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Research Article

EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *GARCINIA INDICA* LINN FRUIT RIND

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ABSTRACT

Objective: The aim was to evaluate cardioprotective activity of aqueous extract of *Garcinia indica* Linn fruit rinds.

Methods: Wistar rats were divided into different groups. Two doses 250 mg/kg and 500 mg/kg b.w, p.o of the *G. indica* fruit rind extract (GIFE) were subjected for the evaluation of cardioprotective activity against isoproterenol (ISO) and ischemia reperfusion injury (IRI) induced myocardial damage in rats. Propranolol (10 mg/kg b.w, p.o) was used as a standard drug. The influence of prophylactic treatment was analyzed by quantification of biomarkers and antioxidants, physical parameters, and histopathological observations.

Results: Both GIFE-250 and GIFE-500 showed a significant reduction in creatine kinase (CK) MB fraction, CK-N-acetylcyseine, lactate dehydrogenase in the extract treated rats when compared with positive control. Both the doses showed increase in superoxide dismutase and catalase levels. Significant level of percentage recovery in terms of heart rate and developed tension were seen in all treated groups in IRI model. Cardioprotective effect was also confirmed by histopathology of hearts which showed less necrosis in extract treated rats when compared to untreated rats of toxic control group. The results obtained were comparable with that of the standard. Thus, investigational finding concludes that, the administration of high dose of GIFE was the most effective in alleviating the abnormal conditions induced by ISO and IR.

Conclusion: The present study concluded that *G. indica* Linn fruit rinds were found to be effective against ISO and IR induced myocardial damage in rats.

Keywords: Cardioprotective, *Garcinia indica,* Ischemia reperfusion injury, Isoproterenol, Propranolol.

INTRODUCTION

Ischemia-reperfusion injury (IRI) is a major pathological factor in the natural course of ischemic heart disease, myocardial ischemia develops when coronary blood supply to the myocardium is reduced, either in terms of absolute flow rate or relative to increased tissue demand. The most serious consequence of diminished blood flow to the heart is irreversible myocardia, which occurs when ischemia is extended beyond 20 minutes [1]. Myocardial ischemia, followed by reperfusion results in myocardial IR injury and is characterized by decreased myocardial function, increased incidence of arrhythmias and the development of tissue necrosis. The reperfusion period is beneficial to the heart, but is still associated with myocardial injury [2]. Reperfusion of ischemic myocardium can result in new cellular damage by calcium overload, acidosis, and oxidative stress. A consensus still exists that reactive oxygen species play a significant role in the pathogenesis of cardiac reperfusion injury [3]. Myocardial IR is clinically, relevant in situations such as myocardial infarction, coronary angioplasty, thrombolytic therapy, coronary revascularization and heart transplantation.

According to WHO 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost entirely on traditional medical practices, in particular, herbal medicine for their primary health care needs [4]. Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals [5]. Therefore, several attempts have been made to prevent and treat cardiovascular diseases with various drugs and chemicals by using several antioxidant principles.

Garcinia indica Linn belongs to family Clusiaceae, is a medicinal plant having a long history of usage in Ayurveda. Fruits of *G. indica* were proved for its antibacterial, anticancer, antioxidant, anti-diarrheal, hepatoprotective and anti-inflammatory activity. *G. indica* fruit consists of phytochemical constituents such as alkaloids, flavonoids, tannins, alkaloids, glycosides, saponins, etc. [6]. However, there is a paucity of scientific data for the cardioprotective activity of *G. indica*. Based on its use in Ayurveda for the treatment of cardiac ailments [7]. It is hypothesized that *G. indica* may possess cardioprotective potential, which needs to be investigated. Hence, this study was designed to evaluate the cardioprotective activity of aqueous extract of *G. indica* Linn fruit rind using isoproterenol (ISO) induced myocardial necrosis in rats and IRI in rats.

METHODS

Collection and identification of plant materials

The fruits of *G. indica* Linn used for the present study were collected from Local Ayurvedic Pharmacy, Mangalore, in March 2014. The fruits samples were authenticated by Mr. M Dinesh Nayak (Green Belt) Botnist, MSEZL, Mangalore, Karnataka. The fruit rinds were dried under shade. The shade dried fruit rinds were pulverized separately into a coarse powder by a mechanical grinder and were used for preparation of aqueous extract.

