RPS Research in Pharmaceutical Sciences, October 2015; 10(5): 388-396
Received: Oct 2014
Accepted: Dec 2014

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Evaluation of cardioprotective effect of aqueous extract of *Garcinia indica* Linn. fruit rinds on isoprenaline-induced myocardial injury in *Wistar* albino rats

Kaksha J. Patel[1], Ashwin K. Panchasara[1], Manish J. Barvaliya[1], Bhargav M. Purohit[1,*], Seema N. Baxi[2], Vishal K. Vadgama[1], and C. B. Tripathi[1]

[1]*Department of Pharmacology, Government Medical College, Bhavnagar-364001, Gujarat, India.*
[2]*Department of Pathology, Government Medical College, Bhavnagar-364001, Gujarat, India.*

Abstract

In the present study,cardioprotective effect of aqueous extract of *Garcinia indica* Linn. fruit rinds in isoprenaline-induced myocardial infarction in *Wistar* albino rats was evaluated. *In vitro* total phenolic, total flavonoid content and 2, 2'-diphenyl-1-picrylhydrazyl hydrate radical scavenging activity was measured. *In vivo* effect of aqueous extract of *G. indica* was evaluated in *Wistar* albino rats by isoprenaline-induced myocardial injury model. Thirty six rats were randomly divided in 6 groups. Rats were treated with *G. indica* 250 mg/kg and 500 mg/kg doses for 21 days and myocardial injury was produced by subcutaneous injection of isoprenaline 85 mg/kg on day 20 and 21. Carvedilol 1 mg/kg for 21 days served as active control. Electrocardiogram parameters, cardiac injury markers (serum troponin-I, uric acid, lactate dehydrogenase, creatinine kinase-MB, aspartate aminotransferase and alanine aminotransferase), oxidative stress markers (superoxide dismutase, catalase and malondialdehyde level) and histopathological changes were evaluated in each group and compared using appropriate statistical tests. *In vitro* evaluation of aqueous extract showed significant antioxidant property. Isoprenaline produced significant myocardial ischemia as compared to normal control group (P<0.05). Administration of *G. indica* in both the doses did not significantly recover the altered electrocardiogram, cardiac injury markers, oxidative stress markers and histopathological myocardial damage as compared to disease control group (P>0.05). The aqueous extract of *G. indica* was not found to be cardioprotective against myocardial injury. Further study with more sample size and higher dose range may be required to evaluate its cardioprotective effect.

Keywords: Carvedilol; Cardioprotective; *Garcinia indica*; Isoprenaline; Myocardial infarction

INTRODUCTION

Ischemic heart disease is a major non communicable disease and it has become an important problem worldwide. According to WHO, it will be a leading cause of mortality worldwide by 2020 (1). Among ischemic heart disease, acute myocardial infarction (MI) is the most alarming one and it occurs due to imbalance between coronary blood supply and myocardial demand. Free radical mediated myocardial damage is an important etiological mechanism that is associated with increased level of reactive oxygen species and/or inadequate antioxidant defense system (2).

Isoprenaline (ISO), a nonselective β agonist produces infarct like necrosis of myocardium (3). ISO-induced MI is the most widely used model to evaluate cardioprotective effect of various drugs (4). Administration of ISO in high doses produces myocardial lesions similar to MI in human.

Modern medicines are effective in preventing heart diseases but their use is limited due to various adverse effects (5). Many dietary plants and food antioxidants are increasingly being recognized as health promoter in heart diseases (6). *Garcinia indica* choisy belongs to the family Clusiaceae/Guttiferae. It is an evergreen tree that is commonly found along the west coast of India (7). The dried outer rind of the fruit of *G. indica* is known as 'kokum'. In Charak Samhita, it has been mentioned as cardiotonic

*Corresponding author: B.M. Purohit
Tel: 0091 9725024978, Fax: 0091 2782422011
Email: purohitbhargav@rediffmail.com

agent (8). Many other therapeutic uses of kokum for scalds and chaffed skin, sunstroke, dysentery, allergic skin rashes, burns, mucous diarrhoea, piles, anorexia, liver tonic and to allay thirst have been mentioned in traditional Indian medicine (9).

