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Antioxidant Effect of *Garcinia indica* Linn Fruit Extract against Isoprenaline Hydrochloride Induced Myocardial Necrosis in Rats

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ABSTRACT

The present study was designed to investigate the antioxidant effect of *Garcinia indica* Linn fruit extract on heart weight, lipid peroxidation; enzymatic and non-enzymatic antioxidants against isoprenaline hydrochloride (ISO) induced myocardial infarction (MI) in rats. Group II rats treated with Isoprinosine (25 mg/kg. b.w. i.p.) recorded significant (p<0.05) increase in heart weight and cardiac lipid peroxidation whereas there was significant decrease in enzymatic and non-enzymatic antioxidants. Pretreatment with *Garcinia indica* fruit extract (250 mg/kg b.w and 500 mg/kg b.w. per day/p.o.) for 30 consecutive days followed by ISO injections on 29th and 30th days in group III and IV rats showed that significant decrease in heart weight and cardiac lipid peroxidation where as the enzymatic antioxidant and nonenzymatic activities, were significantly increased when compared to ISO treated group II. Hence, the present study suggested that the fruit extract of *Garcinia indica* was effective as cardioprotective and antioxidant agent.

Keywords: *Garcinia indica*, isoprenaline hydrochloride, cardioprotective, antioxidant.

INTRODUCTION

Myocardial infarction (MI) is caused due to an interruption in blood supply to any part of heart, resulting in death of cardiac tissue (Myocardial necrosis; MN). Consequences of MI include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity. [1] Cardiovascular diseases (CVDs) have a high prevalence in developing and developed countries and MI accounts for majority of deaths and disabilities. [2] It has also been suggested that heart failure subsequent to myocardial infarction may be associated with antioxidant deficit as well as increased myocardial oxidative stress. [3]

Isoprenaline [L-b-(3,4-dihydroxyphenyl)-a-isopropylaminoethanol hydrochloride], a β-adrenergic agonist has been reported to cause oxidative stress in the myocardium, which results in infarct like necrosis of heart muscle similar to those observed in myocardial infarction in human beings. [4] It induces myocardial necrosis by a multiple step mechanism. The primary disturbance of isoprenaline induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which in turn would have lead to the higher lipid accumulation in the myocardium. [5] Increased lipolysis and peroxidation of endogenous lipids also play a major role in the cytotoxic action of isoprenaline. [6] A considerable body of clinical and experimental evidence now exists suggesting the involvement of free radical mediated oxidative process in the pathogenesis of isoprenaline-induced myocardial infarction. [7] Therefore, Isoprenaline-induced myocardial injury serves as a well standardized model to study the beneficial effects of many drugs and cardiac functions. [8] Recent research has shown that medicinal plants with antioxidant properties are also able to impart cardioprotection. World Health Organization (WHO) estimates that 80% of total world's population presently uses medicines of herbal origin for primary health care. Hence, WHO has recommended the use of herbal medicines as an alternative medicine, especially in developing countries. [9]

The extract *Garcinia indica*, popularly known as *Kokum* or *Mangosteen* has been valued in the Indian subcontinent, Africa and China for its sweet and sour taste. It has traditionally been used as a seasoning, a snack, or steeped in syrup for a refreshing drink. In addition, it has been recommended by the Ayurvedic system of medicine for the treatment of ailments such as heat strokes, infections and edema. The major chemical constituents of the fruit extract include citric acid, hydroxycitric acid (HCA), hydroxycitric acid lactone and oxalic acid in addition to the benzophenone derivatives garcinol and its isomer isogarcinol. [10] These, constituents have shown hypocholesterolemic, antiobesity activity and antioxidant behavior.

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In the present study, an attempt has been made to assess the preventive effects of *Garcinia indica* Linn fruit extract on myocardial antioxidant defense system in isoprenaline-induced myocardial infarction in rats.

