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Research Article

CARDIOPROTECTIVE ACTIVITY OF *GARCINIA INDICA* LINN. FRUIT EXTRACT ON ISOPRENALINE HYDROCHLORIDE INDUCED CARDIO TOXICITY IN RATS

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ABSTRACT

Objective: The present study was undertaken to investigate the cardioprotective effect of ethanolic extract of *Garcinia indica* linn fruit.

Methods: The ethanolic extract of *Garcinia indica* were studied for their cardioprotective effect on isoprenaline hydrochloride (25mg/kg. b.w.) induced cardio toxicity on wistar albino rats. The degree of cardio protection was measured by assessing biochemical parameters such as AST, ALT, LDH, CPK, CK-MB in serum and heart tissue homogenate and membrane bound ATPase (Na^+K^+ ATPase, Mg^{2+} ATPase, Ca^{2+} ATPase) in tissue homogenate.

Results: The ethanolic extract at a dose of 250mg/kg b.w., 500mg/kg b.w. produced significant ($p<0.005$) protective activities in group III and IV rats when compared to isoprenaline hydrochloride induced rats (group II).

Conclusion: Finally we concluded that *Garcinia indica* fruit extract exerts equipotent cardioprotective activity in the experimental model of isoprenaline hydrochloride induced myocardial necrosis in rats.

Keywords: *Garcinia indica*, Isoprenaline hydrochloride, Cardioprotective, Membrane bound enzyme.

INTRODUCTION

Myocardial infarction or heart attack is the leading cause of death for both men and women all over the world. It occurs when blood supply is insufficient to the myocardium, death of myocardial muscle occurs, a condition known as ischemia. Prolonged ischemia of the myocardium leads to necrosis, which is referred as myocardial infarction [1].

Isoprenaline, a synthetic catecholamine has toxic effect on the myocardium. Amongst the various mechanisms proposed to explain isoprenaline-induced cardiac damage, generation of

highly cytotoxic free radical through auto-oxidation of catecholamines has been implicated as one of the important causative factor. In the present study, isoprenaline was administered at a dose of 25 mg/kg b.w., i.p. for inducing myocardial infarction [2].

The medicines currently used to treat myocardial infarction have many side effects. Dietary factors play a key role in the development of various human diseases, including cardiovascular diseases. Common belief that, herbal formulations are safer than modern drugs has lead to increasing use of herbal preparations. World Health Organization (WHO) estimates that 80% of total world's population presently uses medicines of herbal origin for primary health care [3]. Hence, WHO has recommended the use of herbal medicines as an alternative medicine, especially in developing countries [4].

Garcinia indica Linn belonging to family Clusiaceae commonly called as 'Kokum' is found in Maharashtra and particular in Konkan, Goa and the western region of India. Fruits of *Garcinia indica* have been suggested in the Indian system of medicine for a number of diseases. These include its usefulness as an infusion, in skin rashes caused by allergies, to relieve sunstroke, remedy for dysentery, an appetizer, liver tonic, to allay thirst and as a cardio tonic. The outer rind of the fruits of *Garcinia indica* has been shown to be antioxidant activity. The fruit rind contains polyisoprenylated benzophenones (garcinol), its isomer isogarcinol, xanthochymol, Hydroxycitric acid and isoxanthochymol. Garcinol has antioxidative, chelating, free radical scavenging, antiglycation, anticancer, anti-inflammatory and antiulcer activities. Hydroxycitric acid has been patented for use as a hypocholesterolaemic agent [5]. Based on traditional uses we selected this plant for the study. The purpose of present study was to evaluate cardioprotective activity of *Garcinia indica* Linn fruit extracts in Isoprenaline intoxicated rats.

MATERIALS AND METHOD

Chemicals

Isoprenaline was procured from Sigma Chemical Co., St. Louis, MO, USA while the assay kits used for biochemical assays were products of beacon diagnostics. All other chemicals and reagents used in the study were of analytical grade.