Chemicals and instruments

All chemicals and solvents used in the study were of analytical grade. The DL-ISO hydrochloride (Sigma Aldrich, Germany), all estimation kits creatine kinase-MB fraction (CK-MB), CK-N-acetylcyseine (NAC), lactate dehydrogenase (LDH) (Agapee) were obtained from their respective distributers. Instruments like U.V (Shimadzu), Semi auto analyzer (Mispa-plus), Physiograph (INCO, India) were used for the present study.

Preparation of *G. indica* fruit extract (GIFE)
The powdered material was subjected to batch extraction using warm water as a solvent. The powdered material (1250 g) of *G. indica* fruit rind were packed and extracted by using a water solvent for 36 hrs by maceration process. The extract was concentrated by using rotary flash evaporator. The concentrated extract was then air-dried at room temperature, weighed and percentage yield was calculated.

Experimental animals
Healthy Wistar albino rats (150-200 g) of either sex were used for the experiment. They were maintained under standard conditions (temperature 22±2°C, relative humidity 60±5% and 12 hrs light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (Approval No. SCP/CPCSEA/P02/F150/2013).

ISO induced myocardial necrosis in rats [8]
Wistar rats of either sex weighing between 150 and 200 g were divided into five groups of six animals each.

Group I: Normal control (saline, 1 ml/kg for 30 days).
Group II: Toxic control ISO (85 mg/kg, i.p.) on 29th and 30th day.
Group III: Rats pre-treated with propranolol (10 mg/kg, p.o.) 7 days before the induction of myocardial infarction with ISO (85 mg/kg, i.p).
Group IV: Rats were pretreated with low dose of *G. indica* extract (250 mg/kg, b.w.) orally for 28 days before the induction of myocardial infarction with ISO (85 mg/kg, i.p) on 29th and 30th day of experimental period.
Group V: Rats were pretreated with low dose of *G. indica* extract (250 mg/kg, b.w.) orally for 28 days before the induction of myocardial infarction with ISO (85 mg/kg, i.p) on 29th and 30th day of experimental period.

Blood sample collection
48 hrs after the first dose of ISO, the animals were anesthetized with ketamine (75 mg/kg, i.p.) and xylazine (8.0 mg/kg, i.p.) and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of biomarkers.

Biochemical assay in the serum and heart tissue homogenate
Estimation of serum enzymes: LDH, CK-MB and CK-NAC in serum were estimated using commercially available kit (Agapee assay kit) [9]. Animals were sacrificed, and three hearts were used for the preparation of homogenate to estimate antioxidants superoxide dismutase (SOD) and catalase (CAT). Remaining three hearts were embedded in formal saline for histopathological examination.

Isolated rat heart preparation for IRI [10]

Perfusion technique and perfusion medium

For the perfusion of isolated rat heart, rats were injected with 92 units of heparin (i.p) 15 minutes prior to the administration of anesthetics ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p). The heart was exposed and excised quickly.

The heart was immediately placed in China dish containing carbogenated K-H solution. The preparation was lightly squeezed to remove as much as blood possible. The heart was mounted on a modified Langendorff's apparatus through the aorta and perfused with modified Krebs–Heinseleit (KH) buffer solution (pH 7.4) bubbled with carbogen (95% O_2 + 5% CO_2); at a constant flow rate of 5.5 ml/minutes using a regulated perfusion pump (peristaltic pump).