The fruit rind contains polyisoprenylated benzophenones garcinol, its isomer isogarcinol, xanthochymol, isoxanthochymol and hydroxycitric acid (10). Hydroxycitric acid and garcinol are the main ingredients in kokum with antioxidant effect (11). Other compounds with potential antioxidant properties in kokum are citric acid, malic acid, polyphenols, carbohydrates, anthocyanin pigments and ascorbic acid (12). Various plants have been found to have a cardioprotective effect due to their antioxidant properties (13,14). So, this study was planned to evaluate cardioprotective effect of aqueous extract of *G. indica* on isoprenaline induced MI in *Wistar* albino rats.

MATERIALS AND METHODS

All experiments were performed after prior permission from Institutional Animal Ethics Committee (IAEC), Government Medical College, Bhavnagar, Gujarat, India. (IAEC No.28/2012, dated: 27/10/2012). Care of experimental animals was taken as per the guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Government of India, New Delhi, India.

Experimental animals

Wistar albino rats (250 ± 50 g) of both sexes and female mice (25-30 g) were procured from central animal house of the institution.

They were housed in standard transparent polypropylene cages with wheat husk bedding, renewed every 24 h. They were kept under controlled room temperature and humidity (18 to 29 °C; 30 to 70%) in a 12 h light-dark cycle. Animals were acclimatized for one week to laboratory conditions before starting the experiment. The rats were given standard laboratory diet and water *ad libitum*.

Drugs and chemicals

Aqueous extract of *G. indica* was a generous gift from Leopard Investments Ltd, Mumbai. ISO, Gallic acid, quercetin, urethane and 2, 2'-diphenyl-1-picrylhydrazyl hydrate (DPPH) were obtained from the Sigma Chemical Company, St. Louis, MO, USA. Carvedilol was purchased from Selleck chemicals, Munich, Germany. ISO solution was freshly prepared in normal saline at the time of injection. *G. indica* was dissolved in distilled water.

Acute toxicity study

Acute toxicity study of *G. indica* extract was conducted using female *swiss* albino mice (25-30 g) in accordance with Organization for Economic Co-operation and Development guideline No. 423.

Determination of total phenols and flavonoids

Total phenolic content was determined according to Folin-Ciocalteu method (15). Flavonoid content in the extract was determined by a colorimetric method (16).

2, 2'-diphenyl-1-picrylhydrazyl hydrate radical scavenging assay

In this assay, free radical scavenging activity of *G. indica* was determined by measuring the bleaching of purple-colored methanol solution of DPPH (17).

Experimental groups

Total 36 *Wistar* albino rats were allocated randomly into 6 groups using rando software (6 animals per group). MI was induced in rats by giving ISO (85 mg/kg) subcutaneously (s.c.) for two subsequent days, on day 20 and 21 at the interval of 24 h. Distribution of study groups were as follow:

Group 1 (Normal control), Rats were given distilled water orally for 21 days and normal saline s.c.

Group 2 (ISO control), Rats were given distilled water orally for 21 days and ISO (85 mg/kg) s.c.

Group 3 (GI control), Rats were given *G. indica* extract 500 mg/kg orally for 21 days and normal saline s.c.

Group 4 (GI low dose), Rats were given *G. indica* extract 250 mg/kg orally for 21 days and ISO (85 mg/kg) s.c.

Group 5 (GI high dose), Rats were given *G. indica* extract 500 mg/kg orally for 21 days and ISO (85 mg/kg) s.c.

Group 6 (Active control), Rats were given carvedilol (1 mg/kg) orally for 21 days and ISO (85 mg/kg) s.c.

Outcome measures

After dosing and administration of ISO, the following parameters were recorded to evaluate the cardioprotective effect.

Electrocardiography recording

At the end of 21 days, 48 h after the first dose of ISO, all the animals were anaesthetized with intraperitoneal injection of urethane (125 mg/100 g, i.p) and was given stabilization period of 30 min before recording of electrocardiography (ECG). ECG was recorded with the help of student's physiograph and ECG coupler [Inco Ambala Co, Haryana, India; paper speed-50 mm/sec, sensitivity- 200 µV/cm, gain-maximum]. The electrodes constructed from 26 gauge hypodermic needle were attached to both front and hind paw. One precordial lead was used and was placed in a position corresponding to V4 in human. Heart rate, RR interval, QT interval, corrected QT interval and QRS inteval were calculated and compared between the groups.

