Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against isoproterenol-induced oxidative stress in rats

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Antioxidant activity of ethanolic extract of fruits of *Terminalia chebula* (500 mg/kg body wt, orally for 30 days) against isoproterenol-induced oxidative stress was investigated in rats. The levels of serum lipid peroxides, iron, ascorbic acid, vitamin E, plasma iron-binding capacity, and the activities of ceruloplasmin and glutathione were assayed, in addition to the activities of the antioxidant enzymes — glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase in the heart tissue. Administration of isoproterenol increased the levels of lipid peroxides and iron, with corresponding decrease in the activities of the enzymic and non-enzymic antioxidants. The pre-treatment with ethanolic extract of fruits significantly prevented the alterations induced by isoproterenol, and maintained a near normal antioxidant status. Results suggest that the cardioprotective effect of *T. chebula* fruit may partly be attributed to its antioxidant properties.

**Keywords:** *Terminalia chebula*, isoproterenol, myocardial infarction, lipid peroxidation, antioxidant.

**IPC Code:** A61P9/00, A61P9/10

The fruits of *Terminalia chebula* (Retz) commonly known as *Kadukkai* in Tamil and *Haritaki* in Sanskrit are an indigenous drug, advocated for cardiac disorders and enters into the composition of a number of herbal formulations. The extracts of *T. chebula* have demonstrated hypolipidemic and anti-inflammatory activities. The fruit extract exhibits negative inotropic and chronotropic response and exerts muscarinic effect on the heart muscle, and hence recommended for ischemic heart diseases. In our preliminary study, ethanolic extract of the fruits was found to ameliorate the effect of isoproterenol on myocardial marker enzymes, supporting its role as a promising cardioprotective agent.

The generation of reactive oxygen species (ROS), the relative deficit in myocardial endogenous antioxidant status, as well as higher oxidative stress have been implicated in the pathogenesis of myocardial infarction. The positive inotropic and chronotropic response of isoproterenol cause a severe oxidative stress in the myocardium through increased lipid peroxidation. Isoproterenol is reported to induce pathophysiological changes, resulting in infarct-like necrosis, comparable to that of human myocardial infarction. In the present study, the antioxidant potential of ethanolic extract of *T. chebula* fruits against isoproterenol-induced oxidative stress has been investigated.

**Materials and Methods**

**Materials**

Isoproterenol, epinephrine, 1,1’, 3,3’ tetramethoxy propane, NADPH, bovine serum albumin, glutathione (reduced) were purchased from Sigma Chemical (St. Louis, Mo, USA). All other chemicals used were of the analytical grade.

Fruit powder of *T. chebula* was a gift from Rumi Herbal Research Institute (Pvt.) Limited, Chennai. The powder (1 kg) was soaked in ethanol (95%) for 7 days with intermittent shaking and filtered. The filtrate was evaporated under vacuum drier and the brown mass residue obtained was stored at -4°C. The weighed amount of residue was dissolved in 0.9% saline and used (administered orally to group III and IV rats).

**Animal treatment**

Adult male albino rats of Wistar strain (weighing 120-150 g) were fed with commercial pellet and water *ad libitum* and were maintained under standard laboratory conditions with 12:12 hr light:dark cycle. The rats were divided into four groups of six animals each. Group I, normal rats; group II, rats administered with isoproterenol (20 mg/100 g body wt s.c twice at an interval of 24 hr) in saline; group III, rats pre-treated with ethanolic extract of *T. chebula* fruit...
(500 mg/kg body wt, orally for 30 days); and group IV, rats pre-treated with ethanolic extract of fruit (500 mg/kg body wt, orally for 30 days), and administered with isoproterenol (20 mg/100 g body wt s.c, twice at an interval of 24 hr) on the 29th and 30th day of treatment with ethanolic extract of fruit.