The pressure of the perfusion fluid exhibits retrograde flow in the aorta, where it closes the leaflets of the aortic valve. A fine thread was tied to the apex of the heart and passed through a thermostatically controlled water-jacketed moist chamber and through a pulley to the force transducer. In the thermostatic chamber, the heart was allowed to equilibrate for 10 minutes then the initial pre ischemic period of 15 minutes and then regular recording were taken for perfusion period of 15 minutes. Measurement of contractile force was done with force displacement transducer and recorded in the physiograph. After the initial pre ischemic period of 15 minutes, the heart was subjected to KH solution. The recording of contractile force and heart rate (HR) was done. After pre ischemic period, the heart was subjected 15 minutes of global no-flow ischemia by blocking the flow of drug incorporated in carbogenated modified KH solution. Perfusate was collected at post ischemic and was used for the determination of diagnostic marker enzymes. The heart was removed and weighed, subjected for either heart tissue homogenate preparation or histopathological investigation. Susceptibility or vulnerability of the ischemic heart to reperfusion injury was estimated by comparing the pre and post reperfusion effect among the treatment group with respect to control.

Wistar rats of either sex weighing between 150 and 200 g were divided into four groups of four animals each.

Group I: IRI control.
Group II: Propranolol -10 mg/kg was given for last 7 days and subjected to IRI.
Group III: GIFE (low dose: 250 mg/kg) GIFE was given for 28 days and subjected to IRI.
Group IV: GIFE (high dose: 500 mg/kg) GIFE was given for 28 days and subjected to IRI.

The heart was allowed to stabilize for initial 15 minutes and after stabilization the heart was subjected to a period of 15 minutes of global no-flow ischemia by blocking the complete energy and oxygen supply, by switching off the flow of physiological salt solution (PSS) followed by reperfusion for 15 minutes.

The following parameters were estimated:

- HR, biomarkers, *in-vivo* antioxidant and histopathological study was done.

Statistical analysis
All data were expressed as mean±standard error mean. The statistical significance between groups was compared using one-way ANOVA, followed by Dunnett's (multiple comparison tests).

RESULTS

ISO induced myocardial necrosis in rats

Relative heart weight (ROW) analysis in ISO induced myocardial necrosis

ROW was decreased in ISO treated animals when compared to normal control. There was extremely significant increase in heart weight in propranolol pre-treated animals. The animals pre-treated with GIFE-250 showed moderately significant increase in heart weight. The results are summarized in Table 1.

Effect on serum CK-MB, CK-NAC and LDH in ISO induced myocardial necrosis
Serum levels of cardio specific enzymes such as CK-MB, CK-NAC and LDH, were elevated in ISO treated animals. When compared to normal control. The prophylactic treatment with propranolol showed extremely significant reduction in marker enzyme CK-MB. The animals pre-treated with GIFE-250 showed less significant reduction in CK-MB level, whereas GIFE-500 treated animals showed extremely significant reduction in marker enzyme CK-MB level as compared to the positive control group.

The prophylactic treatment with propranolol showed extremely significant reduction in marker enzymes CK-NAC level. The animals pre-treated with GIFE-250 showed moderately significant in CK-NAC level whereas GIFE-500 treated animals showed extremely significant

reduction in marker enzyme CK-NAC level when compared to the positive control group.

The prophylactic treatment with propranolol showed extremely significant reduction in marker enzyme LDH levels. The animals pre-treated with GIFE-250 showed extremely significant reduction in marker enzyme LDH level where as GIFE-500 treated animals showed extremely significant reduction in marker enzyme LDH levels as compared to the positive control group. The results are summarized in Table 2.

Effect on antioxidant parameters like SOD and CAT in ISO induced myocardial necrosis

Animals in ISO treated group developed a myocardial damage observed as decrease in CAT and SOD when compared to normal control. Animals treated with propranolol showed extremely significant value. Pre-treated with GIFE-250 showed moderately significant increase in SOD. Whereas, GIFE-500 showed extremely significant increase in SOD as compared to positive control.

Animals treated with propranolol showed extremely significant increase in CAT. Pre-treated with GIFE-250 showed moderately significant increase in CAT. Whereas, GIFE-500 showed extremely significant increase in CAT as compared to positive control. Results are summarized in Table 3.

IRI model

The biomarkers such as LDH, CK-MB and CK-NAC were estimated in perfusate, while SOD, catalase were estimated in heart tissue homogenate. The biomarkers and antioxidants activity of propranolol, GIFE-250 and GIFE-500 was compared with IRI control.

Effect of propranolol and GIFE on perfusate CK-MB, CK-NAC and LDH in IR induced myocardial injury

Levels of cardio specific enzymes like CK-MB, CK-NAC and LDH in perfusate were elevated in IRI.