Collection of plant material

Garcinia inidica fruits were collected in and around Goa. The fruits samples were authenticated by Dr.K.Arumugasamy, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu. Fruits were cut open and the seeds were separated from the pulp. Then the fruit rinds were allowed to dry in the shade. The fruit rinds were cut into pieces and shade dried at room temperature. The dried fruit rinds were subjected to size reduction to coarse powder by using mixer grinder. The coarsely powdered sample was kept under refrigerator under 4°C.

Preparation of extract

30 gram of *Garcinia inidica* fruit rinds powder was extracted with 250ml of ethanol in a soxhlet apparatus. The extract was dried at room temperature till semisolid mass was obtained, The sweet scented, chocolate colored semisolid residue formed after the complete dryness was dissolved in water (250mg/kg.b.w.and500mg/kg.b.w.) respectively.

Experimental animals

Male albino wistar rats (120-150 g) used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages under standard environmental conditions (12H dark /12H light cycles; temp., 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experiment use. The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethical committee.

Induction of myocardial infraction

Isoprenaline [25 mg (dissolved in physiological saline)/ kg b.w. / day] was administered intrapretonially on 29th and 30th day of experimental period for the induction of myocardial infarction.

Experimental designs

A total of 24 rats were randomly divided into four groups of 6 animals each. Group I, normal control group I (Control): animals were fed with standard pellet diet for 30 days. Group II (IP) induced group: Isoprenaline [25 mg (dissolved in physiological saline)/ kg b.w. / day] was administered intrapretonially on 29th and 30th day of experimental period. Groups III rats were pretreated with low dose of *Garcinia indica* extract (250mg / kg b.w.) orally for 30 days before the induction of myocardial infarction, IV rats animals were pretreated with high dose of *Garcinia indica* extract (500mg / kg b.w.) orally for 30 days before the induction of myocardial infarction..

Sample collections

Twelve hours after the second injection of IP, the rats were sacrificed by ether anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and an aliquot of the blood was collected and centrifuged. The serum was carefully aspirated with a Pasteur pipette into sample bottles. The heart was dissected out and immediately washed with ice cold 0.9% saline and homogenate was prepared in 0.1 N Tris HCl buffer (pH 7.4). The homogenate was centrifuged and the clear supernatant and serum collected were used for the biochemical analysis.

Biochemical assay in the serum and heart tissue

Estimation of serum enzymes: lactate dehydrogenase (LDH) by the method of King (1965) [6], creatine phosphokinase (CPK) by the method of Okinaka *et al*. (1961) [7], aspartate transaminase (AST) and alanine transaminase (ALT) by the method of Bergmeyer and Bernt (1974) [8] and creatine phosphokinase-MB (CK-MB) in serum were estimated using commercially available kit (Beacon assay kit).

Assay of Membrane bound enzymes

Tissue homogenate pallet obtained after centrifugation was resuspended in ice cold Tris buffer (10mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of Na^+K^+ ATPase was assayed by Bonting *et al*., 1970 [9], Ca^{2+} ATPase was assayed using the method of Hjerken and Pan, 1983[10] and Mg^{2+} ATPase was assayed using the method of Ohinishi *et al*., 1982 [11]. Protien was estimated according to the method of Lowry *et al*., 1951 [12].

Statistical analysis

The values were expressed as mean ± SD. Data were analyzed for the statistical significance by one way analysis of variance (ANOVA) followed by the group means were compared with Dunnet`s multiple comparison test using a statistical software SPSS version 10 and value of $P<0.05$ was considered to indicate a significant difference between the groups.