Biochemical estimations

After recording of ECG, blood was collected through retro-orbital plexus using capillary tube. The serum was separated by centrifugation and used for the estimation of lactate dehydrogenase (LDH, measured by UV kinetic method), creatinine kinase-MB (CK-MB, measured by Immuno inhibition method), troponin I (measured by Immuno-fluorescence method), aspartate *aminotransferase* (AST, measured by UV kinetic method), alanine *aminotransferase* (ALT, measured by UV kinetic method) and uric acid (measured by Uricase pap method). The manufacturer for the kit used for Troponin I is Toshos Corporation, Tokyo, Japan. All other biochemical parameters were measured by kits of Instrumentation Laboratory, Lexington, USA.

After blood collection, rats were sacrificed and heart was dissected out with mid abdominal incision. It was blotted in filter paper, weighed and further proceeded for histopathology or antioxidant analyses. From all groups, 3 hearts were used for histopathological analysis and 3 hearts were used for antioxidant estimation.

Antioxidant estimation in heart tissue homogenate preparation

Hearts were washed in ice cold saline followed by in 0.25 M sucrose solution and were finely sliced. Homogenate was prepared in chilled tris-HCl buffer solution [10% w/v, 0.1 M, pH 7.4]. The homogenate was then centrifuged at 5000 rpm at 4 °C using compact high speed refrigerated centrifuge (Kubota 6500, Japan). The clear supernatant obtained was used for the estimation of superoxide dismutase (SOD) by the method of Misra and Fridovich (18), catalase by the method of Hugo e. Aebi (19) and marker of lipid peroxidation malondialdehyde (MDA) by the method of Slater and Sawyer (20).

Heart weight to body weight ratio

In each group, heart weight to body weight ratio was calculated. Body weight was the weight of animal on the day of sacrification.Heart weight was measured after washing it in heart in ice cold saline after removal from the body, squeezing out the blood and blotted on the filter paper.

Histopathological analysis

Three hearts from each group were preserved in 10% formalin, processed and embedded in paraffin wax. 5-6 µm thick sections were cut and stained with Hematoxyline and Eosin (H & E) stain and observed with light microscope to evaluate myocardial injury. All slides were coded and analyzed blindly by the trained pathologist from our institute without knowledge of treatment plan. Histological observations for oedema, infiltration and necrosis were categorised in to 5 different grades ranging from grade 0 to 4. Grade 0 indicates no change and +1, +2, +3, +4 indicates slight, mild, moderate and severe histopathological changes, respectively.

Statistical analysis

ll the values were presented as Mean ± SEM. Data were checked for normal distribution using the Kolmogorov–Smirnov test. Parametric data were analyzed by one way ANOVA followed by Tukey- Kramer multiple comparison test and Non parametric data were analyzed by Kruskal Wallis followed by Dunn's multiple comparison test using GraphPad InStat (version 3.00, GraphPad Software, California USA). A *P* value <0.05 was considered as statistically significant.

RESULTS

In toxicity study, no mortality was observed in mice treated up to 5000 mg/kg of aqueous extract of *G. indica*. Accordingly, 1/10 (500 mg/kg, orally) and 1/20 (250 mg/kg, orally) doses were selected for screening of cardioprotective activity.

During *in vitro* evaluation of antioxidant property of aqueous extract of *G. indica*, we found that extract had total phenolic content 252.87 mg/g GAE (Gallic Acid Equivalent), total flavonoid content 34 mg/g QUE (Quercetin Equivalents) and IC_{50} value for DPPH scavenging activity 231.85 ± 21.56 µg/ml.

As shown in Table 1, rats in ISO group showed significant increase in cardiac injury markers like troponin-I, LDH, CK-MB, ALT, AST and uric acid as compared to normal control group (*P*<0.05). In test groups, administration of *G. indica* extract could not significantly restore the elevated parameters due to ISO administration (*P*>0.05), whereas, carvedilol in active control group significantly restored the cardiac injury markers (*P*<0.05) (Table 1).

Table 1. Comparison of cardiac injury markers: serum Troponin-I, LDH, CK-MB, ALT, AST and Uric acid level between experimental groups.