MATERIALS AND METHODS

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 indica 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, Tamil Nadu. 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 at 4°C.

Preparation of extract

Thirty gram of *Garcinia indica* fruit rinds powder was extracted with 250 ml 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 (250 mg/kg b.w .and 500 mg/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 intraperitoneally 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 (ISO) induced group: Isoprenaline [25 mg (dissolved in physiological saline)/ kg b.w. / day] was administered intraperitoneally on 29th and 30th day of experimental period. Groups III rats were pretreated with low dose of *Garcinia indica* extract (250 mg/ g b.w.) orally for 28 days and 29th, 30th days isoprenaline was administered intraperitoneally. Group IV rats animals were pretreated with high dose of *Garcinia indica* extract (500 mg/kg b.w.) orally for 28 days before the induction of myocardial infarction.

Sample collections

Twelve hours after the second injection of ISO, the rats were sacrificed by ether anaesthetization and 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.

Cardiac endogenous antioxidant activites

Cardiac tissue pieces from control and treated groups were weighed and homogenized (10% w/v) in chilled Tris buffer (10 mM, pH7.4), centrifuged at 10,000 rpm for 20 min in high speed cooling centrifuge (01°C). Clear supernatant was used for assaying superoxide dismutase (SOD; Mishra and Fridovich, 1972) [11], catalase (CAT; Aebi, 1984) [12], Glutathione peroxidase (GPX Paglia and Valentine (1967) [13], Vitamin-C (Omaye *et al.* (1971)) [14], Reduced glutathione (GSH; Beutler *et al.*, 1963) [15] and lipid peroxidation (LPO; Buege and Aust, 1978). [16]

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.

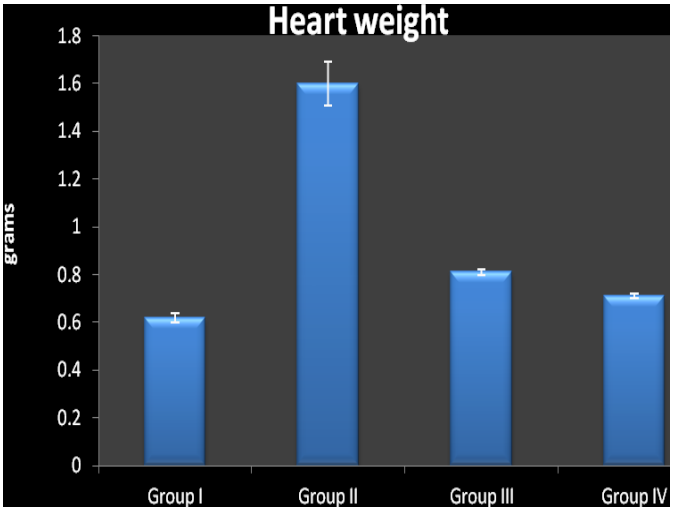

Fig. 1: Effect of ethanolic extract of *Garcinia indica* on heart weight

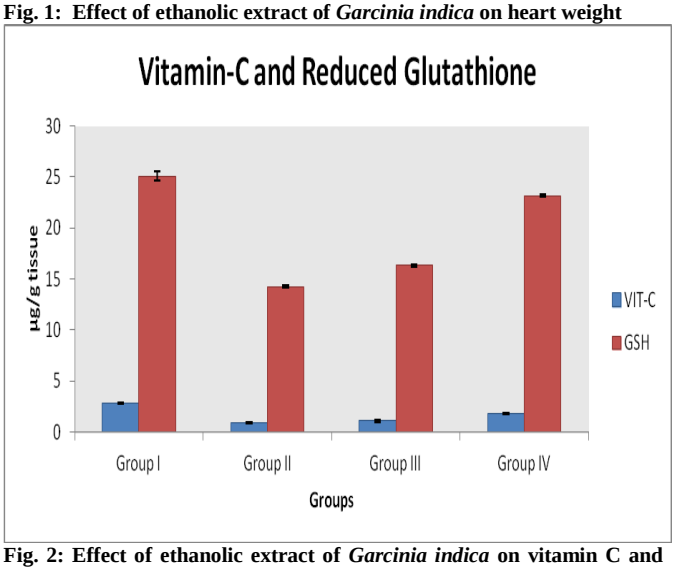

Fig. 2: Effect of ethanolic extract of *Garcinia indica* on vitamin C and reduced glutathione