RESULTS

Biochemical parameters

Table 1 shows the activities of marker enzymes of cardiac function (LDH, AST, ALT, CK-MB and CPK) in the serum of control and experimental rats. The administration of isoprenaline resulted in significant ($p<0.005$) increase in the serum levels of heart marker enzymes in group II rats. Pretreatment with *Garcinia indica* (250 mg / kg b.w.) extract in Group III rats showed a significant ($p<0.05$) decrease in the activities of above mentioned cardiac marker enzymes when compared with Group II rats. Group IV rats also pretreated with *Garcinia indica* (500 mg / kg b.w.) extract showed the values near normal to control rats (group I). The results were observed in dose dependent manner when compared with IP treated rats and **Table 2** shows the activities of marker enzymes of cardiac function (LDH, AST and ALT) in the tissue homogenate of control and experimental rats**.** The administration of isoprenaline in rats resulted in significant ($p<0.05$) decrease in the levels of heart marker enzymes. However, pretreatment with ethanolic extract of *garcinia indica* (250 mg / kg b.w.) in Group III rats showed a significant ($p<0.05$) decrease in the activities of above mentioned cardiac marker enzymes when compared with Group II rats. Group IV rats also pretreated with *Garcinia indica* (500 mg / kg b.w.) extract which shows the values near to control rats.

Table 1: The effect of ethanolic extract of *Garcinia indica* on the activities of marker enzymes in serum of normal and experimental rats

Treatment	LDH	AST	ALT	CK-MB	CPK
Group I	92.91±7.72	76.65±6.52	47.15±3.84	118.30±9.50	84.10±4.09
Group II	242.23±21.81a*	215.02±13.08a*	163.47±10.72a*	488.15±31.12a*	316.38±23.25a*
Group III	199.23±16.05b*	121.08±10.19b*	100.17±7.12b*	297.66±24.48b*	185.59±11.14b*
Group IV	123.27±9.65c*	86.67±5.84c*	61.58±5.13c*	181.97±13.08c*	115.39±9.22c*

Values are mean ± SD of six animals

($p<0.05$), analysis of variance for multiple comparison by Dennett's test.

a: Group II vs. I; b: Group III vs. II; c: Group IV vs. II.

Units

LDH – U/L, AST – IU/L, ALT– IU/L, CK-MB – U/L, CPK – U/L

Table 2: The effect of ethanolic extract of *Garcinia indica* on the activities of marker enzymes in heart tissues of normal and experimental rats

Treatment	LDH	AST	ALT
Group I	182.36±15.84	349.18±22.38	345.39±25.80
Group II	101.72±8.99a*	124.26±9.88a*	86.08±5.13a*
Group III	118.35±10.06b*	188.44±15.99b*	203.21±13.07b*
Group IV	172.93±10.53c*	316.60±21.90c*	319.84±19.10c*

Values are mean ± SD of six animals

($p<0.05$), analysis of variance for multiple comparison by Dennett's test.

a: Group II vs. I; b: Group III vs. II; c: Group IV vs. II.

Units

LDH – U/L, AST – IU/L, ALT– IU/L

Table 3: The effect of ethanolic extract of *Garcinia indica* on membrane bound enzymes in heart tissues of normal and experimental rats.

Treatment	Na^+K^+ATPase	Mg^{2+} ATPase	Ca^{2+} ATPase
Group I	5.36±0.05	3.61±0.04	1.85±0.04
Group II	2.81±0.04a*	8.20±0.04a*	7.62±0.04a*
Group III	3.38±0.05b*	6.22±0.03b*	4.47±0.11b*
Group IV	4.18±0.06c*	4.61±0.08c*	3.32±0.03c*

Values are mean ± SD of six animals

($p<0.05$), analysis of variance for multiple comparison by Dennett's test.

a: Group II vs. I; b: Group III vs. II; c: Group IV vs. II.