Groups	Troponin-I (ng/ml)	LDH (IU/l)	CK-MB (IU/l)	AST (IU/l)	ALT (IU/l)	S. uric acid (mg/dl)
Group 1 (Normal control)	0.17 ± 0.03	349.83 ± 51.28	469.33 ± 38.99	238.33 ± 6.08	76 ± 3.12	1.38 ± 0.17
Group 2 (ISO control)	1.31 ± 0.21##	1290 ± 50.33#	1828.33 ± 46.43#	287.5 ± 7.23#	175.16 ± 3.11#	2.61 ± 0.19#
Group 3 (GI control)	0.25 ± 0.09	356 ± 23.62	468.83 ± 35.07	236 ± 4	79.33 ± 4.05	1.43 ± 0.13
Group 4 (GI 250 mg/kg)	1.05 ± 0.14	1267.5 ± 86.82	1669 ± 110.21	279.5 ± 4.95	171.33 ± 5.14	2.23 ± 0.16
Group 5 (GI 500 mg/kg)	1.3 ± 0.21	1258 ± 46.76	1653.33 ± 106.26	275 ± 7.64	170.83 ± 3.8	2.25 ± 0.08
Group 6 (Active control)	0.21 ± 0.05**	901.66 ± 42.38*	1494.83 ± 49.90*	245.16 ± 8.28*	141.5 ± 3.93*	1.53 ± 0.16*

Data are expressed as Mean ± SEM (N = 6 for each group).GI; *Garcinia indica*. #*P*< 0.05 as compared to normal control by one way ANOVA followed by Tukey Kramer's multiple comparison test. ##*P*<0.05 as compared to normal control by Kruskal Wallis followed by Dunn's multiple comparison test. **P*<0.05 as compared to isoprenaline control by one way ANOVA followed by Tukey Kramer's multiple comparison test. ***P*<0.05 as compared to isoprenaline control by Kruskal Wallis followed by Dunn's multiple comparison test. AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, LDH; Lactate dehydrogenase, CK-MB; Creatine Kinase-MB.

Table 2. Comparison of electrocardiography parameters between experimental groups.

Groups	HR (beats/min)	QRS (s)	QT (s)	RR interval (s)	QTc (s)
Group1 (Normal control)	290.66 ± 13.81	0.056 ± 0.006	0.093 ± 0.016	0.206 ± 0.009	0.207 ± 0.039
Group 2 (ISO control)	443.16 ± 19.83#	0.036 ± 0.006	0.12 ± 0.014	0.136 ± 0.006#	0.328 ± 0.043
Group 3 (GI control)	296.16 ± 15.54	0.05 ± 0.008	0.086 ± 0.019	0.203 ± 0.01	0.204 ± 0.034
Group 4 (GI 250mg/kg)	413.5 ± 20.28	0.04 ± 0.008	0.116 ± 0.006	0.146 ± 0.006	0.304 ± 0.011
Group 5 (GI 500mg/kg)	422.33 ± 18.8	0.035 ± 0.007	0.103 ± 0.012	0.143 ± 0.006	0.273 ± 0.031
Group 6 (Active control)	341.5 ± 11.8*	0.053 ± 0.006	0.09 ± 0.004	0.176 ± 0.006*	0.213 ± 0.008

Data are expressed as Mean ± SEM (N = 6 for each group). GI; *Garcinia indica,* HR; Heart rate, QTc; Corrected QT interval. #*P*<0.05 as compared to normal control by one way ANOVA followed by Tukey Kramer's multiple comparison test. **P*<0.05 as compared to ISO control by one way ANOVA followed by Tukey Kramer's multiple comparison test.

Table 3. Comparison of body weight, heart weight, heart weight/body weight ratio between experimental groups.

