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CARDIOPROTECTIVE EFFECTS OF *GARCINIA INDICA* FRUIT RIND EXTRACT AND GARCINOL IN ISOPRENALINE HYDROCHLORIDE INDUCED CARDIOTOXICITY IN RATS

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ABSTRACT

The aim of the study was to investigate the potential effect of ethanolic extract of *Garcinia indica* and its active principle (Garcinol) against isoprenaline hydrochloride (ISO) induced cardiotoxicity in rats. The cardiotoxicity was induced by myocardial infarction through subcutaneous administration of isoprenaline hydrochloride (25mg/kg b.w) for two consecutive days. The ethanolic extract of *G.indica* (250mg and 500mg/kg b.w) and garcinol (5mg/kg b.w) were given as pre-treatment for 28 days through oral gavage. A significant ($P<0.05$) increase in the activities of the serum marker enzymes (creatine phosphokinase, creatine kinase-MB, lactate dehydrogenease, aspartate transaminase and alanine transaminase), a prominent expression of LDH 1 and LDH 2 isoenzymes, increased levels of serum total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglycerides (TG), and decreased level of high density lipoprotein (HDL) were observed in ISO-induced rats. The levels of TC, TG and free fatty acids (FFA) increased whereas phospholipids (PL) significantly ($P<0.05$) decreased in the heart tissue of ISO-induced rats. Further myocardium infarct size was observed by staining the heart tissue with triphenyltetrazolium chloride (TTC). Pre-treatment with *G.indica* and garcinol significantly inhibited the effect of isoprenaline. Moreover, biochemical findings were supported by histopathological observations. The present study provide evidence for the first time, that *G.indica* and garcinol pre-treatment ameliorated myocardial injury in ISO-induced myocardial infarcted rats and exhibited significant cardioprotection.

Keywords: Garcinol, Cardiotoxicity, Isoprenaline hydrochloride, Myocardial infarction, Triphenyltetrazolium chloride.

1. INTRODUCTION

Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. It is well recognized that ischemic tissue generates oxygen-derived free radicals and other reactive species which bring about oxidative damage of membrane lipids, proteins, carbohydrates and DNA, leading to qualitative and quantitative alterations of the myocardium [1]. Cardiovascular disease (CVD) is the prime cause of death worldwide and is the world's largest killer, claiming 17.1 million lives a year. Myocardial infarction (MI) is the main causes of death from CVD. MI commonly known as heart attack, which arrests the cardiac function, and leads to morbidity and mortality in not only developing countries like India but also in developed countries like U.S. MI is invariably followed by several biochemical alterations such as hyperlipidemia, lipid peroxidation (LPO), free radical damage etc., leading to qualitative and quantitative alterations of myocardium. Oxygen free radicals are the mediators of heart tissue injury [2]. Isoprenaline hydrochloride, a synthetic catecholamine, has been reported to cause infarct like necrosis of the cardiac muscle in doses exceeding the physiological concentrations. Excessive production of free radicals resulting from oxidative metabolism of catecholamines is one of the mechanisms proposed to explain the ISO-induced injury to myocardial cells. ISO-induced myocardial damage in rats is a widely used experimental model for evaluation of cardioprotective effect of various herbal drugs, because the pathophysiological changes following ISO administration in rats are comparable to those taking place during MI in humans [3]. The medicines currently used to treat myocardial infarction have many side effects. Dietary factors play a key role in curing and preventing various human diseases, including cardiovascular diseases. Common

belief that, herbal formulations are safer than modern drugs has lead to increasing use of herbal preparations. The prophylactic and therapeutic effect of many plant extracts such as *Azadirachta india*, *Camellia sinesis*, *Allium sativum*, *Ginkgobiloba*, *Cocos nucifera*water, *Withania somnifera* in reducing isoprenalin-induced cardiovascular toxicity [4].

