acute and chronic periods ISO-induced rats (groups 3 and 4).

#### 3.4. Effect of luteolin on tissue antioxidants in the myocardial infarction rats

Table 3 depicts the effect of luteolin on heart tissue antioxidants (SOD, CAT, GSH and GPx) of control and experimental rats. The tissue antioxidant levels (SOD, CAT, GSH and GPx) were significantly ($p < 0.05$) decreased in acute and chronic periods of ISO-induced rats (groups 3 and 4) as compared to the control rats (group 1). However, pre-treatment with luteolin (group 2) increased the circulating antioxidants near to normal levels. Luteolin administration to acute and chronic periods of ISO-induced rats (groups 5 and 6) were significantly ($p < 0.05$) increased the levels of tissue antioxidants (SOD, CAT, GSH and GPx) as compared to the unsupplemented acute and chronic periods ISO-induced rats (groups 3 and 4).

#### 4. Discussion

Epidemiological studies indicate that IHD, especially MI, will contribute to the major disease burden worldwide in the year 2020 and in fact, MI is considered the most leading cause of death worldwide even with the huge improvement in clinical care and the wide use of various health care innovations. Myocardial infarction is characterized by cardiac dysfunction, increased lipid peroxidation, altered activities of cardiac injury markers and depletion of endogenous antioxidants.

Lipid peroxidation, is a type of free radical-mediated propagation of oxidative insult to polyunsaturated fatty acid (PUFA) involving several types of free radicals and termination occurs through enzymatic means or scavenging of free radical by antioxidants. It is an important pathogenic event in myocardial necrosis and accumulation of lipid hydroperoxides reflects the damage of the cardiac constituents. In the present study we observed the increased levels of lipid peroxidation (TBARS) in serum, erythrocyte and heart tissue of acute and chronic periods after ISO-induced rats (Groups 3 and 4) when compared to control rats, which may lead to the lipid peroxidation products injure blood vessels, causing increased adherence and aggregation of platelets to the injured site, and it could attribute to...
irreversible myocardial membrane damage, which was observed during myocardial infarction.  

The decreased level of TBARS in luteolin pretreated rats (group 4) when compared to control (Group 1) and isoproterenol induced rats (Groups 3 and 4). This might be due to enhanced activities of antioxidant enzymes. Luteolin has shown to efficiently scavenge hydroxyl and peroxyl radicals and contributes to the defense against lipid peroxidation.  

The free radical scavenging and anti-lipidperoxidative activity of luteolin revealed in heart supports the earlier reports, demonstrated the protective effect of luteolin against oxidative damages of the organs including heart.  

The formation of free radicals and accumulation of lipid peroxides is one of the possible biochemical mechanisms for the myocardial damage caused by this catecholamine. Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. Hence, these antioxidants are expected to be consumed by enhanced radical reactions. Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase are the first line cellular defense against oxidative injury, decomposing O₂ and H₂O₂ before their interaction to form the more reactive hydroxyl radical (OH•). The equilibrium between these enzymes is essential for the effective removal of oxygen stress in intracellular organelles. However, in pathological conditions like MI, the generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system.

SOD and CAT are anti-peroxidative enzymes that protect the cellular constituents against oxidative damage. SOD and CAT are two important enzymatic antioxidants that act against toxic oxygen free radicals such as superoxide (O₂⁻) and hydroxyl (OH•) ions in the biological system. They are involved in the direct elimination of reactive oxygen metabolites, which is probably one of the most effective defenses of the living body against diseases. In the present study there is decreased levels of SOD and CAT in the heart tissue and serum as observed at acute and chronic periods ISO-induced rats (Groups 3 and 4). This may be due to increased production of reactive oxygen radicals which are capable of reducing the activities of these enzymes and it’s loss of function and integrity of the myocardial membrane, which is usually observed during myocardial necrosis. This can leads to the accumulation of superoxide anion which further damages the myocardium. It may also due to an excessive formation of superoxide anions. A decrease in SOD activity can result in the decreased removal of superoxide anions which can be harmful to the myocardium. The activities of H₂O₂ scavenging enzymes CAT and GPx were also decreased significantly. The decline in these enzyme levels may be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in an inactivation of the H₂O₂ scavenging enzymes.

Luteolin pre-treatment (Group 2) prevented decline of the myocardial SOD and CAT activities when compared to control group I and ISO-induction rats (Groups 3 and 4). Present findings are also strongly supportive of its antioxidant activity against ISO-induced oxidative stress. Luteolin was reported to scavenge superoxide radicals and hydrogen peroxide. Because of these activities, it was expected that luteolin could decrease the workload of SOD, CAT and reduce the free radical mediated inactivation of enzymes and thereby maintaining the activities of enzymatic antioxidants.

Glutathione (GSH) is important in protecting the myocardium against free radical mediated injury and thus a reduction in the cellular GSH content could impair recovery after short period of ischemia. In the present study it was observed that acute and chronic periods of ISO induction rats (groups 3 and 4) showed a significant decrease in myocardial GSH and GPx activity it may be due to the increased oxidative stress. Glutathione has the ability to manage oxidative stress with adaptional changes in enzymes regulating its metabolism. Increased concentrations of reactive oxygen species have been implicated in the development of cardiac diseases.

Luteolin pre-treatment (Group 2) prevented decline of the myocardial GPx and GSH activities in acute and chronic periods of ISO-induction rats (Groups 3 and 4). Present study findings also strongly supportive of its antioxidant activity against ISO-induced oxidative stress. Decreased activities of GPx and GSH may be due to loss of enzymes after membrane disruption by the events of lipid peroxidation. GPx, GSH are ‘SH’ dependent enzymes, and their ‘SH’ groups can react with ISO oxidation products, quinones and isoprenochrome, leading to enzyme inactivation. The inhibition of these enzymes not only reflects oxidative stress, but also exposes the cells to further oxidative damage, since GPx and GSH are essential enzymes in cellular protection against ROS. Luteolin administration changes the levels of GPx and GSH by restoring reduced glutathione level and counteracting the free radicals produced by ISO.

5. Conclusion

In our experiment, we found that luteolin protected myocardium from isoproterenol-induced myocardial functional and structural injury via favourably improved hemodynamic, biochemical, histopathological and biochemical parameters suggesting its cardioprotective action.

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References


