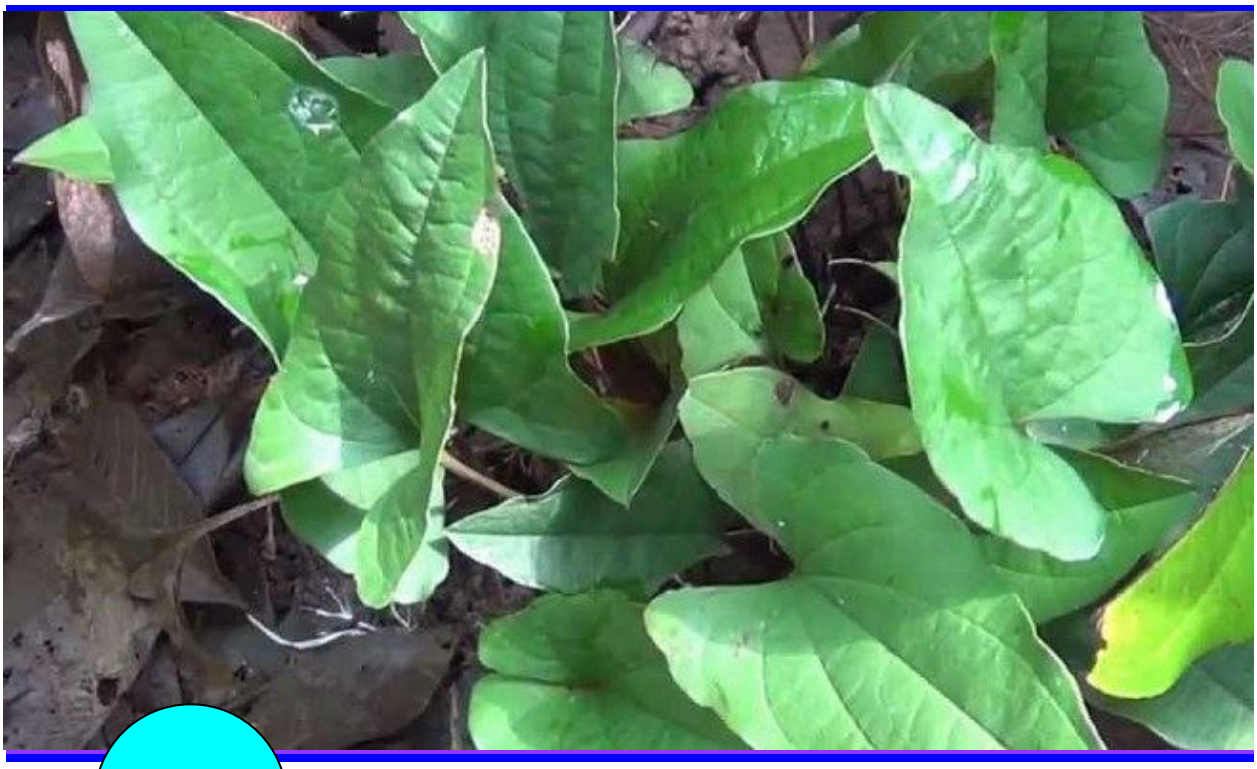

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Cardioprotective effect of *Trichopus zeylanicus* against myocardial ischemia induced by isoproterenol in rats

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Abstract

The cardioprotective effect of *Trichopus zeylanicus* leaves against isoproterenol -induced myocardial ischemia was studied. Wister strain rats were pretreated with *T. zeylanicus* leaves (500 mg/kg body weight) for 28 days and then intoxicated with isoproterenol (20 mg/100 g, i.p. for 2 consecutive days). Cardioprotection was assessed by estimating plasma and heart aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine phosphokinase. Troponin T was estimated in serum, and the levels of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) were analyzed in heart and plasma. In isoproterenol-treated group, shrinkage of cardiac markers in plasma and elevated lipid peroxidation were accompanied by decreased content of reduced glutathione in heart and plasma. The prior administration of *T. zeylanicus* significantly ($p<0.001$) prevented the isoproterenol-induced alterations and restored the cardiac markers. These findings indicate the cardioprotective activity of *T. zeylanicus* during isoproterenol-induced myocardial ischemia.

Introduction

Trichopus zeylanicus Gaerten Trichopodaceae, is a wild plant, a rare genus, small glabrous herb growing in the Agasthyar hilly forests of Kerala, India. The tribal inhabitants (Kani tribe) of this area call this plant ''Arogyappacha'' meaning the greener of health, and use this plant as a health tonic and rejuvenator. This information is based on ethno-medico-botanical investigations (Pushpangadan et al., 1988; Evans et al., 2002). Earlier studies on *T. zeylanicus* in experimental animals have shown several pharmacological activities such as hepatoproctection, anti-ulcer activity, anti-fatigue, anti-inflammatory activity, antioxidant activity, aphrodisiac activity, anti-stress, enhancement of swimming performance and immunomodulation (Sharma et al., 1989; Subramoniam et al., 1997; Singh et al., 2001; Singh et al., 2005). In the present study, an attempt has been made to assess the cardio protective effects of *T. zeylanicus* leaves on cardiac function in isoproterenol-induced myocardial infarction in rats.

