Cardioprotective Activity of Alcoholic Extract of *Tinospora cordifolia* in Ischemia-Reperfusion Induced Myocardial Infarction in Rats

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Received February 11, 2005; accepted September 13, 2005

It has been suggested that the beneficial effects of reperfusing the myocardium might be in part reversed by the occurrence of reperfusion injury. Oxidative stress was suggested to be implicating in the pathogenesis of ischemia-reperfusion (I/R) injury. Many antioxidative plants were shown to be cardioprotective in experimental models of myocardial ischemia-reperfusion (I/R) injury. The present study was designed to investigate the effects of pretreatment with alcoholic extract of *Tinospora cordifolia* in an in vivo rat model. The model adopted was that of surgically-induced myocardial ischemia, performed by means of left anterior descending coronary artery occlusion (LAD) for 30 min followed by reperfusion for another 4 h. Infarct size was measured by using the staining agent TTC (2,3,5-triphenyl tetrazolium chloride). Lipid peroxide levels in serum and in heart tissue were estimated spectrophotometrically by the methods developed by Yagi and Ohkawa et al. respectively. A lead II electrocardiogram was monitored at various intervals throughout the experiment. A dose dependent reduction in infarct size and in lipid peroxide levels of serum and heart tissue were observed with the prior treatment of *T. cordifolia* with various doses for 7 days compared to control animals. Hence, the present study suggests the cardioprotective activity of *T. cordifolia* in limiting ischemia-reperfusion induced myocardial infarction.

Key words cardioprotection; *Tinospora cordifolia*; ischemia-reperfusion; myocardial injury

Vigorous global research is underway in an effort to develop pharmacological means to control morbidity and mortality arising from ischemic heart disease. It has been suggested that the beneficial effects of reperfusing the myocardium might be in part reversed by the occurrence of reperfusion injury. Oxidative stress was suggested to be implicating in the pathogenesis of ischemia-reperfusion (I/R) injury. During I/R injury, a number of events that predispose the heart to formation of reactive oxygen species (ROS) may occur. After reperfusion, these events can set off a cascade of other biochemical and molecular sequence such as the xanthine dehydrogenase/xanthine oxidase (XD/XO) conversion, leading to production of ROS. Oxidative reperfusion injury was suggested to be a central mechanism of the cellular damage affecting all organs and tissues after ischemia; however the mechanisms, which trigger and modulate this damage have been partially characterized. Since reperfusion injury is associated with an imbalance of oxidative stress and antioxidant defense system then, theoretically, it would be possible to limit oxidative damage and ameliorate disease progression by supplementing antioxidants. Indeed, many antioxidative plants and their isolated active components have been reported to be cardioprotective in isoproterenol-induced myocardial infarction. Our previous studies also suggested the cardioprotective activity of *Hydrocotyle asiatica* against ischemia-reperfusion injury.

*Tinospora cordifolia* (*T. cordifolia*) belong to menispermaeae popularly known as “Giloya” which is a Hindu mythological term that refers to the heavenly elixir that have saved celestial beings from oldage and kept them eternally young. In the Ayurvedic system of medicine as a tonic, vitalizer and as a remedy for diabetes and metabolic disorders. Scientific reports describing immuno-modulatory, anti-diabetic, anti-inflammatory, hepatoprotective, anti-leprotic and anti-allergic activities are available. Antioxidant activity of *T. cordifolia* and inhibition of lipid peroxidation have been reported. The hypolipidemic action of *T. cordifolia* greatly enhances its heart disposition. The potential therapeutic value of *T. cordifolia* in myocardial I/R injury has yet to be investigated. Therefore, the present study, aims at investigating the potential protective effects of *T. cordifolia* against ischemia-reperfusion induced myocardial infarction in rats and the results were compared with ACE inhibitor ramipril as positive control, which proved to be the most successful class of drugs in reducing post myocardial infarct size.

MATERIALS AND METHODS

**Plant Extract** Freshly collected *T. cordifolia* whole plant was dried under shade and the dried material was milled to obtain a coarse powder. The alcoholic extract of the powder was prepared by the process of continuous extraction (Soxhlation), such that 1 g of alcoholic extract equivalent to 4.57 g of crude drug was obtained.

**Animals** Laboratory bred Sprague Dawley rats of either sex weighing 200—350 g were selected. The rats were maintained under standard laboratory conditions at 25±2°C, relative humidity 50±15% and normal photo period (12 h dark/12 h light) were used for the experiment. Commercial pellet diet (Ratan Brothers, India) and water were provided *ad libitum*. Rats were randomly divided into four groups, each consisting of five animals.

