Isoprenaline-induced myocardial infarction in rats: protective effects of hesperidin.

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ABSTRACT

Myocardial infarction is amongst the most common causes of death worldwide. The present study aimed to investigate the cardioprotective effect of hesperidin (200 mg/kg) either individually or in combination with atorvastatin (10 mg/kg), as a reference standard, in isoprenaline-induced myocardial infarction in rats. Markers chosen to assess cardiac damage included serum activity of creatine kinase-MB (CK-MB) and serum level of cardiac troponin-I (cTn-I), oxidative stress biomarkers including cardiac contents of malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) as well as serum levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10). Furthermore, ECG monitoring and histologic examinations of cardiac tissues were done. Isoprenaline increased CK-MB activity as well the levels of cTn-I, inflammatory and oxidative stress biomarkers. In addition, it produced ST segment elevation and degenerative changes in heart tissues. The obtained data revealed that pretreatment with hesperidin alone or in combination with atorvastatin significantly decreased the elevated activity of serum CK-MB as well as serum levels of cTn-I, CRP, TNF-α and IL-10 coupled by a reduction in cardiac lipid peroxides and NO content. Moreover, both treatments resulted in marked improvement in isoprenaline-induced ECG and histopathologic changes. In conclusion, hesperidin can be regarded as a promising cardioprotective natural agent in myocardial infarction when used alone or combined with atorvastatin.

Key Words: Hesperidin, isoprenaline, myocardial infarction, C-reactive protein, cytokines, oxidative stress.

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1. INTRODUCTION

Myocardial infarction (MI), the most dreaded sequel among coronary heart diseases, is the rapid development of myocardial necrosis because of total deprivation of blood supply to an area of cardiac muscle for an appreciable period of time (Vennila and Pugalendi, 2010). Inflammation is a key process involved in mediating myocardial tissue damage after an ischemic event (Tawfik et al., 2010). Neutrophils infiltrate to the infarcted area and can promote myocardial cell damage through the release of proteolytic enzymes and/or production of reactive oxygen species (ROS). Inflammation may also increase the risk of recurrent ischemic events by destabilizing atherosclerotic plaques and making them prone to rupture (Jordan et al., 1999).

Isoprenaline, a β-adrenergic agonist, has been reported to produce MI in large doses (Padmanabhan et al., 2008). Upon auto-oxidation, isoprenaline generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids causing severe damage to myocardial membrane. Hence, it is widely used as a model for induction of MI in rats (Panda and Naik, 2009).

Hesperidin is a citrus bioflavonoid with wide biological and pharmacological properties including anti-carcinogenic, antioxidative, vascular protective and lipid-lowering activities (Morand et al., 2011; Wang et al., 2011). It is a potent anti-inflammatory agent with reported protection against in vivo focal myocardial ischemia/reperfusion injury-induced arrhythmias and apoptosis (Gandhi et al., 2009).
Atorvastatin is a cholesterol-lowering agent acting by competitive inhibition of the rate limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA reductase. The effects of statins extend beyond their lipid lowering actions; they possess anti-inflammatory and antioxidant effects and can protect the myocardium against ischemic injury (Kapur and Musunuru, 2008; Balakumar and Mahadevan, 2012).

The present study aimed to investigate the cardioprotective effects of pretreatment with hesperidin either alone or in combination with atorvastatin, as a reference standard, in isoprenaline-induced MI in rats

2. MATERIALS AND METHODS

2.1. Animals

Male Wistar albino rats weighing 150-200 g were used in the present study. They were purchased from the Egyptian Company for Production of Vaccines, Sera and Drugs (EGYVAC; Cairo, Egypt) and allowed free access to water and standard pellet chow obtained from El-Nasr Chemical Company (Cairo, Egypt). Rats were kept under constant conditions with 12/12 h light/dark cycles and housed in plastic cages in the animal house at October University for Modern Sciences and Arts (MSA University). The study was carried out according to the guidelines of the Ethics Committee, Faculty of Pharmacy, Cairo University.