Garcinia indica (Family: Guttiferae; Clusiaceae), a slender ever-green tree, is endemic to the west coast of India. It has many culinary, pharmaceutical and industrial uses. The dried outer rind of the fruit of *G. indica* is popularly known as kokum and is used for imparting flavour and taste to curries. Many therapeutic effects of the fruit have been described in Ayurveda, which include its usefulness in skin ailments, such as rashes caused by allergies; in treatment of burns, scalds and chaffed skin; as a remedy for dysentery and mucous diarrhoea; as an appetizer and a good liver tonic; as a cardio tonic and for bleeding, piles, tumours and heart diseases [5]. Garcinol is a polyisoprenylated benzo-phenone derivative from the fruit rind of *Garcinia indica*. This compound is structurally similar to a well-known anti-oxidant, curcumin, which contains both phenolic hydroxyl groups and β-dike tone moiety. Several studies have demonstrated that garcinol exhibited significant anti-oxidative properties and possessed inhibitory activity on lipid peroxidation [6]. In view of this, we have discovered that *G.indica* and garcinol possess cardioprotective activity in isoprenaline induced cardiotoxic myocardial infarcted rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Isoprenaline was procured from Sigma Chemical Co., St. Louis, MO, USA and Garcinol were purchased from Enzo Life Sciences, Inc.10 Executive Blvd Farmingdale, NY 11735, USA. The purity of Garcinol is ≥ 90% (HPLC).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.

2.2. Collections of plants

Garcinia inidica fruits were collected in and around Goa. The fruits samples were authenticated by Dr. K. Arumugasamy, Associate professor, 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 at 4°C.

2.3. Preparation of extract

Thirty (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. The residue was used for further analysis.

2.4. HPTLC analysis

The isolated fraction 75:25 (petroleum ether: ethyl acetate) from *G.indica* using column chromatography and standard garcinol were subjected to HPTLC. The given samples were centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis. Five (5) µl of the test solution and 3µl (3µg of garcinol) of standard solutions were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin through developing chamber (after saturated with solvent vapour) with respective mobile phase (garcinol) and the plate was developed in the respective mobile phase toluene- ethyl acetate-formic acid (4 : 1 : 0.5) up to 90 mm. The developed plate was dried in hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV366 nm. The developed plate was sprayed with respective spray reagent (1% vanillin in 10% ethanolic sulphuric acid reagent) and dried at 100°C in hot air oven. The plate was photo-documented in day light mode and UV366nm using photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning: Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at daylight 254 nm. The peak table, peak display and peak densitogram were noted.

2.5. 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.

2.6. Induction of myocardial infraction

Isoprenaline (25 mg dissolved in physiological saline/ kg b.w / day) was administered subcutaneously on 29th and 30th day of experimental period for the induction of myocardial infarction [7].

2.7. Experimental designs

Animals were grouped into five, each group consisting of six animals. Group I, control rats received standard diet and water *ad libitum*. In Group II, the rats were administered with ISO (25 mg/kg body weight suspended in 1ml of 0.9% saline) s.c., (subcutaneously) for two consecutive days on 29th and 30th day of experimental period. Group III rats pre-treated with *G.indica* (250mg/kg body weight orally suspended in 1ml of water), Group IV rats pre-treated with *G.indica* (500mg/kg body weight orally suspended in 1ml of water) and Group V rats pre-treated with garcinol (5 mg/kg body weight orally suspended in 1ml of olive oil) for 28 days. Group III, IV and V rats were administered with ISO (25 mg/kg body weight) s.c., for two consecutive days on 29th and 30thday.

2.8. Sample collections

Twelve hours after the second injection of ISO, 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, the clear supernatant and the serum collected were used for the biochemical analysis.

2.9. Estimation of cardiac markers

Estimation of serum cardiac marker enzymes: creatine phosphokinase (CPK) by the method of Okinaka *et al*., 1961 [8], creatine phosphokinase-MB (CK-MB) in serum were estimated using commercially available kit (Beacon assay kit), lactate dehydrogenase (LDH) by the method of King, 1965 [9], aspartate transaminase (AST) and alanine transaminase (ALT) by the method of [10] were estimated.

2.10. Separation of serum LDH isoenzymes by Agarose Gel Electrophoresis

LDH isoenzyme was separated by agarose gel electrophoresis using the standard method [11].

2.11. Estimation of serum lipid profiles

Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were analysed using commercially available kits (Reckon diagnostics, Baroda, India). Very low density lipoproteins (VLDL) and low density lipoprotein (LDL) were calculated [12].