Groups	Heart weight (g)	Body weight (g)	Heart weight / Body weight (× 10^{-3})
Group 1 (Normal control)	0.91 ± 0.043	272.5 ± 10.30	3.37 ± 0.19
Group 2 (ISO control)	1.21 ± 0.049#	257.8 ± 6.16	4.7 ± 0.09##
Group 3 (GI control)	0.93 ± 0.029	275.0 ± 8.94	3.43 ± 0.22
Group 4 (GI 250 mg/kg)	1.00 ± 0.048	261.6 ± 6.14	3.83 ± 0.22
Group 5 (GI 500 mg/kg)	1.04 ± 0.100	258.3 ±14.24	3.99 ± 0.25
Group 6 (Active control)	0.95 ± 0.043*	277.5 ± 8.92	3.45 ± 0.14**

Data are expressed as Mean ± SEM (N=6 for each group). GI; *Garcinia indica*, #*P*<0.05 as compared to normal control by one way ANOVA followed by Tukey Kramer's multiple comparison test. ## *P*<0.05 as compared to normal control by Kruskal Wallis followed by Dunn's multiple comparison test. **P*<0.05 as compared to isoprenaline control by one way ANOVA followed by Tukey Kramer's multiple comparison test. ***P*<0.05 as compared to isoprenaline control by Kruskal Wallis followed by Dunn's multiple comparison test.

Subcutaneous ISO administration caused marked increase in heart rate and decrease in RR interval as compared to normal control group (*P*<0.05; Table 2). Heart rate and RR interval was significantly restored in carvedilol group (*P*<0.05), whereas they were not restored in test groups (*P*>0.05).

The rats in ISO control group showed marked increase in heart weight and heart weight/body weight ratio but ratio was decreased in active control group (Table 3). SOD and catalase level were decreased and thiobarbituric acid reactive substance (TBARS) level was increased significantly in ISO control group as compared to normal control group (*P*<0.05). In *G. indica* treated groups, the levels of antioxidant enzymes was not restored (*P*>0.05) (Table 4).

Average grading of hisopathological findings are shown in Table 5. Fig. 1 shows histopathological texture of myocardium in study groups. Administration of ISO significantly caused necrosis, oedema and neutrophilic infiltration in myocardium as compared to normal control group. Myocardium of animals treated with *G. indica* extract and carvedilol showed improvement as compared to ISO control group. However, it could not achieve the statistical significance. The *G. indica* control group was comparable to normal control group in relation to all observed parameters.

Table 4. Comparison of biomarkers of oxidative stress between experimental groups.

Groups	SOD (Unit/g of tissue)	Catalase (μmol of H_2O_2 consumed/min/g of tissue)	TBARS (nmol MDA/g of tissue)
Group 1 (Normal control)	13.55 ± 0.62	2.66 ± 0.24	1.65 ± 0.12
Group 2 (ISO control)	5.38 ± 0.31$^{\#}$	0.54 ± 0.02$^{\#}$	3.82 ± 0.21$^{\#}$
Group 3 (GI control)	12.16 ± 0.31	2.22 ± 0.35	1.53 ± 0.21
Group 4 (GI 250 mg/kg)	6.12 ± 0.93	1.1 ± 0.17	2.55 ± 0.25
Group 5 (GI 500 mg/kg)	7.56 ± 0.22	1.08 ± 0.19	2.17 ± 0.13
Group 6 (Active control)	11.73 ± 0.64	1.98 ± 0.48	2.04 ± 0.13

Data are expressed as Mean ± SEM (N=3 for each group). GI; *Garcinia indica*, $^{\#}P<0.05$ as compared to normal control by Kruskal Wallis followed by Dunn's multiple comparison test. SOD; superoxide dismutase, TBARS; thiobarbituric acid reactive substance.

Table 5. Comparison of histopathological score between experimental groups.

Groups	Oedema	Infiltration	Necrosis
Group 1 (Normal control)	0 ± 0	0 ± 0	0 ± 0
Group 2 (ISO control)	2.33 ± 0.44$^{\#}$	2.33 ± 0.44$^{\#}$	2.66 ± 0.16$^{\#}$
Group 3 (GI control)	0 ± 0	0 ± 0	0 ± 0
Group 4 (GI 250 mg/kg)	0.83 ± 0.33	0.66 ± 0.16	0.83 ± 0.33
Group 5 (GI 500 mg/kg)	0.5 ± 0	0.5 ± 0.28	0.5 ± 0.28
Group 6 (Active control)	0.16 ± 0.16	0.16 ± 0.16	0.33 ± 0.33

Data are expressed as Mean ± SEM (N=3 for each groups). GI; *Garcinia indica*. $^{\#}P<0.05$ as compared to normal control by Kruskal Wallis followed by Dunn's multiple comparison test.