Units

Na^+K^+ATPase -Micromoles of Pi liberated/min/mg protein; Mg^{2+} ATPase -Micromoles of Pi liberated/min/mg protein; Ca^{2+} ATPase- Micromoles of Pi liberated/min/mg protein**.**

Effect of *G. indica* on membrane bound enzymes

The levels of membrane bound ATPase (Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase) in the heart of the control and experimental rats are represented in **table 3**,

It was evident that, a significant ($p<0.05$) decrease in the activity of Na^+ K^+ ATPase whereas a significant ($p<0.05$) increase in the activities of Ca^{2+} ATPase and Mg^{2+} ATPase in isoprenaline administered rats when compared to control group. Pretreatment with *Garcinia indica* (both 250, 500 mg /kg b.w.) showed a significant ($p<0.05$) increase in the activity of Na^+K^+ATPase and a significant ($p<0.05$) decrease in the activities of Ca^{2+} ATPase and Mg^{2+} ATPase as compared to isoprenaline treated group. This effect is due to the membrane stabilizing properties of *Garcinia indica.*

DISCUSSION

Diseases including cardiac diseases have been linked to oxidative stress which is initiated by the reaction of free radicals with biological macromolecules such as proteins, lipids and DNA [13]. Generally antioxidants, preferably from natural sources, have been considered as effective treatments [14]. The present study has clearly demonstrated that the ethanolic extract of *garcinia inidica* has antioxidant activity which could prevent the occurrence of heart related diseases.

The serum enzymes namely LDH, AST, ALT, CPK and CK-MB serve as sensitive indices to assess the severity of myocardial infarction [15]. The increased activities of these enzymes following injection of IP as observed in this study confirmed the onset of myocardial necrosis [16]. Pretreatment with the extract of *garcinia inidica* lowered the elevated activities of the enzymes comparable to the control. This is an indication of the protective action of the extract in reversing cardiac damage. Similar observation was reported by Vishal *et al*., (2010) [17] using *Lagenaria siceraria* fruit powder in IP-induced myocardial injury in rats. The reversal of these enzyme activities by pretreatment with the extract indicates its therapeutic potential against myocardial infarction.

Membrane bound enzymes play a significant role in maintaining ion levels within the myocytes. Any alteration in the properties of these enzymes is known to affect the function of heart. Failure of the cell membrane to maintain normal trans- membrane ionic distribution through ion pumps is considered to be a major event in pathogenesis of ischemia and arrhythmia [18]. Na^+K^+ ATPase are the '_SH' group containing enzyme that is responsible for the active transport of Na^+ and K^+ across cell membrane. Reduction in activity levels of Na^+K^+ ATPase in IP treated rats might be due to enhanced lipid peroxide at ion by free radicals as reported earlier [19]. Also, reduced activity levels of Mg^{2+} ATPase and Na^+K^+ ATPase in IP treated group may be responsible for creating ionic imbalance and eventually damage the membrane proteins. Cardiac cytosolic calcium level is a key factor involved in maintaining normal activity levels of many enzymes [20]. IP induced myocardial necrosis has been reported to enhance adenylate cyclase activity, resulting in increased formation of cAMP [21]. During IP induced β-adrenergic stimulation, cAMP phosphorylates several sites on the C terminal chains of the calcium channels resulting in the channel opening up [22]. This may be the reason for enhanced activity of Ca^{2+} ATPase in IP-induced myocardial necrosis rats in the present study. Intra cellular Ca^{2+} overload is known to generate various reactive oxygen species (ROS) and Ca^{2+} surge in combination with ROS is responsible for contractile dysfunction of the ischemic myocardium [23]. In our study, *garcinia indica* treatment could effectively prevent IP induced reduction in activity levels of $Mg^{2+}$and Na^+K^+ ATPases and elevate Ca^{2+} ATPase activity. This result may be due to the membrane stabilizing properties of *garcinia indica*.

Hence, it can be summarized that pre-treatment with extract provide cardioprotection by inhibiting the formation of free radicals generated during oxidation of catecholamine thus inhibiting peroxidation of membrane lipids and preventing subsequent leakage of enzymes. Also, pre-treatment improvemed the status of enzymatic antioxidants that further contributes to its overall cardio protective property. Further studies are needed to determine the mechanism by which plant acts on the myocardium to beneficially affect the cardiovascular system.

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