2.12. Estimation of heart tissue lipids

Tissue lipids were extracted by the method of Folch et al. [13]. To a known volume of plasma or tissue homogenate, 10.0 ml of chloroform-methanol (2:1, v/v) mixture was added and mixed well for 30 min and was filtered through Whatmann filter paper (No. 42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept undisturbed overnight. The lower phase containing the lipid was drained off into pre-weighed beakers. The upper phase was re-extracted with more of chloroform-methanol mixture; the extracts were pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3.0 ml of chloroform-methanol (2:1) mixture and aliquots were taken for the estimation of lipids. Total cholesterol [TC] [14], triglycerides [TG] (Rice, [15], free fatty acids [FFA] (Horn and Menahan, [16], and phospholipids [PL] [17].

2.13. Triphenyl tetrazolium chloride (TTC) macroscopic enzyme-mapping assay

TTC test used for macroscopic enzyme mapping of the ischemic myocardium was a modification of the method described [18], done without the addition of an exogenous substrate [19].

2.14. Histopathological studies

Animals were sacrificed on the day of withdrawal of blood; hearts were removed, washed immediately with saline and then fixed in 10% formalin. The hearts stored in 10% formalin were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histoarchitectural changes.

2.15. 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.

3. RESULTS

3.1. HPTC Analysis

Green, greenish grey coloured zones at visible light mode present in the given standard garcinol and sample track observed in the chromatogram after derivatization (fig.1). This confirmed the presence of garcinol in the given sample when compared with standard garcinol. The Rf value of the given sample (0.62) was much neared to the Rf value (0.59) of standard garcinol (table 1).

Fig. 1: HPTLC chromatogram of visible light isolated garcinol and Standard garcinol

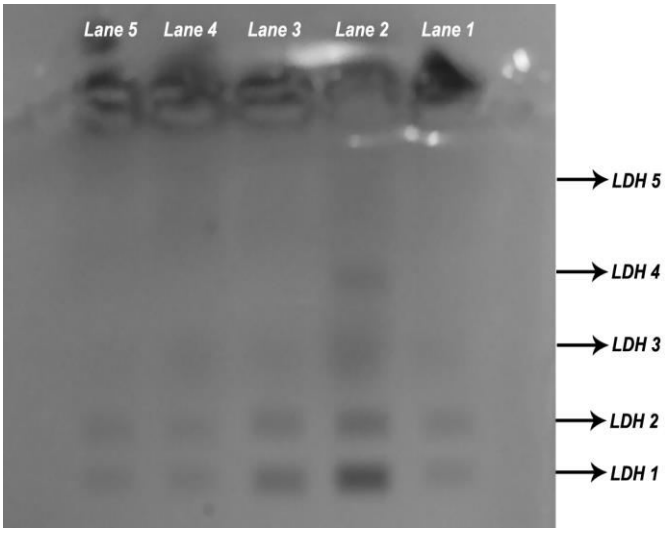
Table 1: Track showed standard Baseline display (Scanned at 254 nm)

Track	Rf	Height	Area	Assigned substance
Sample	0.62	433.8	18525.3	Garcinol
STD (Garcinol)	0.59	242.3	6369.4	Garcinol standard

3.2. Effect of *G. indica* and garcinolon serum cardiac marker enzymes and LDH isoenzymes

Table 2 shows the effect of *G.indica* and garcinol on the activities of CPK, LDH, CK-MB, AST and ALT in myocardium of control and experimental rats. A significant ($p<0.05$) increase was observed in the ISO-induced rats (Group II) when compared to control rats (Group I). In drug treated group III, IV and V significant ($p<0.05$) decrease in the activities of CPK, LDH, CK-MB, AST and ALT were observed when compared to ISO- induced rats (group II) (250mg and 500mg/kg b.w) and garcinol (5mg/kg b.w) pre-treatment on the activities of cardiac marker enzymes (CPK, LDH, CK-MB, AST and ALT) in the serum of control and ISO-induced rats.

Agarose gel electrophoresis separation patterns of serum LDH isoenzymes of control and experimental rats are depicted in fig. 3. ISO-induction (Group II) caused an increased expression of LDH isoenzymes, predominatly LDH 1 (lane 2), when compared to control rats in lane 1 (Group I). The LDH 1 in *G. indica* at a dose of 250mg/kg b.w (Group III) pre-treated rats was found to be less prominent than in ISO-induction (Group II). *G. indica* at a dose of 500mg/kg b.w (Group IV) and garcinol at a dose of 5mg/kg b.w pre-treated rats (Group V) showed faint bands of LDH isoenzymes which were comparable with those of control rats.