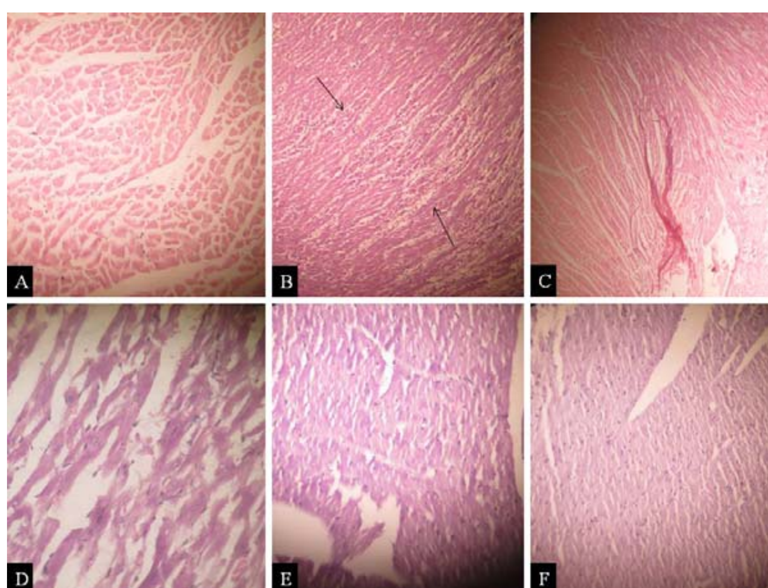

Fig. 1. Histopathological comparisons between the various study groups. A; Normal control group, B; Isoprenaline control group, C; *Garcinia indica* control group, D; *Garcinia indica* low dose, E; *Garcinia indica* high dose, F; Active control group. Arrows indicates marked infiltration of neutrophils and mononuclear inflammatory cells and necrosis of the myocardium.

DISCUSSION

In vitro evaluation showed a good antioxidant property of aqueous extract of *G. indica* fruit rind. So, we evaluated cardio protective effect of *G. indica* extract *in vivo* by ISO-induced myocardial infarction model in *Wistar* albino rats.

ISO is a synthetic catecholamine and nonselective β agonist. It is proven that catecholamine, if given in high doses, produces necrosis of heart muscle (21). Exact mechanism of ISO induced MI is not proven yet. Intracellular calcium overload due to massive influx (22), hypoxemia due to increased cardiac work and O_2 demand, free oxygen radical generation by auto oxidation of catecholamines, alteration of myocardial cell membrane permeability due to lipid peroxidation, interruption of mitochondrial oxidative phosphorylation by free fatty acid and changes in electrolyte contents are various proposed mechanisms. Among them, oxidative stress and free radical mediated injury appears to be the most important mechanism for ISO-induced MI (23).

In the present study, ISO caused significant myocardial damage as indicated by cardiac injury markers, ECG abnormalities, oxidative stress markers, heart weight and histopathological analysis. In the ISO control group significant increase in cardiac injury markers indicates necrosis of myocardium and leakiness of plasma membrane (24). The level of uric acid was also significantly increased. This could be due to proteolysis and excessive degradation of purine nucleotides (25). After injection of ISO, enormous amount of reactive oxygen species are generated due to auto-oxidation which reacts with polyunsaturated fatty acids present within cell membrane initiating chain of lipid peroxidation (26). The level of malondialdehyde, a marker of lipid peroxidation was increased and level of antioxidant enzymes like SOD and catalase was decreased in ISO group which indicates oxidative stress.

The plant phenolic compounds and flavonoids provide defense against oxidative stress (27). Flavonoids act as good antioxidants because of their free radical scavenging activity and protect tissue against free radical mediated lipid peroxidation and also chelate metal ions (28). Garcinol is a potent antioxidant present in *G. indica* which has structural similarity to curcumin as it contains both phenolic hydroxyl groups as well as a β-diketone moiety (29). It is a free radical scavenger (30). In our study, administration of aqueous extract of *G. indica* in a dose of 250 and 500 mg/kg for 21 days could not restore the altered parameters produced by ISO. This suggests the lack of cardio protective effect of aqueous extract of *G. indica* at given doses despite of its antioxidant property.