The animals were anaesthetized with pentobarbital sodium (35 mg/kg, ip) on 30th day of the treatment period. Blood was drawn from the external jugular vein of the rat and collected in two different test tubes with and without anticoagulant. Serum was separated by centrifugation at 2500 × g. The animals were subsequently sacrificed and hearts dissected, washed in ice-cold saline and homogenized in Tris-HCl buffer (0.1 M, pH 7.4). The homogenate was centrifuged and supernatant obtained was used for the assay of various enzymes.

Estimations
Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), ascorbic acid, and vitamin E were estimated by previously described methods. Ceruloplasmin, plasma iron-binding capacity (PIBC), and iron content of serum were determined as described. Lipid peroxides (LPO) in the serum and heart were estimated using thiobarbituric acid reaction. Protein was estimated by the method of Lowry et al.

Statistical analysis
The data were analyzed using one-way ANOVA, followed by Bonferroni’s multiple comparison test. The results from experimental groups were compared with respective control group and p values <0.01 were considered statistically significant.

Results and Discussion
The levels of lipid peroxides (LPO) in the serum and heart tissue of the control and experimental groups are shown in Fig. 1. The levels LPO were significantly increased (p<0.01) in the serum and heart tissue of isoproterenol-administered (group II) animals. Pre-treatment with ethanolic extract of T. chebula fruits significantly prevented the rise in their levels.

Oxidative stress in pathophysiological conditions involves increased production of reactive oxygen species (ROS) and may play an important role in heart failure. Isoproterenol has been reported to induce lipid peroxidation and alter heart antioxidant defense system through formation of free radicals. Increased lipid peroxidation may lead to cardiomyocytes damage and degeneration of cellular membranes in tissues. Thus, the observed increase in the levels of LPO in group II rats could be due to the damage induced by isoproterenol. Fruit extract of T. chebula has been reported to prevent hemolysis, scavenge superoxide anions, peroxide radicals, and thus prevents MDA formation, as well as mitochondrial lipid peroxidation in in vitro studies. T. chebula is found to be active against the effect of free radicals on biological membranes and prevents platelet aggregation.

The level of iron was found to be significantly elevated (p<0.01), with a concomitant decrease in the levels of ascorbic acid, vitamin E, PIBC, and the activities of GSH and ceruloplasmin in the serum of isoproterenol-intoxicated (group II) animals (Table 1). Group IV animals showed near normal levels of LPO, iron, PIBC, ascorbic acid, vitamin E and ceruloplasmin activity (p<0.01), when compared with group II animals. Increased levels of free iron and low PIBC have been reported to be associated with free radical formation. Besides promoting the lipid peroxidation, free iron increases the risk of myocardial infarction through elevation of blood haematocrit and haemoglobin concentration. This increases viscosity of the blood and has a thrombogenic effect. Significant decrease in the levels of ascorbic acid, vitamin E, and ceruloplasmin in isoproterenol-intoxicated rats correlates well with the previous findings. Ascorbic acid and ceruloplasmin have been reported to inhibit iron induced lipid peroxidation and thus reduce levels of...
free circulating iron. Thus, the free radical scavenging property of T. chebula extract could have maintained the near normal level of non-enzymic antioxidants in group IV animals.

The effect of fruit extract on the antioxidant enzymes in the heart tissue is shown in Table 1. In group II animals, the activities of GSH, GPx, GR, SOD and CAT were significantly decreased (p<0.01), compared to group I. The decreased levels of GSH in group II animals may probably be due to the increased utilization or lower expression of GSSG to GSH. The unavailability of GSH reduces the activities of GR, GPx and its isofrom GST. Group IV animals pretreated with ethanolic extract of T. chebula fruits retained the activity of the antioxidant enzymes at near normal levels. No significant change was observed in group III animals, when compared with normal rats. The two key enzymes SOD and CAT decrease during the peak period of infarction due to peroxidative insult. T. chebula has been reported to restore the activity of SOD from radiation-induced damage.