Electrophorotogram showing the effect of G.indica and garcinolon LDH-isoenzymes in the serum of control and experimental rats. Lanes 1: Control; 2: ISO; 3: ISO+ G.indica (250mg/kg b.w.); ISO + G.indica (500mg/kg b.w.); 5: ISO + garcinol (5mg/kg b.w.).

Fig. 2: Effect of *G. indica* and garcinolon LDH isoenzymes

3.3. Effect of *G. indica* and garcinol on lipid profiles in serum and heart tissue

The table 3 represents the serum lipid profiles of control and experimental rats. It is evident that there was significant ($p<0.05$) increase in the levels of TC, TG, LDL and VLDL, and significant ($p<0.05$) decrease in the level of HDL in ISO-induced rats (Group II). HDL and LDL are significant variables for Coronary Heart Disease. Pre-treatment with *G. indica* fruit extract group of rats (Group III and IV) and garcinol pre-treatment rats (Group V) show decreased levels of serum cholesterol, TG, LDL, VLDL and increased levels of HDL when compared to ISO treated group (Group II). Increased levels of serum HDL was observed in rats pre-treated with *G. indica* and garcinol facilitates the transport of cholesterol from peripheral tissues to the liver for the catabolism and excretion from the body in ISO treated rats.

Table 4 represents the levels of myocardial tissue lipids in control and experimental rats. ISO-induced rats (Group II) showed significant ($p<0.05$) increase in the levels of TC, TG, FFA and a significant ($p<0.05$) decrease in PL when compared to control rats (Group I). *G. indica* pre-treated Group III (250mg/kg b.w) and Group IV(500mg/kg b.w) showed significant ($p<0.05$) decrease in the levels of TC, TG, FFA and a significant ($p<0.05$) increase in PL when compared to control rats (Group II) and garcinol (5mg/kg b.w) administration (Group V) brought the levels of myocardial tissue lipids to near normality.

Table 2: The effects of ethanolic extract of *G.indica* and garcinolon the activities of marker enzymes in serum of control and experimental rats.

Groups	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	CK-MB (IU/L)	CPK (IU/L)
I	92.91±7.72	76.65±6.52	47.15±3.84	118.30±9.50	84.10±4.09
II	242.23±21.81a*	215.02±13.08a*	163.47±10.72a*	488.15±31.12a*	316.38±23.25a*
III	199.23±16.05b*	121.08±10.19b*	100.17±7.12b*	297.66±24.48b*	185.59±11.14b*
IV	123.27±9.65c*	86.67±5.84c*	61.58±5.13c*	181.97±13.08c*	115.39±9.22c*
V	120.15±6.33d*	80.34±6.58d*	50.47±4.10d*	147.76±7.13d*	117.38±5.05d*

Values are mean ± SD of six animals, ($p<0.05$), analysis of variance for multiple comparison by Dunnet's test, a: Group II vs I; b: Group III vs II; c: Group IV vs II; d: Group V vs II.

Table 3: Effect of *G.indica* and garcinol on lipid profiles in serum of control and experimental rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	68.41 ± 5.21	39.15 ± 6.71	43.76 ± 4.45	45.68 ± 5.25	7.83 ± 1.34
II	103.33 ± 3.08a*	83.07 ± 5.91a*	26.78 ± 3.36a*	90.14 ± 6.38a*	16.61 ± 1.18a*
III	80.29 ± 5.21b*	66.40 ± 7.34b*	32.29 ± 6.13b*	84.34 ± 6.64b*	13.28 ± 1.47b*
IV	76.38 ± 7.81c*	50.00 ± 4.57c*	38.71 ± 5.04c*	65.73 ± 7.76c*	10.00 ± 0.91c*
V	72.76 ± 5.54d*	42.99 ± 2.70d*	40.49 ± 1.99d*	54.60± 2.88d*	8.60± 0.54d*

Values are expressed as mean ± SD of six animals, ($p<0.05$), analysis of variance for multiple comparison by Dunnet's test. a: Group II vs I; b: Group III vs II; c:Group IV vs II; d: Group V vs II.