The prophylactic treatment with propranolol showed extremely significant reduction in marker enzyme CK-MB. The animals pre-treated with GIFE-250 showed less significant reduction in CK-MB level where as GIFE-500 treated animals showed extremely significant reduction in marker enzyme CK-MB level as compared to IRI control group.

Table 1: Effects of GIFE and propranolol on ROW in isoproterenol induced myocardial infarction

Groups	Treatment	ROW (g/100 g)
Normal control	Saline	0.34±0.016
Positive control	ISO 85 mg/kg, s.c.	0.24±0.014
Standard	Propranolol 10 mg/kg, p.o	0.32±0.014***
Low dose	GIFE-250 mg/kg, p.o	0.27±0.033**
High dose	GIFE-500 mg/kg, p.o	0.30±0.016***

All the values are in absorbance mean±SEM, % inhibition shown in parentheses, n=6, ns: p>0.05, **p<0.05, ***p<0.001 one-way ANOVA followed by Dunette's test compared to positive control, ROW: Relative heart weight, GIFE: *Garcinia indica* fruit rind extract, ISO: Isoproterenol, SEM: Standard error mean

The prophylactic treatment with propranolol showed extremely significant reduction in marker enzymes CK-NAC level. The animals pre-treated with GIFE-250 showed less significant in CK-NAC level where as GIFE-500 treated animals showed extremely significant reduction in marker enzyme CK-NAC level as compared to IRI control group.

The prophylactic treatment with propranolol showed extremely significant reduction in marker enzyme LDH levels. The animals pre-treated with GIFE-250 showed moderately significant reduction in marker enzyme LDH level where as GIFE-500 treated animals showed extremely significant reduction in marker enzyme LDH levels as compared to IRI control group. The results are summarized in Table 4.

Effect of propranolol and GIFE on SOD and CAT in IR induced myocardial injury

Animals in IRI control group developed a myocardial damage observed as decrease in CAT and SOD. Animals treated with standard (propranolol) showed extremely significant increase in SOD. Pre-treated with GIFE-250 showed extremely significant increase in SOD. Pre-treated with GIFE-500 showed extremely significant increase in SOD as compared to IRI control group. Animals treated with propranolol showed extremely significant increase in CAT. Pre-treated with GIFE-250 showed extremely significant increase in CAT. Pre-treated with GIFE-500 showed extremely significant increase in CAT as compared to IRI control group.

Results are summarized in Table 5.

Effect on developed tension and HR

Global no flow ischemia for 15 minutes resulted in fall in developed tension and HR compared to pre ischemic condition. Oral pre-treatment of animals with propranolol showed extremely significant increase in percentage recovery of developed tension and HR compared to IRI control. Similarly, less significant increase in percentage recovery of diastolic time (DT) and significant increase in HR were observed in group treated with GIFE-250 when compared to IRI control. Oral pre-treatment of GIFE-500 revealed in extremely significant increase in

Table 3: Effect on antioxidant parameters like SOD and CAT in ISO induced myocardial necrosis

Groups	Treatment	SOD (Abs at 560 nm)	CAT (Abs at 620 nm)
Normal control	Saline	0.81±0.02	0.46±0.02
Positive control	ISO 85 mg/kg, s.c.	0.14±0.06	0.13±0.02
Standard	Propranolol 10 mg/kg, p.o	0.62±0.02*** (+87.66)	0.34±0.01*** (+87.76)
Low dose	GIFE 250 mg/kg, p.o	0.26±0.03** (+81.33)	0.21±0.02** (+52.84)
High dose	GIFE 500 mg/kg, p.o	0.56±0.03*** (+85.82)	0.39±0.02*** (+73.53)

All the values are in absorbance mean±SEM, % inhibition shown in parentheses, n=6, **p<0.01, ***p<0.001 one-way ANOVA followed by Dunette's test compared to positive control, SOD: Superoxide dismutase, CAT: Catalase, GIFE: *Garcinia indica* fruit rind extract, ISO: Isoproterenol, SEM: Standard error mean, Abs: Absorbance