2.2. Drugs and chemicals

Isoprenaline hydrochloride and hesperidin were purchased from Sigma-Aldrich (MO, USA); whereas atorvastatin was obtained kindly from Pfizer Pharmaceutical Company (Egypt). All other chemicals used were of analytical grade and purchased from Sigma-Aldrich (MO, USA) or El-Nasr Chemical Company (Cairo, Egypt).

2.3. Induction of myocardial infarction

MI was induced in rats by subcutaneous injection of 100 mg/kg isoprenaline hydrochloride dissolved in saline once daily for two successive days. The selected route and dose were chosen from published literatures (Priscilla and Prince, 2009; Kumaran and Prince, 2010).

2.4. Experimental design

Rats were randomly allocated into 5 groups (n=8). The first group received 1% tween 80 (p.o.) for 14 days and served as control group. The second group received 1% tween 80 (p.o.) for 14 days and isoprenaline (100 mg/kg; s.c.) in the last 2 days and served as isoprenaline control group. One of the remaining three groups received hesperidin (200 mg/kg; p.o.) for 14 days. Another group was treated with atorvastatin (10 mg/kg; p.o.) for 14 days. The last group received a combination of hesperidin and atorvastatin for 14 days. All of these three groups received isoprenaline (100 mg/kg; s.c.) in the last 2 days of treatment.

After 24 hours from the last injection of test agents, the animals were anesthetized with urethane (1.5 g/kg; i.p.) and subjected to ECG monitoring. Thereafter blood samples were collected via the retro-orbital plexus where sera were separated by centrifugation at 3000 rpm for 15 min and used for estimation of creatine kinase-MB (CK-MB) activity as well as cardiac troponin-I (cTn-I), C-reactive protein (CRP), interleukin-10 (IL-10) and tumor-necrosis factor-alpha (TNF-α) levels. Rats were sacrificed by decapitation then the hearts were rapidly isolated, washed with ice-cold saline and each heart was divided into two equal halves. One half was homogenized in phosphate buffer to prepare 20% (w/v) homogenate that was used for the estimation of cardiac reduced glutathione (GSH), lipid peroxides and nitrite contents. The other half was preserved in 10% formalin for histological examination.

2.5. ECG monitoring

After 24 hours from the last dose of isoprenaline hydrochloride, rats were anesthetized with urethane, placed in the supine position on a board and ECG was recorded continuously with standard artifact free lead II (right fore limb to left hind limb). Needle electrodes were inserted subcutaneously into paw pads of each rat, and connected to Biocare ECG 101 (Shenzhen Biocare Electronics Co., China). The ECG was recorded to determine duration and amplitude of the P wave, QRS complex and ST segment alterations.

2.6. Biochemical assays

The activity of CK-MB in serum was determined using Stanbio CK-MB diagnostic kit (USA) according to manufacturer procedure. Serum cTn-I level was measured by enzyme linked immunoassay (ELISA) technique using a standard kit (Glory Science Co., Ltd, USA) following manufacturer procedure.

Lipid peroxidation in cardiac tissues was estimated by determination of thiobarbituric acid reactive substances content that was evaluated as malondialdehyde (MDA) in heart homogenate using a standard kit purchased from Biodiagnostic (Egypt) following manufacturer procedure.

Cardiac GSH content was determined using a commercial kit (Biodiagnostic, Egypt). Cardiac nitrite was determined as an index of nitric oxide (NO) content in heart homogenate using a commercial kit.
Serum TNF-α and IL-10 were determined by ELISA technique using standard kits (RayBiotech, Inc., USA). Serum CRP was measured using specific immunoassay kit (Immunospec Corporation, CA, USA) following manufacturer procedure.

2.7. Histopathologic assessment of myocardial damage

Autopsy samples of hearts were taken from the different groups and fixed in 10% formalin prepared in saline. Washing was done in tap water then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathologic examination using the electric light microscope.

2.8. Statistical analysis

Data were expressed as mean ± S.E.M. Comparisons between means of different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. The level of significance was taken as \( p < 0.05 \). SPSS statistical software package version 16 (SPSS Inc., Chicago, IL) was used to carry out all statistical tests.