Materials and Methods

Chemicals

Isoproterenol hydrochloride, thiobarbituric acid, 2,4-dinitrophenyl hydrazine, and glutathione were purchased from Sigma Chemical, Mumbai. All other reagents and chemicals used in this study were of analytical grade with high purity.

Animals

Wister strain male albino rats, weighing 100-120 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28 ± 2°C, humidity

60-70%, 12 hours light/dark cycle). The animals were allowed a standard feed and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. The study protocol was carried out as per the rules and regulation of the institutional animal's ethics committee.

Plant material and preparation of extract

T. zeylanicus leaves were collected in the month of April and May from the Agasthyar hilly forests of Kerala, India. The collected leaves were identified and authenticated by a botanist Prof. V. Mahesh, Department of Microbiology, Marudhupandiyar College, Thanjavur, Tamil Nadu, India. The collected leaves were open-air-dried under the shade, pulverized in to a moderately coarse powder (using pestle and mortar). Three hundred grams of the powered leaves were extracted with ethanol (70%) for 48 hours. A semi-solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract was dissolved in distilled water just before oral administration. The yield of the extract was found to be 28% (w/w).

Induction of myocardial infarction

The myocardial infarction was induced in experimental rats by intraperitoneal injection of isoproterenol hydrochloride 20 mg/100 g body weight, dissolved in physiological saline, for two consecutive days (Prabhu et al., 2005).

Experimental protocol

The rats were randomly divided onto three groups with six rats each. Group I, normal animals received with standard fed and water to allowed *ad libitum* throughout the experimental period. Group II, rats were orally fed 0.9% normal saline once daily for 28 days and in addition received isoproterenol (20 mg/100 g body weight) on the 29th and 30th day at an interval of 24 hours. Group III, rats were pretreated with extract (500 mg/kg body weight) for a period of 28 days and in addition received isoproterenol (20 mg/100 g body weight) on the 29th and 30th day at an interval of 24 hours.

On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg/kg). The blood was collected with and without EDTA as anticoagulant. Plasma and serum were separated by centrifugation. Heart was excised immediately and immersed in physiological saline. It was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate, plasma and serum were used for the estimation of various biochemical parameters.

Biochemical analysis

The activities of aspartate aminotransferase and alanine aminotransferase were estimated by the method of Reitman and Frankel (1957). Lactate dehydrogenase and creatine phosphokinase activities were determined by the method of King (1965) and Okinaka et al. (1961) respectively. The level of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) were estimated by the method of Beuge and Aust (1978) and Moron et al. (1979). Troponin T was estimated by the method of Bhaskar and Rao (2002). The protein content was estimated by the method of Lowry et al. (1951).

Statistical analysis

Values were expressed as mean ± SD and statistical significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons (Harvey and Paige, 1998). Statistical analysis carried out by MS-Windows based graph pad Instat software (GraphPad Software, USA) 3 version was used. A value of $p<0.001$ was considered statistically significant.

Results

Administration of isoproterenol caused a significant ($p<0.001$) increase in lipid peroxidation in plasma and heart tissue of Group II rats as compared with that of Group I rats (Table I). This was paralleled by significant reduction in the level of reduced glutathione in the heart tissue and plasma of Group II rats as compared with that normal control rats. In Group III rats, the prior administration of *T. zeylanicus* significantly prevented the isoproterenol-induced lipid peroxidation in plasma and heart tissue and maintained the level of reduced glutathione at near normal in Group I rats. Significant ($p<0.001$) rise observed in the activities of diagnostic marker enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK)] and troponin T in plasma (Table II) and decreased activities of ALT, AST, LDH and CPK in heart of Group II

Table I

Levels of TBARS and reduced glutathione (GSH) in plasma and heart tissue of of rats

Groups	Plasma		Heart	
	TBARS	GSH	TBARS	GSH
I	2.8 ± 0.2	13.3 ± 0.6	0.9 ± 0.1	6.5 ± 0.6
II	6.3 ± 0.3[a]	9.6 ± 0.6[a]	1.9 ± 0.1[a]	3.5 ± 0.4[a]
III	2.6 ± 0.2	13.7 ± 0.7	0.9 ± 0.1	6.8 ± 0.9

Results are mean ± SD for 6 animals. Values expressed: Plasma lipid peroxide-nmol/dL, Reduced glutathione-mg/dL; Heart lipid peroxide-nmol/mg protein, Reduced glutathione-µg/g wet tissue; [a]$p<0.001$ significantly different compared with Group I control animals