**Substances** 2,3,5-Triphenyl tetrazolium chloride (TTC) was purchased from BDH chemicals Ltd. (England) and 1,1,3,3-tetraethoxypropane was purchased from Sigma Chemical company (St. Louis, U.S.A.). Thiopentone sodium is generously supplied by Abbott Lab (I) Ltd. (Ankleshwar, India). All other chemicals used were of analytical grade.

**Experimental Protocol** Rats were randomly divided...
into six groups, each consisting of five animals. Alcoholic extract of T. cordifolia whole plant, suspended in 1% sodium CMC vehicle was fed by oral gavage everyday at a fixed time (10.00 a.m.) for 7 d in three different doses. Group-1 (Sham control animals) and Group-2 (control animals) treated with vehicle orally for 7 d. Group-3, 4 and 5 were treated with alcoholic extracts of T. cordifolia at doses 250 mg kg⁻¹, 500 mg kg⁻¹ and 1000 mg kg⁻¹ respectively for 7 d. Group-6, treated with ramipril at a dose of 2 mg kg⁻¹ for 7 d and served as positive control. On day 8th, 2 h after the above treatments, the rats were subjected to the following evaluation tests.

**In Vivo Studies of Myocardial Ischemia Reperfusion Surgical Preparation** Rats were anaesthetised with thiopentone sodium (30 mg kg⁻¹, i.p.), tracheotomized and ventilated with room air by a Techno positive pressure respirator (Animal respirator, Crompton Parkinson Ltd., England). A left thoracotomy and pericardiotomy were performed and left anterior descending coronary artery (LAD) was dissected free above the first diagonal branch and the artery and was ligated below the origin of left circumflex artery with the help of a silk thread (4-0). The artery was occluded for 30 min by a knot. The silk thread was removed after 30 min. with the help of two knot releasers to allow reperfusion of the heart for succeeding 4 h. The sham control animals were subjected to the entire surgical procedure and thread was passed beneath the coronary artery but the LAD coronary artery was not ligated. A lead II electrocardiogram was monitored throughout the study by using Cardiart 408 (BPL) with sensitivity 20 mm mV⁻¹ at a paper speed 50 mm s⁻¹. Heart rates were expressed as beats min⁻¹.

**Quantification of Infarct Size** In all the groups after sacrificing the animal by injecting 2.56 M potassium chloride directly into the left ventricle, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and was weighed. It was sliced parallel to the atrioventricular groove to 2–3 mm thick sections and the slices were incubated in 1% TTC (2,3,5-triphenyl tetrazolium chloride) solution prepared in pH 7.4 phosphate buffer for 30 min at 37 °C.22 In viable myocardium TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue dark red.23 The infarcted myocardium that does not take TTC stain where the dehydrogenase enzymes are drained off, remains pale in color.24 The pale necrotic myocardial tissue was separated from the stained portions and weighed on an electronic balance (Dhona 200D). The unstained portion of the left ventricle is used for estimating lipid peroxides in the heart tissue.

**Biochemical Estimations** In all the groups before sacrificing the animal at the end of 4 h of reperfusion, 2 ml of blood sample was collected from the left ventricle for the estimation of MDA (malondialdehyde) levels in blood serum. Serum MDA levels were estimated by the method developed by Yagi.25 Tetraethoxypropane (in amounts of 0.5, 1, 2, 4, 6, 8 and 10 nmol) served as an external standard. MDA levels in serum were expressed as nmol ml⁻¹. Lipid peroxide levels in heart tissue were estimated by the method developed by Ohkawa et al.26 MDA levels in heart tissue were expressed as nmol g⁻¹ tissue.

**Statistical Analysis** The results are expressed as mean±S.D. Differences in infarct size, serum and tissue MDA levels were determined by factorial one-way analysis of variance. Individual groups were compared using Dunnet’s ‘t’ test. Differences with p<0.05 were considered statistically significant.