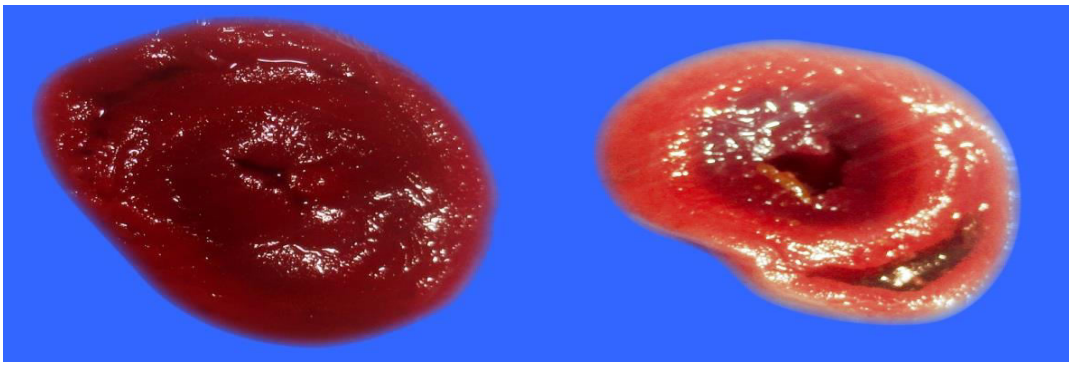
Table 4: Effect of *G.indica* and garcinol on lipid profiles in heart of control and experimental rats

Groups	Cholesterol (mg/g)	Triglycerides (mg/g)	Free fatty acids (mg/g)	Phospholipids (mg/g)
Group I	0.12 ± 0.01	0.33 ± 0.03	1.00 ± 0.05	0.18 ± 0.02
Group II	0.51 ± 0.02 a*	1.862 ± 0.04 a*	5.16 ± 0.03 a*	0.06 ± 0.04 a*
Group III	0.42 ± 0.02 b*	1.042 ± 0.04 b*	2.01 ± 0.08 b*	0.10 ± 0.03 b*
Group IV	0.21 ± 0.01 c*	0.678 ± 0.14 c*	1.29 ± 0.08c*	0.15 ± 0.03 c*
Group IV	0.16 ± 0.02 d*	0.583 ± 0.04 d*	1.10 ± 0.72d*	0.13± 0.02d*

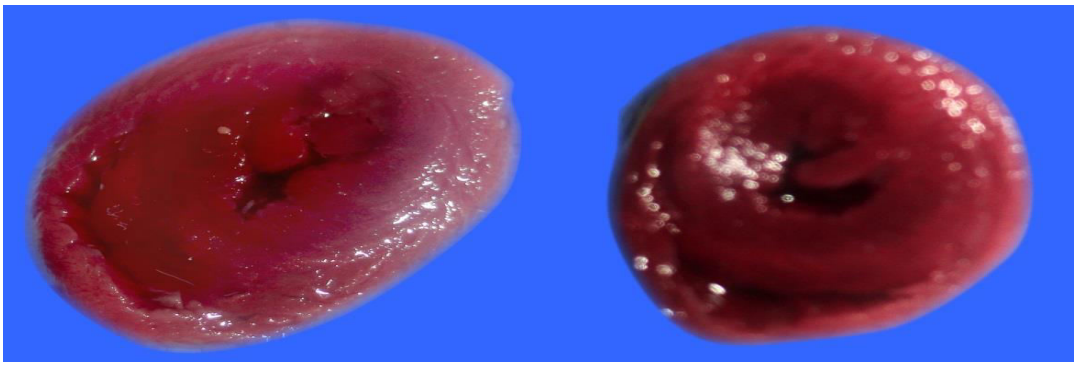
Values are expressed as mean ± SD of six animals, ($p<0.05$), analysis of variance for multiple comparison by Dunnet's test. a: Group II vs. I; b: Group III vs. II; c: Group IV vs. II. d: Group V vs. II.