3. RESULTS

3.1. Effect of hesperidin and atorvastatin either individually or in combination on ECG in isoprenaline-induced myocardial infarction in rats

Subcutaneous injection of isoprenaline for two successive days induced MI represented by positive T wave, ST segment elevation and a decrease in R wave amplitude as compared to control group (Figure 1). Pretreatment with hesperidin, atorvastatin or their combination resulted in a reduction in ST segment elevation. Moreover, marked increase in R wave amplitude were noticed in rats pretreated with hesperidin either alone or combined with atorvastatin as compared to the rats injected with isoprenaline alone (Figure 1).

3.2. Effect of hesperidin and atorvastatin either individually or in combination on biochemical analysis in isoprenaline-induced myocardial infarction in rats

Isoprenaline injection resulted in significant increased activity of serum CK-MB and serum level of cTn-I by 52.5% and 349.44%, respectively as compared to control group. Pretreatment with hesperidin alone or in combination with atorvastatin decreased serum activity of CK-MB by 21.8% and 22.7% as well as serum level of cTn-I by 53.12% and 50.90%, respectively as compared to isoprenaline control group; meanwhile pretreatment with atorvastatin decreased the elevated cTn-I level by 49.51% as compared to isoprenaline control group (Figures 2 and 3).

Isoprenaline increased cardiac MDA and nitrite contents by 39.3% and 161.2%, respectively with a parallel decrease in GSH content by 42.84% as compared to control rats. Pretreatment with hesperidin alone or in combination with atorvastatin decreased MDA content by 23.3% and 20%, respectively with a parallel increase in GSH content by 34.3% and 31.2%, respectively as compared to isoprenaline control group. Regarding cardiac nitrite content, hesperidin alone or combined with atorvastatin decreased it by 37.7% and 29.1%, respectively as compared to isoprenaline control group. Atorvastatin decreased MDA by 17.6% but it increased nitrite content by 26.9% as compared to isoprenaline control group (Table 1).

Serum levels of CRP, TNF-α and IL-10 were increased significantly in rats injected with isoprenaline by 87.7%, 62.6% and 49.1%, respectively as compared to the control group, while pretreatment of rats with hesperidin, atorvastatin, or their combination showed reduction of CRP by 28%, 30.1% and 38.1%; TNF-α by 25.3%, 22% and 27.6%; and IL-10 by 22.6%, 31.4% and 36.1%, respectively as compared with isoprenaline control group (Table 2).

3.3. Effect of hesperidin and atorvastatin either individually or in combination on histopathologic changes in isoprenaline-induced myocardial infarction in rats

Hearts from rats injected with isoprenaline showed multiple focal areas of myocardial cell degeneration with edema and inflammatory cells infiltration. A marked improvement was only noticed in rats pretreated with hesperidin. On the other hand, pretreatment with atorvastatin or its combination with hesperidin resulted in mild improvement of heart tissue as compared to isoprenaline control group (Figure 4).
Table (1): Effect of hesperidin and atorvastatin either individually or in combination on cardiac contents of malondialdehyde (MDA), reduced glutathione (GSH) and nitrite in isoprenaline-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g wet tissue)</th>
<th>GSH (mg/g wet tissue)</th>
<th>Nitrite (µmol/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline; s.c.)</td>
<td>116.08 ± 3.20</td>
<td>13.33 ± 0.73</td>
<td>7.83 ± 0.28</td>
</tr>
<tr>
<td>Isoprenaline (100 mg/kg; s.c.)</td>
<td>161.71* ± 6.44</td>
<td>7.62* ± 0.61</td>
<td>20.45* ± 0.38</td>
</tr>
<tr>
<td>Hesperidin (200 mg/kg; p.o.)</td>
<td>123.96@ ± 5.91</td>
<td>10.23*@ ± 0.40</td>
<td>12.75*@# ± 0.38</td>
</tr>
<tr>
<td>Atorvastatin (10 mg/kg; p.o.)</td>
<td>133.25@ ± 6.65</td>
<td>9.47*@ ± 0.30</td>
<td>25.95*@ ± 0.60</td>
</tr>
<tr>
<td>Atorvastatin + Hesperidin</td>
<td>129.37@ ± 6.43</td>
<td>10.00*@ ± 0.44</td>
<td>14.50*@# ± 0.36</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM (n = 8)
*Significantly different from control group at p < 0.05
@Significantly different from isoprenaline control group at p < 0.05
#Significantly different from atorvastatin treated group at p < 0.05