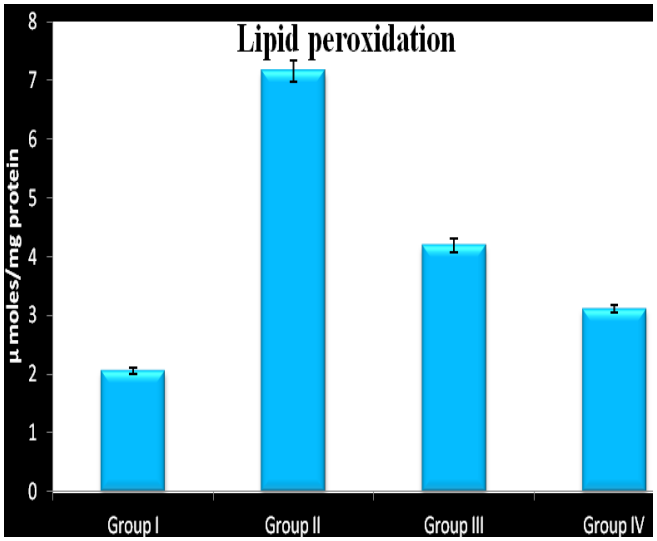

Fig. 3: Effect of ethanolic extract of *Garcinia indica* on LPO

Table 1: Effect of ethanolic extract of *Garcinia indica* on enzymatic antioxidant in heart

Groups	SOD	CAT	GPX
Group I	1.07±0.05	8.17±0.14	9.80±0.19
Group II	0.52±0.03a*	2.66±0.09a*	5.81±0.15a*
Group III	0.84±0.04b*	4.87±0.08b*	7.18±0.25b*
Group IV	1.03±0.05c*	6.89±0.07c*	8.77±0.14c*

RESULTS

Heart weight

The heart weights of control and experimental rats were recorded and the results are depicted in the Fig. 1.

From the Fig. 1, it was evident that isoprenaline hydrochloride induction significantly (p<0.05) increased the heart weight when compared to normal control rats (group I). Pretreatment with low and high dose (250 mg / kg b.w. and 500 mg / kg. b.w.) of Garcinia *indica* significantly (p<0.05) decreased the heart weight in group III and IV rats respectively, when compared to group II rats. This could be due to the reduction of the degree of the damage in the myocardium by *Garcinia indica*.

Enzymatic and non enzymatic antioxidants

Enzymatic antioxidants

Table 1 represents the activities of enzymic antioxidants such as superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX) in heart of control and experimental rats**.**

From the Table 1, it was evident that the levels of enzymatic antioxidants in heart tissue were significantly decreased (p<0.05) in ISO induced rats when compared to control rats. Pretreatment with *Garcinia indica* showed a significant (p<0.05) increase in the levels enzymatic antioxidants in both group III and group IV rats when compared to group II rats. The reduced levels of antioxidants in group II animals may be due to the accumulation of free radicals in cardiac tissue. Fruit extract pretreated groups rats exhibited normal level of the above mentioned antioxidants; this proves cardio protective effect of *Garcinia indica*.

Non enzymatic antioxidants

The levels of non enzymatic antioxidants vitamin C and reduced glutathione in heart of control experimental rats are shown in the Fig. 2.

From the figures, it was evident that the levels of non enzymatic antioxidants like vitamin C and reduced glutathione in heart tissue were significantly decreased (p<0.05) in group II rats when compared to group I rats. Pretreatment with *Garcinia indica* showed these levels to near normal range in group III and group IV rats in dose dependent manner.