3.4. TTC macroscopic enzyme-mapping assay

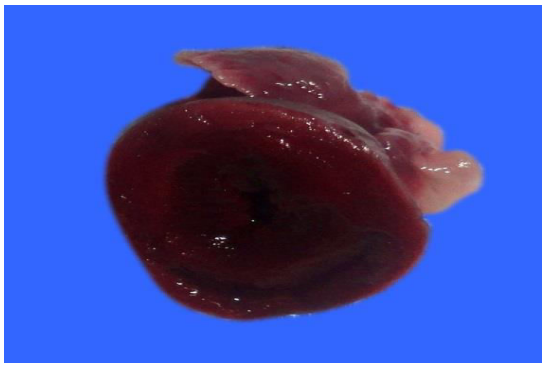
Fig. 3 (a-e) portrays the histochemical approach to detect the myocardial changes in the heart of control and ISO-induced rats through macroscopic enzyme mapping assay. A soaring proportion of infarct size with decreased staining was observed in ISO-induced rats (fig. 3b). *G. indica* at a dose of 250mg/kg b.w administered rats showed very less improvement to reduced infarct size with reduced staining when compared to ISO-induced rats (fig. 3c) and 500mg/kg b.w of *G. indica* administered rats (fig. 3d) illustrated a moderately low infarct size with reduced staining when compared to ISO administered rats. Garcinol (5mg/kg b.w) administered rats (fig. 3e) show highly reduced infarct size when compared to induced group.



a) Control **b) Induced**



c) *G. Indica* 250mg/kg **d) *G. Indica* 500mg/kg**



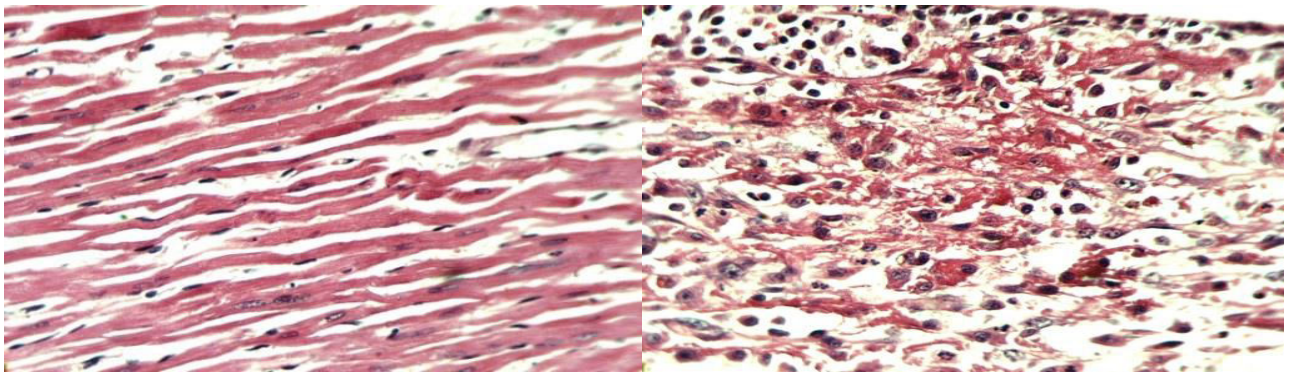
e) Garcinol g mg/kg

Control rat showing completely viable myocardial tissue. (b) ISO induced rat heart showing soaring proportion of infarct size with reduced stain. (c) ISO + G.indica (250mg/kg b.w) administered rat showing heart tissue with less activity to reduced infarct size.(d)ISO + G.indica (500mg/kg b.w) administered rat showing heart tissue with reduced infarct size (e) ISO + garcinol(5mg/kg b.w) administered rat showing heart tissue highly reduced infarct size.

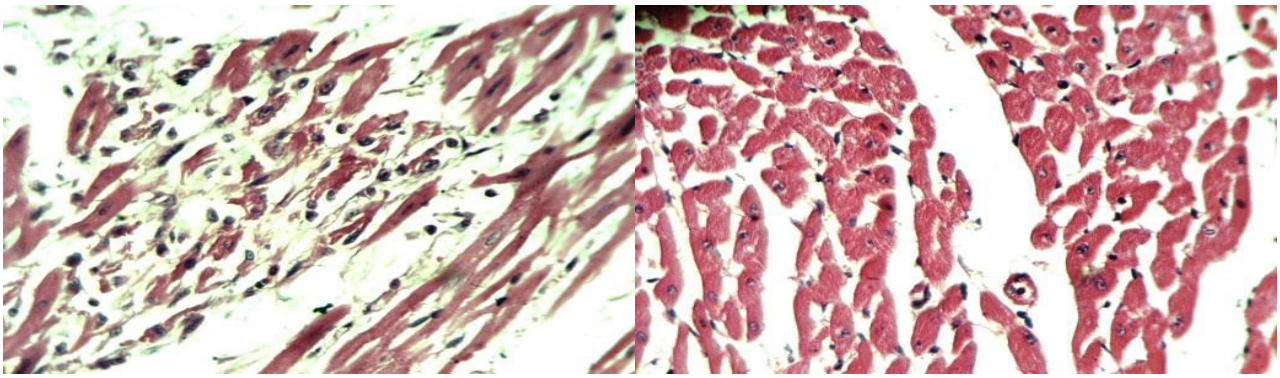
Fig. 3: Effect of *G.indica* and garcinol on macroscopic enzyme mapping of heart in control and ISO-induced rats