Table (2): Effect of hesperidin and atorvastatin either individually or in combination on serum levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) in isoprenaline-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CRP (µg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline; s.c.)</td>
<td>1.54 ± 0.04</td>
<td>82.55 ± 1.69</td>
<td>85.97 ± 3.38</td>
</tr>
<tr>
<td>Isoprenaline (100 mg/kg; s.c.)</td>
<td>2.89* ± 0.20</td>
<td>134.22* ± 6.14</td>
<td>128.15* ± 10.20</td>
</tr>
<tr>
<td>Hesperidin (200 mg/kg; p.o.)</td>
<td>2.08*@ ± 0.12</td>
<td>100.24*@ ± 3.43</td>
<td>99.13*@ ± 6.69</td>
</tr>
<tr>
<td>Atorvastatin (10 mg/kg; p.o.)</td>
<td>2.02*@ ± 0.09</td>
<td>104.73*@ ± 3.27</td>
<td>87.94*@ ± 7.09</td>
</tr>
<tr>
<td>Atorvastatin + Hesperidin</td>
<td>1.79*@ ± 0.10</td>
<td>97.17*@ ± 2.56</td>
<td>81.92*@ ± 2.45</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM (n = 8)
*Significantly different from control group at p < 0.05
@Significantly different from isoprenaline control group at p < 0.05
Fig. 1: Lead II ECG trace pattern of: (A) Control rat showing regular ECG pattern with defined P, QRS and T waves; (B) Isoprenaline control rat showing positive T wave, ST segment elevation and decreased R wave amplitude; (C) Hesperidin-treated rat showing a decrease in ST segment elevation and an increase in R wave amplitude; (D) Atorvastatin-treated rat showing a decrease in ST segment elevation and an increase in R wave amplitude and (E) Atorvastatin + hesperidin-treated rat showing a marked decrease in ST segment elevation and an increase in R wave amplitude.

Fig. 2: Effect of hesperidin (200 mg/kg) and atorvastatin (10 mg/kg) either individually or in combination on serum activity of creatine kinase-MB (CK-MB).
Each bar represents mean ± SEM (n=8).
*Significantly different from normal group at p < 0.05
@Significantly different from isoprenaline control group at p < 0.05
#Significantly different from atorvastatin-treated group at p < 0.05
Fig. 3: Effect of hesperidin (200 mg/kg) and atorvastatin (10 mg/kg) either individually or in combination on serum level of cardiac troponin-I (cTn-I).
Each bar represents mean ± SEM (n=8).
*Significantly different from normal group at p < 0.05
@Significantly different from isoprenaline control group at p < 0.05

Fig. 4: Effect of hesperidin (200 mg/kg) and atorvastatin (10 mg/kg) either individually or in combination on isoprenaline-induced histopathologic changes in heart tissue. (A) Section in cardiac tissue of a control rat showing normal architecture of heart tissue, being composed of muscle cells, cardiomyocytes (my), with one centrally placed nucleus; (B) Section in cardiac tissue of an isoprenaline control rat showing multiple focal areas of myocardial cell degeneration (d) with edema (e) and inflammatory cells infiltration; (C) Section in cardiac tissue of a hesperidin-treated rat showing a noticeable preservation from the deleterious effects of isoprenaline; (D) Section in cardiac tissue of an atorvastatin-treated rat showing edema and inflammatory cells infiltration in focal manner at the myocardium; (E) Section in cardiac tissue of a rat treated with hesperidin + atorvastatin showing a decrease in focal areas of edema and inflammatory cells infiltration as compared to isoprenaline control group.
4. DISCUSSION

Isoprenaline, a potent β-adrenergic agonist, increases the myocardial oxygen demand by mixture of its positive inotropic and chronotropic actions. Administration of isoprenaline in high doses to animals produces ‘infarct-like’ lesions in the heart similar to those present in MI in humans (Vennila and Pugalendi, 2010). In the current study, MI was induced in rats by subcutaneous administration of isoprenaline hydrochloride in a dose of 100 mg/kg for two successive days.