A recently published study by Kumar and coworkers (31) showed cardio protective effect of ethanolic extract of *G. indica* in doses of 250 and 500 mg/kg. This is in contrast to the present study. ISO causes dose dependant myocardial damage. Correlation between injected dose and severity of necrosis produced by ISO has been reported (32). Subcutaneous injection of ISO in various doses (21.2, 42.5, 85, 170, 340, 680 mg/kg) has shown differences in severity of myocardial necrosis. Doses above 85 mg/kg, cause more mortality in experimental animals. 85 mg/kg dose produces massive necrosis in apex and/or papillary muscles and adjacent sub-endocardial parts of left ventricles with an incidence of mortality around 10%. Doses less than 85 mg/kg produce cardiac necrosis with 100% incidence but extent and severity decreases with decreasing dose of ISO. In majority studies, 85 mg/kg ISO is used to induce MI. Hence, we administered 85 mg/kg ISO s.c. to produce MI. Whereas in study conducted by Kumar and colleagues, 25 mg/kg ISO intraperitoneally was used. This may be the reason for contradicting results in the present study. It may also be possible due to the different standards of extracts used in both the studies.

In vitro evaluation showed that aqueous extract of *G. indica* has dose dependent antioxidant potential (33). Various studies on *G. indica* for hepato protective evaluation have been conducted in a dose of 400 mg/kg and 800 mg/kg (34,35). Hepatoprotective effect was found better with dose 800 mg/kg (34,35).

In the present study, doses of 250 and 500 mg/kg of GI were not effective in restoring the myocardial damage caused by ISO may be because of lower dose range selection.

CONCLUSION

Aqueous extract of *G. indica* in doses of 250 mg/kg and 500 mg/kg for 21 days did not show cardioprotective effect in *Wistar* rats. Further evaluation can be planned with higher doses of aqueous extract of *G. indica*.

ACKNOWLEDGMENTS

Authors would like to thank Dr. Tejas K. Patel, Assistant Professor, Pharmacology, GMERS Medical College, Gotri, Vadodara, Gujarat, India for critically scrutinizing the manuscript. They are also thankful to Mr. Praveen Parmar, Ph.D Scholar (Chemistry) who helped a lot in execution, conduction and analyses of antioxidant activity of herbal extract. Special thanks to Central Salt Marine and Research Institute (CSMCRI), Bhavnagar, Gujarat, India for providing facility to carryout antioxidant analysis of heart tissue homogenate.

REFERENCES

1. Abubaker S, Shanmukha I, Jyoti TM, Gupt K. Cardioprotective effect of *Spathodea campanulata* bark on isoproterenol induced myocardial infarction in rats. Asian Pac J Trop Dis. 2012;2:S1-S5.
2. Boudina S, Laclau MN, Tariosse L, Daret D, Gouverneur G, Bonoron-Adèle S, *et al.* Alternation of mitochondrial function in a model of chronic ischemia *in- vivo* in rat heart. Am J Physiol Heart Circ Physiol. 2002;282:H821-H831.
3. Wexler BC. Myocardial infarction in young vs old male rats; pathophysiologic changes. Am Heart J. 1978;96:70-80.
4. Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, *et al.* Development of heart failure following isoproterenol administration in the rat: role of rennin angiotensin system. Cardiovasc Res. 1998;37:91-100.
5. Rajadurai M, Prince PS. Comparative effects of *Aegle marmelos* extract and alphatocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction. Singapore Med J. 2005;46:78-81.
6. Upaganlawar A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: Protective role of natural products. J Pharmacol Toxicol. 2011;6:1-17.
7. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary, 2nd ed. New York, NY: Springer; 2007. p. 278–279.
8. Sharma RK, Dash B. Charaka samhita-text with english translation and critical exposition based on Chakrapani Datta's Ayurveda dipika. India: Chowkhamba Varanasi; 2003.
9. Darji K, Shetgiri P, D'mello PM. Evaluation of antioxidant and antihyperlipidemic activity of extract of *Garcinia indica.* Int J Pharm Sci Res. 2010;1:175-181.
10. Chattopadhyay SK, Kumar S. Identification and quantification of two biologically active polyisoprenylated benzophenones xanthochymol and isoxanthochymol in Garcinia species using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;844:67-83.
11. Liao CH, Ho CT, Lin JK. Effects of garcinol on free radical generation and NO production in embryonic rat cortical neurons and astrocytes. Biochem Biophys Res Commun. 2005;329:1306-1314.
12. Devasagayam TA, Mishra A, Bapat MA, Tilak JC. Antioxidant activity of *Garcinia indica* (kokam) and its syrup. Currt Sci. 2006;91:90-93.
13. Haleagrahara N, Varkkey J, Chakravarthi S. Cardioprotective effects of glycyrrhizic acid against isoproterenol-induced myocardial ischemia in rats. Int J Mol Sci. 2011;12:7100-7113.
14. Fathiazad F, Matlobi A, Khorrami A, Hamedeyazdan S, Soraya H, Hammami M, *et al.* Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L.(basil)against isoproterenol induced myocardial infarction in rats. Daru. 2012;20:87.
15. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol. 2000;72:35-42.
16. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull (Tokyo). 1988;36:2090-2097.
17. Amin I, Zamaliha MM, Chin WF. Total antioxidant activity and phenolic content in selected vegetables. Food Chem. 2004;87:581-586.
18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-3175.
19. Aebi H. Catalase *in-vivo*. Methods Enzymol. 1984;105:121-126.
20. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fractions *in vitro*. General features of the systems used. Biochem J. 1971;123:805-814.