3.5. Histopathology

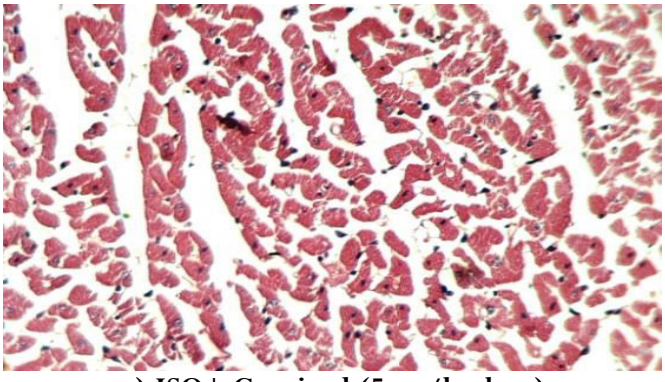
The histoarchitecture of cardiac tissue of control group appeared to be normal as there was no visible necrotic damage to the myocytes (fig. 4a). However, extensive myocyte membrane damage, myo- necrosis, fibroblastic proliferation and infiltration of inflammatory cells was observed in ISO group (fig. 4b). In ISO + *G.indica* 250mg/kg b.w pre-treated myocardium showed mild activity (fig. 4c) and ISO + *G.indica* 500mg/kg b.w pre-treated myocardium showed decreased degree of necrosis (mild) and less infiltration of inflammatory cells (fig. 4d). In Garcinol (5mg/kg b.w) pre-treated group, there was much less extent of infiltration of inflammatory cells, myonecrosis, vacuolar changes and oedema as compared to ISO group (fig. 4e).



a) Control **b) Induced**



c) ISO+*G.indica* (250mg/kg b.w) LD **d) ISO+*G.indica* (500mg/kg b.w) HD**



e) ISO+ Garcinol (5mg/kg b.w)

(a) photomicrograph showing normal architecture of normal rat heart. (b) Photomicrograph of rat heart subjected to ISO induced myonecrosis with myophagocytosis and lymphatic infiltration. Vacuolar changes and oedema are prominent with chronic inflammatory cells visible (HXE 200).(c, d and e) photomicrograph of rat heart of G.indica and garcinol preteated groups showing less degree of myonecrosis and infiltration of inflammatory cells (HXE).

Fig. 4: Histoarchitecture of cardiac tissue of treated rats

4. DISCUSSION

Qualitative HPLC and HPTLC fingerprints have been utilized to standardize many medicinal plant extracts [20]. Standardization of herbal products/drugs is more challenging than synthetic drugs. In most of the cases the biological activity is due to synergistic effect of all chemical constituents of the plant. The qualitative fingerprint analysis of garcinol from *Garcinia indica* by HPTLC in the present study is useful for further future investigations. ISO, a synthetic catecholamine and beta adrenergic agonist, has been found to cause severe oxidative stress in the myocardium resulting in infarct-like necrosis of the heart muscles. ISO-induced myocardial necrosis is the most authenticated model of MI in rats [21].

In the present investigation, we observed a decrease in the activities of myocyte injury markers like CK, CK-MB, LDH, AST and ALT in hearts of ISO administered rats. When myocardial cells are damaged due to deficient oxygen supply or glucose, the integrity of cell membrane gets disturbed and it might become more porous that results in the leakage of these enzymes. Oral pre-treatment of *G. indica* (250mg and 500mg/kg b.w) and garcinol (5mg/kg b.w) restored the activities of myocardial marker enzymes. This could be due to protective effect of *G. indica* and garcinol on the myocardium, reducing the myocardial damage and thereby restricting the leakage of CK, CK-MB, LDH, AST and ALT. Similar observation was reported [22]. Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the cellular membrane [23]. In this study isoprenaline administration raised TC, TG, LDL and VLDL cholesterol and decreased HDL cholesterol level in the serum of Group II animals. Interestingly, treatment with *G. indica* (250mg/kg b.w and 500mg/kg b.w) and garcinol (5mg/kg b.w) reversed the effects of isoprenaline. Increased TC, TG, LDL and VLDL cholesterol and decreased HDL cholesterol are associated with raised risk for myocardial infarction. High level of circulating cholesterol, triglycerides and their accumulation in heart tissue are associated with cardiovascular damage. Hypertriglyceridemic patients at a risk for cardiovascular disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol, which causes myocardial membrane damage [24]. Hypertriglyceridemia seen in isoprenaline treated rats is a condition observed in ischemic heart disease. The anti-hypertriglyceridemia activity of *G.indica* and garcinol, signify that the myocardial membrane is protected against isoproterenol induced damage.