Mechanisms proposed to explain isoprenaline-induced cardiac damage are varied and include generation of highly cytotoxic free radicals, increased calcium overload, and mitochondrial injury or dysfunction (Dhalla et al., 1992; Rathore et al., 2000).

Hence in the present study, the increase in cardiac MDA content (an indicator for lipid peroxidation) and the decrease in cardiac GSH content in rats injected with isoprenaline could be expected. The current results are in line with the work of other investigators (Trivedi et al., 2006; Panda and Naik, 2009; Gayathri et al., 2011).

Isoprenaline generated free radicals are known to initiate peroxidation of membrane-bound polyunsaturated fatty acids leading to damage of the structural and functional integrity of the myocardium with consequent changes in membrane permeability. Myocyte death or altered membrane permeability causes the cytosolic contents to eventually diffuse to the systemic circulation, where they may be detected as markers of the ischemic heart disease. This accounts for elevation of serum activity of CK-MB and serum level of cTn-I in the current experiment several hours following isoprenaline administration which is in accordance with previous reports (Buja, 1998; Vennila and Pugalendi, 2010).

Inflammation is a key process involved in mediating myocardial tissue damage after an ischemic event. In the current study, isoprenaline produced a significant increase in the serum levels of CRP and the pro-inflammatory cytokine TNF-α, effects that are in accordance with the work of other investigators (Cusack et al., 2002; Tawfik et al., 2010). The infarcted region undergoes local necrosis and myocyte apoptosis resulting in complement activation, free radicals generation and an accumulation of cellular debris. Phagocytosis of the resultant cellular debris by macrophages and neutrophils triggers the inflammatory cytokines as TNF-α (Frangogiannis et al., 2002). The anti-inflammatory cytokines as IL-10 are also produced and tend to modulate the inflammatory pathways, an effect that is supported by a previous report by Pasqui et al. (2005).

An elevation in cardiac NO content (expressed as nitrite) was observed in the present study after isoprenaline injection. NO is generated from L-arginine through nitric oxide synthase (NOS) isoenzymes. Three forms of NOS isoenzymes exist: neuronal NOS (nNOS or NOS-1), macrophage or inducible NOS (iNOS or NOS-2), and constitutive or endothelial NOS (eNOS or NOS-3). Endothelium-derived NO is widely recognized as a mediator of vasodilatation with important anti-inflammatory and antithrombotic properties (Landmesser et al., 2004). In macrophages and several other cell types, inflammatory mediators induce the transcriptional activation of the iNOS gene, resulting in accumulation of iNOS and generation of increased quantities of NO. While basal production of NO via eNOS modulates cardiomyocytes contractility and blood flow distribution (Kirkeboen et al., 1999), high levels of NO production via iNOS are associated with dilated cardiomyopathy and congestive heart failure (Ishibashi et al., 2008). Isoprenaline was demonstrated to increase iNOS expression in rats (Buttros et al., 2009) which may account for the observed increase in cardiac nitrite content.

ECG monitoring of isoprenaline-treated rats showed positive T wave and ST segment elevation coupled with marked decrease in R wave amplitude that reflect the isoprenaline-induced myocardial ischemia and infarction. ECG pattern alterations by isoprenaline were previously demonstrated by previous investigators (Prince and Sathy, 2010; Tawfik et al., 2010). Histopathologic examination of isoprenaline-treated rats revealed infiltration of inflammatory cells along with myocyte degeneration. These results are in accordance with previous studies (Vennila and Pugalendi, 2010; Gayathri et al., 2011). These observations along with biochemical changes in cardiac enzymes and cytokines confirm the severity of myocardial injury.

In the present work, pretreatment with hesperidin (200 mg/kg) before isoprenaline resulted in significant cardioprotection reflected by the reduction in the elevated activities of serum cardiac enzymes like CK-MB and the serum level of cTn-I parallel to alleviation of histopathologic and ECG pattern changes induced by isoprenaline. These results are in accordance with other investigators (Abdel-Raheem and Abdel-Ghany, 2009; Gandhi et al., 2009; Kakadiya and Shah, 2010; Selvaraj and Pugalendi, 2010).