21. Vivek B, Wilson E, Nithya SV, Velmurugan C, Kannan M. Cardioprotective activity of *shilajit* in isoproterenol - induced myocardial infarction in rats: A biochemical and histopathological evaluation. Int J Res Phytochem Pharmacol. 2011;1:28-32.
22. Bloom S, Davis DL. Calcium as a mediator of isoproterenol induced myocardial necrosis. Am J Pathol. 1972;69:459-470.
23. Rahmathulla SB, Lakshmi Devi K. Origination and development of isoproterenol-induced myocardial infarction in male wistar rats. Int Res J Pharm. 2013;4:26-35.
24. Sabeena Farvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK. Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. Pharmacol Res. 2004;50:231-236.
25. Iriama. Uric acid in Ischaemic tissues. Jikeikai Med J. 1987;34:145-168.
26. Priscilla DH, Prince PS. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem Biol Interact. 2009;179:118-124.
27. Amjad L, Shafighi M. Evaluation of antioxidant activity, phenolic and flavonoid content in *Punica granatum* var. Isfahan Malas flowers. Intl J Agri Crop Sci. 2013;5:1133-1339.
28. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, *et al*. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr. 2002;76:560-568.
29. Singh T, Kasture SB, Mohanty PK, Jaliwala Y, Karchuli M. *In vitro* antioxidative activity of phenolic and flavonoid compounds extracted from fruit of Garcinia indica. Int J Pharm Lif Sci. 2011;2:613-616.
30. Sutar RL, Mane SP, Ghosh JS. Antimicrobial activity of extracts of dried kokum (*Garcinia indica* C). Int Food Res J. 2012;19:1207-1210.
31. Kumar V, Gurusamy K, Virndha CA. Cardioprotective activity of *Garcinia indica* Linn. fruit extract on isoprenaline hydrochloride induced cardio toxicity in rats. Int J Pharm Pharm Sci. 2013;5:242-245.
32. Rona G, Chappel CI, Balazs T, Gaudry R. An infarct like myocardial lesion and other true manifestation produced by isoproterenol in the rat. AMA Arch Pathol. 1959;67:443-455.
33. Rawri R, Bharathi K, Jayaveera KN, Asdaq S. *In vitro* antioxidant properties of *Garcinia indica* Linn alcoholic fruits extracts. J Pharm Sci Innov. 2013;2:9-12.
34. Panda VS, Ashar HD, Sharan A. Antioxidant and hepatoprotective effects of *Garcinia indica* fruit rind in antitubercular drug-induced liver injury in rats. Botanics. 2013;3:29–37.
35. Panda V, Ashar H, Srinath S. Antioxidant and hepatoprotective effect of *Garcinia indica* fruit rind in ethanol induced hepatic damage in rodents. Interdiscip Toxicol. 2012;5:207-213.