Lipid peroxidation (LPO)

The levels of LPO in heart of control and experimental rats are depicted in the Fig. 3.

From the figure it is evident that, ISO administration significantly (p<0.05) increased the level of TBARS in heart tissue of group II rats when compared to control rats (group I). Pretreatment with *Garcinia indica* fruit extract showed the decreased level of TBARS in group III and group IV rats respectively, It is suggested that *Garcinia indica* scavenges the LPO products produced excessively by ISO, and protects the cardiac tissue because of its anti lipid peroxidation effect.

DISCUSSION

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. Generally antioxidants, preferably from natural sources, have been considered as effective treatments. [17] Rats treated with ISO have been reported to undergo increase in heart weight due to increase in water content and development of oedema in intramuscular spaces culminating in extensive necrotic changes and invasion of inflammatory cells. [18] In our study, identical set of changes were observed in ISO group (group II) that recorded significant increase in heart weight. However, non-significant increment in heart weight of ISO and *Garcinia indica* treated group (group III and IV) may be due to prevention of oedema.

Cardioprotective effect of *Garcinia indica* was further supported by increased myocardial enzymatic and non-enzymatic antioxidant activities. Antioxidants play a vital role in scavenging reactive oxygen species and protect the cells from oxidative damage. The generation of reactive oxygen species occurs by the leakage of electrons into oxygen from various systems. Endogenous antioxidant enzymatic defense is a very important source to neutralize the oxygen free radical-mediated tissue injury. [19] Superoxide dismutase and Catalase, the primary free radical scavenging enzymes, are the first line of cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical. Saravanan and Prakash [20] found decreased CAT and SOD activities in ISO-treated rats. The observed decrease in the activities of these enzymes might be due to their increased utilization for scavenging ROS and their inactivation by excessive ISO oxidants.

Glutathione is important in protecting the myocardium against oxygen free radical injury and thus a reduction in cellular glutathione content could impair recovery after short period of ischemia. The observed decrease in reduced glutathione levels might be due to increased utilization in protecting thiol containing proteins from lipid peroxides and from other reactive oxygen species which causes the reduction in the activities of GPx, GSH, and Vitamin-C. Oral treatment with *A. viridis* increased the levels of reduced glutathione and also increased in the in the activities of the above mentioned enzymes in heart of isoproterenol induced cardiotoxic rats was reported by Saravanan *et al*., 2011. [21] The similar observation was also found in our studies.

Lipid peroxidation is a well established mechanism of cellular injury and has been used as an indicator of oxidative stress that leads to pathogenesis of MI. The degree of lipid peroxidation was evaluated by estimating thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes. [22] Significant increases in the levels of lipid peroxidation products implicate an increased production of free radicals in ISO treated rats. Elevation of lipid peroxides in ISO treated rats could be attributed to the accumulation of lipids in the heart and damage to the myocardial membranes. [23] ISO treatment is also known to create an imbalance between enzymatic as well as non-enzymatic antioxidant defence system leading to production of free radicals that induce MN and LPO. [24] Significant decrement of LPO in ISO and *Garcinia indica* treated group further justifies the role of *Garcinia indica* as a potent antioxidant and free radical scavenger. Our results are conformity with reports of Thounaojam *et al*., 2010 who have demonstrated modulation of cellular antioxidant by treatment with natural products. [25] In conclusion, the result of the present study clearly indicates that the protective effect of *Garcinia indica* against isoprenaline-induced myocardial infarction in rats could be related to its effects on antioxidant defense system. The observations highlight that *Garcinia indica* may be one of the promising drug for improving defense mechanisms in the physiological systems against oxidative stress caused during myocardial infarction. The overall cardioprotective effect of *Garcinia indica* is probably related its ability to strengthen the myocardial membrane by its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant property.

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