Table II

Activities of biomarkers in serum of rats

Plasma	Group I	Group II	Group III
ALT	105.0 ± 9.3	278.0 ± 33[a]	121.0 ± 10.4
AST	92.6 ± 9.6	251.0 ± 28.0[a]	98.4 ± 9.2
LDH	189.0 ± 15.8	292.0 ± 29.0[a]	198.0 ± 15.3
CPK	131.0 ± 10.2	268.0 ± 21.0[a]	145.0 ± 9.9
Serum			
Troponin T	6.0 ± 1.4	12.5 ± 2.2[a]	7.2 ± 1.6
Heart			
ALT	36.3± 2.6	16.3 ± 1.6[a]	30.5 ± 2.1
AST	54.3 ± 4.0	38.6 ± 3.1[a]	51.2 ± 3.8
LDH	248.0 ± 10.8	148.0 ± 6.4[a]	231.0 ± 10.4
CPK	188.0 ± 10.7	124.0 ± 8.0[a]	205.0 ± 12.5

Results are mean ± SD for 6 animals; Values expressed: ALT (alanine transaminase), AST (aspartate transaminase) and LDH (lactate dehydrogenase)-µmol pyruvate liberated/hour/liter in plasma and mg protein in heart; CPK-µmol creatine liberated/ hour/liter in plasma and mg protein in heart. Troponin T-mg/dL. [a]p<0.001 significantly different compared with Group I control animals

myocardial infarction-induced rats as compared to Group I control animals. The pretreatment with *T. zeylanicus* significantly reduced the release of these diagnostic marker enzymes and the level of troponin T into the systemic circulation as compared with Group II rats.

Discussion

Lipid peroxidation has been implicated in the pathogenesis of a number of diseases include atherosclerosis, cancer etc. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi et al., 2005). Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia (Handforth, 1962). In the present results showed that the level of lipid peroxides, measure in term of TBARS was significantly increased in plasma and heart of isoproterenol-treated group. *T. zeylanicus* pretreatment in the present study decreases the level of plasma and myocardial lipid peroxides by an apparent direct scavenging of superoxide and hydroxyl radicals and by inactivating the enzyme cyclo-oxygenase (Singh et al., 2005).

GSH status is a highly sensitive indicator of cell functionality and viability. GSH depletion is linked to a number of disease states including cancer, neurodegenarative and cardiovascular diseases. In the present study, the reduction noticed in the level of GSH in plasma and heart of isoproterenol-induced myocardial infarction was either due to increased degradation or decreased synthesis of glutathione. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study. Pretreatment with *T. zeylanicus* prevented the isoproterenol-induced lipid peroxidation and maintained the level of reduced glutathione near normal level in plasma and heart. This is due to anti-oxidant activity of *T. zeylanicus*.

The serum enzymes creatine kinase (CK), LDH, AST, ALT and cardiac specific proteins like troponins serve as sensitive indices to assess the severity of myocardial infarction (Nigam, 2007). In this study, significant decline was shown in the activities of cardiac markers such as ALT, AST, LDH and CK in the heart of acute isoproterenol-treated rats, which is consistent with earlier reports (Kurian et al., 2005). Decreased activities of theses enzymes were due to the leakage from the damaged heart tissues into the blood stream as a result of necrosis induced by isoproterenol in rats. Senthil et al. (2007) observed that these cardio-specific marker enzymes are released from the heart into the blood during myocardial damage due to myofibril degeneration and myocyte necrosis. Significant increase was noticed in the activities of cardiac markers (SGOT, SGPT, LDH and CK) in plasma of isoproterenol -treated rats, which is consistent with earlier reports (Kurian et al., 2005), might be due to enhanced susceptibility of myocardial cell membrane to the isoproterenol mediated peroxidative damage, resulting in increased release of these diagnostic marker enzymes into the systemic circulation.

In the present study, the prior administration of *T. zeylanicus* significantly prevented the isoproterenol-induced elevation in the levels of diagnostic marker enzymes in plasma, indicating the cytoprotective activity of *T. zeylanicus*. Thus, it is possible that likewise *T. zeylanicus* may also prolong the viability of myocardial cell membrane stabilizing action.

Troponin T is a protein found in cardiac tissue. When the myocardial damage occurs, the cytosolic troponins reach the blood stream quickly resulting in a rapid peak of serum troponin (Nigam, 2007). In this study, significant increased level of troponin T found in serum of isoproterenol-treated rats. Increased level of troponin T was due to the leakage from the damaged heart tissues into the blood stream as a result of necrosis induced by isoproterenol in rats. Pretreatment with *T. zeylanicus* to isoproterenol-treated rats restored the level of troponin T in serum indicates the protective action of *T. zeylanicus* against peroxidative damage.

The present results clearly emphasize the beneficial action of *T. zeylanicus* as a cardioprotective herb. *T. zeylanicus* leaves proved to be effective in reducing the extent of myocardial damage, associated lipid peroxidation, thus maintaining, as suggested by biochemical indices, the structure and function of the

myocardium. The potential cardioprotective activity of *T. zeylanicus* may be due to the presence of therapeutic phytochemicals and anti-oxidant action. Further studies are recommended to elucidate the mechanisms of the cardioprotective action of this plant and identification its active agent(s).

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