ISO-induced rats showed a significant increase in the levels of TC, TG and FFA in the heart while the levels of phospholipids decreased in the heart which is finding line with the previous report [25]. Generally, the mechanisms of actions of lipolytic hormones, including ISO, on fat cells are believed to be mediated by the cAMP cascade: lipolytic hormones activate adenylate cyclase, thereby increasing cAMP formation. The cAMP promotes lipolytic activity by activating cAMP dependent protein kinase, which Phosphorylates hormone-sensitive lipase (HSL) [26], resulting in the hydrolysis of stored triacylglycerol thereby showing marked hyperlipidemia. *G. indica* (250mg/kg b.w and 500mg/kg b.w) and garcinol (5mg/kg b.w) administration significantly restored these alterations thereby maintaining the normal fluidity and function of the myocardial membrane. It has been reported that *G. indica* and garcinol is a potent and selective inhibitor of the catalytic subunit of cAMP dependent protein kinase [27] which, in turn, leads to decreased HSL activity thereby reducing the mobilization of FFAs from the fat depots. Measurement of LDH isoenzymes is necessary for greater specificity of cardiac injury, since a non-specific increase of total LDH in serum will cause tissue damage. An increase in the expressions of LDH 1 and LDH 2 bands were observed in ISO-induced rats which are in agreement with the previous findings of Levinson and Hobbs [28]. Voet and Donald have reported that the release of cardiac specific isoenzymes LDH 1 and LDH 2 into the circulation which might be due to the necrosis induced by ISO. *G.indica* and garcinol administration to ISO-induced rats showed decrease in the intensity of LDH 1 and LDH 2-bands and activities of cardiac markers [29].

TTC acts as a proton acceptor for many pyridine nucleotide-linked-dehydrogenases along with cyto-chrome which form an integral part of the inner mitochondrial membrane and make up the electron transport chain [30]. The tetrazolium salt is reduced by the enzymes into a red, lipid soluble formazan. Viable tissues therefore stains deep red while the ischemic zone is known to progress to a dense fibrous scar with no viable muscle fibers, remains unstained. *G. indica* (250mg and 500mg/kg b.w) and garcinol (5mg/kg b.w) administration markedly reduced the myocardial ischemic zone in ISO-induced rats. Histopathological

examination of cardiac tissue in ISO-induced group showed the presence of focal myonecrosis with myophagocytosis and lymphocytic infiltration (myocarditis) in the subendocardial region indicative of infract like lesions as reported other studies [31]. Scrutiny of cardiac tissue of pre-treatment of *G. indica* 250mg/kg b.w. dose also showed myocardial damage to the maximum extent. Although myocardium of *G.indica* at a dose of 500mg/kg b.w. pre-treated group showed moderate oedema and less inflammatory cells, only mild oedema and inflammatory cells were observed in the myocardium of garcinol 5mg/kg b.w. pre-treated group. This data further supported in the conformation of cardioprotective action of *G.indica* and garcinol on ISO-induced MI in rats.

5. CONCLUSION

In conclusion, pre-treatment of *Garcinia indica* and garcinol offers dose dependent protection from MI induced by Isoprenaline in rat heart. Our study provides experimental evidence that *G. indica* 500mg/kg b.w and garcinol 5mg/kg b.w. attenuates the levels of lipids, lipoproteins and augmented the myocardial marker enzymes level and reduced the infract size, preserved histo-architecture and improved cardiac performance following ISO administration.

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Conflict of interest

The authors declare no conflict of interest

Ethical approval

The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethical committee (Approval no.ML-EA-CPCSEA/04-2014/03).

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