Earlier studies showed that the protective effect of T. chebula fruit extracts against oxidative and peroxidative tissue damage might be attributed to its antioxidant potential. The fruit extract contains several phenolic constituents, such as quercitin, gallic acid, catechins, catechol and epigallocatechin, which are potent antioxidants, and are believed to prevent many degenerative diseases, including cardiovascular diseases. The ethanolic extract of T. chebula fruits is reported to contain higher phenolic content and has the ability to strongly inhibit lipid peroxidation in vitro. The present study shows that the significant antioxidant activity of ethanolic extract of T. chebula fruit could have scavenged the superoxide and hydroxyl radicals generated after myocardial ischemia and thus protects the myocardium from injury.

### Acknowledgement

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### References


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<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Isoproterenol-administered)</th>
<th>Group III (Pretreated with T. chebula extract)</th>
<th>Group IV (T. chebula extract + isoproterenol)</th>
<th>ANOVA F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
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<tr>
<td>GSH (mg/dl)</td>
<td>68.33 ± 6.97</td>
<td>40.00 ± 5.54a</td>
<td>67.00 ± 6.63ab</td>
<td>60.00 ± 6.63b</td>
<td>20.50</td>
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<tr>
<td>Ceruloplasmin (mg/dl)</td>
<td>0.940 ± 0.07</td>
<td>0.60 ± 0.04a</td>
<td>0.92 ± 0.07a</td>
<td>0.87 ± 0.06b</td>
<td>34.83</td>
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<tr>
<td>Vitamin E (mg/dl)</td>
<td>12.0 ± 0.27</td>
<td>8.33 ± 0.47a</td>
<td>12.94 ± 1.31*</td>
<td>10.86 ± 0.81b</td>
<td>35.29</td>
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<tr>
<td>Ascorbic acid (mg/dl)</td>
<td>2.28 ± 0.19</td>
<td>1.70 ± 0.16a</td>
<td>2.37 ± 0.23*</td>
<td>2.19 ± 0.16b</td>
<td>14.67</td>
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<td>Iron (µg/dl)</td>
<td>40.98 ± 3.59</td>
<td>60.96 ± 5.96a</td>
<td>43.69 ± 5.76*</td>
<td>44.02 ± 5.03b</td>
<td>18.68</td>
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<tr>
<td>PIBC (µg/dl)</td>
<td>43.38 ± 3.58</td>
<td>34.42 ± 2.16a</td>
<td>42.65 ± 3.91*</td>
<td>41.29 ± 4.91b</td>
<td>7.99</td>
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<td>Heart tissue</td>
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<tr>
<td>GSH (nmol/g of wet tissue)</td>
<td>5.12 ± 0.44</td>
<td>2.80 ± 0.29a</td>
<td>5.30 ± 0.60a</td>
<td>4.60 ± 0.50b</td>
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<td>GPx (µmol of GSH oxidized/min/mg protein)</td>
<td>4.32 ± 0.28</td>
<td>1.89 ± 1.8</td>
<td>4.61 ± 4.6a</td>
<td>4.14 ± 0.41b</td>
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<td>GR (µg of GSSG utilized/min/mg protein)</td>
<td>5.48 ± 0.40</td>
<td>3.78 ± 0.3a</td>
<td>5.50 ± 0.46a</td>
<td>5.01 ± 0.34b</td>
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<td>GST (nM of CDNB conjugated/min/mg protein)</td>
<td>948.33 ± 24.71</td>
<td>727.69 ± 21.33a</td>
<td>942.21 ± 18.56b</td>
<td>921.97 ± 23.92b</td>
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<td>SOD (units/mg protein/min)</td>
<td>3.99 ± 3.11</td>
<td>2.07 ± 0.15a</td>
<td>3.50 ± 0.39a</td>
<td>3.05 ± 0.31b</td>
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<tr>
<td>CAT (µmol of H₂O₂ liberated/mg protein)</td>
<td>4.25 ± 0.27</td>
<td>2.16 ± 0.17a</td>
<td>4.29 ± 0.26a</td>
<td>3.99 ± 0.25b</td>
<td>103.10</td>
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a, b statistically significant from group I and group II respectively at p<0.01 (Bonferroni’s multiple comparison test); b’ statistically significant from group II at p<0.05; *, not significant when compared to group I.
NOTES

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