**RESULTS**

Infarct size was found to be 50.9±4.2 in control animals (Group-2) and statistically significant compared to sham control animals (Group-1). The infarct size was decreased to 34.5±1.2, 27.3±1.5 and 20.8±0.5 with the treatment of alcoholic extract of T. cordifolia at doses 250 mg kg⁻¹, 500 mg kg⁻¹ and 1000 mg kg⁻¹ in test groups respectively and 17.6±0.8 in ramipril (2 mg kg⁻¹) treated groups and the difference was also significant (p<0.05) compared to control (Table 1). MDA levels in serum and heart tissue in control animals were found to be 27.6±3.2 nmol ml⁻¹ (Fig. 1) and 109.2±6.2 nmol g⁻¹ tissue (Fig. 2) respectively and statistically significant compared to sham control group. In Group-3, 4 and 5 the MDA levels in serum were found to be 21.3±1.4, 10.7±1.3, 5.4±1.7 nmol ml⁻¹ and in heart tissue the MDA levels were found to be 64.1±2.9, 53.1±2.2 and 44.7±1.3 nmol g⁻¹ tissue respectively. In Group-6, ramipril significantly decreased the MDA levels in serum and heart tissue and were found to be 4.3±0.4 nmol ml⁻¹ and 30.8±1.9 nmol g⁻¹ tissue respectively. Data for heart rate recorded at various intervals of time during the experiment for all the groups was given in Table 2.

**DISCUSSION**

Since the reperfusion injury is initiated by the treatment of myocardial infarction, it is of importance to limit the extent
membrane lipids and damage their structure and functions.\(^{38}\) Malondialdehyde is one of many products of lipid peroxidation.\(^{39}\) In the present investigation we observed a significant elevation in MDA levels in serum and heart tissue and significant increase in infarct size in the control group as compared to sham control group. The results clearly depicting the injured state of myocardium following ischemia-reperfusion injury. The treatment with alcoholic extract of \textit{T. cordifolia} for 7 d orally ameliorated the elevated malondialdehyde level in serum and heart tissue and significantly reduced the infarct size in a dose dependent manner compared to control animals. In control group, a continuous decrease in heart rate was observed during 30 min coronary artery ligation and throughout the reperfusion period compared to sham control group (Table 2). The groups treated with \textit{T. cordifolia} at doses of 500 and 1000 mg kg\(^{-1}\) produced slight decrease in heart rate during 30 min coronary artery ligation and thereafter gradually increased throughout the reperfusion period and restored to normal value at the end of the 4 h. The protection produced with \textit{T. cordifolia} at doses of 500 and 1000 mg kg\(^{-1}\) depicting its protective activity against ischemia-reperfusion induced injury. The standard drug ramipril significantly decreased the infarct size, MDA levels in serum and heart tissue and protected from fall in heart rate during ischemia-reperfusion period compared to control group. None of the animals showed any signs of intolerance during the course of treatment even at a dose of 1000 mg kg\(^{-1}\) for 7 d and there was no mortality. The present findings suggests that the alcoholic extract of \textit{T. cordifolia} possess a dose dependent cardioprotection against ischemia-reperfusion induced myocardial injury and the cardioprotection may be due to its free radical scavenging activity or indirectly by enhancing the endogenous antioxidant levels or by protecting Mg\(^{2+}\) dependent Ca\(^{2+}\) ATPase enzyme or by antagonizing free radical mediated inhibition of sarcolemmal Na\(^+\)/K\(^+\) exchange inhibitors. Further studies are in progress to identify the possible mechanisms involved in cardioprotective activity of \textit{T. cordifolia} against ischemia-reperfusion injury.

**REFERENCES**


Table 2. Heart Rate (Beat min\(^{-1}\)) Recorded at Various Intervals during Myocardial Ischemia/Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Sham control(^{a})</th>
<th>Control(^{a})</th>
<th>\textit{T. cordifolia}(^{a}) (250 mg kg(^{-1}))</th>
<th>\textit{T. cordifolia}(^{a}) (500 mg kg(^{-1}))</th>
<th>\textit{T. cordifolia}(^{a}) (1000 mg kg(^{-1}))</th>
<th>Ramipril(^{a}) (2 mg kg(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>BO</td>
<td>398±13</td>
<td>385±21</td>
<td>330±42</td>
<td>342±30</td>
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<td>MOP</td>
<td>384±18</td>
<td>362±18</td>
<td>314±30</td>
<td>352±37</td>
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<td>337±17</td>
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<tr>
<td>1h AR</td>
<td>385±12</td>
<td>350±31</td>
<td>308±33</td>
<td>318±32</td>
<td>332±32</td>
<td>330±12</td>
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<tr>
<td>1h AR</td>
<td>380±24</td>
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<td>317±26</td>
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\(^{a}\)Values are expressed as mean±S.D. BO, before occlusion; MOP, middle of LAD occlusion period; IAR, immediately after reperfusion; AR, after reperfusion.

![Graph](image-url)