In addition, hesperidin also ameliorated the altered oxidative stress biomarkers. Hesperidin markedly increased GSH content in heart tissues and decreased the elevated MDA content in isoprenaline-treated rats. The current findings suggest that the cardioprotective effect of hesperidin may be at least in
part due to its antioxidant and free radicals scavenging activities. The present results find support in those of Abdel-Raheem and Abdel-Ghany (2009) and Gandhi et al. (2009).

The mentioned improvement achieved in this study was accompanied by a marked decrease in serum levels of TNF-α, IL-10 and CRP. Similar reduction in TNF-α level was reported (Gandhi et al., 2009; Raza et al., 2011). Hesperidin also reduced the elevated nitrite content, an effect that could be attributed to inhibition of expression of iNOS and decreased NO production (Sakata et al., 2003; Raza et al., 2011).

Pretreatment with atorvastatin (10 mg/kg), in the present study, attenuated the cardiac damage caused by isoprenaline. Atorvastatin decreased ST segment elevation with an increase in the R wave amplitude. Similar findings were previously reported (Tawfik et al., 2010). Atorvastatin also produced a significant decrease in serum cTn-I level. The current results support those of Trivedi et al. (2006) who reported the cardioprotective effect of atorvastatin.

Atorvastatin changes in ECG and cardiac enzymes were coupled by a reduction in lipid peroxidation in heart tissue through the significant decrease in heart MDA content. Similar results were reported by Mahfouz and Kummerow (2005). On the other hand, atorvastatin did not affect the depleted cardiac GSH content, an effect that is supported by Trivedi et al. (2006) who proposed the inability of atorvastatin to compensate the depleted GSH content in heart tissues.

Pretreatment with atorvastatin showed also a marked decrease in the serum levels of CRP, TNF-α and IL-10 owing to atorvastatin documented anti-inflammatory properties (Davignon, 2004; Sun et al., 2009; Balakumar and Mahadevan, 2012). These changes were coupled with a significant increase in cardiac nitrite content. Previous study demonstrated the ability of statins to upregulate eNOS activity and NO production under baseline conditions and after hypoxic conditions through different mechanisms as increasing eNOS mRNA stability (Laufs et al., 1998).

Upregulation of stromal cell-derived factor-1alpha (SDF-1α), a small cytokine belonging to the chemokine family, was shown to exert multiple protective actions including anti-apoptotic effect, amelioration of cardiac remodeling, recruitment of endothelial progenitor cells and neo-vascularization, to improve cardiac function (Hu et al., 2007). A previous study reported that SDF-1α upregulation by atorvastatin in rats with MI was, at least partially, attributable to eNOS up-regulation and NO production and might be considered as another novel mechanism by which atorvastatin confers its pleiotropic effects in cardiovascular diseases (Qiu et al., 2012).

Data of the present work revealed that pretreatment of rats with both hesperidin and atorvastatin decreased serum cardiac markers as CK-MB and cTn-I. These results were further confirmed by ECG monitoring that showed a decrease in ST segment elevation with a marked increase in the R wave amplitude. Histopathologic examination of heart sections from this group showed fewer focal inflammatory cells infiltration in the myocardium. The present results further support those previously reported by other investigators (Gandhi et al., 2009; Tawfik et al., 2010).

Moreover, combined administration of hesperidin and atorvastatin caused enhancement of cardiac GSH, decline in MDA as well as suppression of the cardiac nitrite content. The improvement produced by the latter combination can be attributed to the previously discussed antioxidant activities reported for each of the used agents. These changes were coupled with decrease in systemic inflammation reflected by the decline of CRP, TNF-α and IL-10 in serum owing to their documented anti-inflammatory properties (Gandhi et al., 2009; Sun et al., 2009).

In conclusion, the present study revealed that hesperidin pretreatment either alone or combined with atorvastatin produced noticeable cardioprotection against isoprenaline insults via their antioxidant and anti-inflammatory activities. Daily administration of hesperidin especially when coupled to the hypocholesterolemic drug atorvastatin could protect people at high risk for MI. Further experimental and clinical studies are needed to verify the cardioprotective effects of hesperidin.

5. ACKNOWLEDGMENT

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6. REFERENCES


Protective effects of hesperidin in myocardial infarction