Table 2: Effect of propranolol and GIFE on serum CK-MB, CK-NAC, LDH in ISO induced myocardial necrosis

Groups	Treatment	CK-MB (U/L)	CK-NAC (U/L)	LDH (U/L)
Normal control	Saline	60.30±7.633	154.6±17.32	283.20±22.07
Positive control	ISO 85 mg/kg , s.c	235.60±30.37	326.6±18.94	727.9±34.73
Standard	Propranolol 10 mg/kg , p.o	92.65±10.27***	197.2±15.24***	329.7±18.41***
Low dose	GIFE 250 mg/kg, p.o	205.0±12.19*	296.8±16.71*	552.0±21.80***
High dose	GIFE 500 mg/kg, p.o	114.30±10.87***	224.7±16.69***	435.2±17.94***

All the values are mean±SEM, n=6, ns: p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunette's test compared to positive control. ROW: Relative heart weight, GIFE: *Garcinia indica* fruit rind extract, ISO: Isoproterenol, SEM: Standard error mean, CK-MB: Creatine kinase-MB fraction, CK-NAC: Creatine kinase-N-acetylcyseine, LDH: Lactate dehydrogenase

percentage recovery of DT and HR respectively when compared to IRI control. Results are summarized in Table 6.

DISCUSSION

The current research was designed to evaluate the cardioprotective activity of GIFE against the ISO induced and IR induced myocardial damage in Albino Wistar rats. The findings of the investigations witnessed the beneficial role of aqueous extract of *G. indica* when treated in conditions of anticipated cardiac injury. ISO, a synthetic catecholamine and β-adreno receptor stimulant, is known to cause myocardial damage at higher concentrations [11]. ISO is also known to generate free radical and to stimulate lipid peroxidation, which probably leads to the irreversible damage of the myocardial membrane. ISO administration results in increase in calcium uptake and energy consumption leading to cell death [12]. It is well-established that the biological markers like endogenous enzyme are organ specific and leak from the damaged organ during necrosis. Different cardiac specific biomarker enzymes such as CK-MB, LDH and CK-NAC elevation in serum is due to the leakage from the heart as a result of ISO - induced necrosis [13]. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane. This alters membrane permeability, leading to the loss of function and integrity of myocardial

Table 4: Effect of propranolol and GIFE on perfusate CK-MB, CK-NAC and LDH in IR induced myocardial injury

Groups	Treatment	CK-MB (U/L)	CK-NAC (U/L)	LDH (U/L)
Normal control	Saline	60.6±10.2	85.20±15.3	125.10±20.2
IRI control	IRI	192.8±13.33	243.20±18.82	320.30±14.22
Standard	Propranolol 10 mg/kg, p.o	66.80±12.04***	78.58±0.37***	107.50±7.046***
Low dose	GIFE 250 mg/kg, p.o	166.2±8.265*	212.10±18.05*	276.00±16.16**
High dose	GIFE 500 mg/kg, p.o	115.8±13.02***	139.60±11.10***	203.50±14.90***

All the values are mean±SEM, n=6, ns: p>0.05, *p<0.05, **p<0.01, ***p<0.001 one-way ANOVA followed by Dunette's test compared to positive control. IR: Ischemia reperfusion, GIFE: *Garcinia indica* fruit rind extract, ISO: Isoproterenol, SEM: Standard error mean, CK-MB: Creatine kinase-MB fraction, CK-NAC: Creatine kinase-N-acetylcyseine, LDH: Lactate dehydrogenase

Table 5: Effect of propranolol and GIFE on SOD and CAT in IR induced myocardial injury

Groups	Treatment	SOD (Abs @ 560 nm)	CAT (Abs @ 620 nm)
Normal control	Saline	0.96±0.02	0.57±0.03
IRI control	IRI	0.24±0.02	0.08±0.02
Standard	Propranolol (10 mg/kg, p.o)	0.86±0.02***	0.44±0.02***
Low dose	GIFE 250 mg/kg, p.o	0.56±0.55***	0.24±0.01***
High dose	GIFE 500 mg/kg, p.o	0.74±0.02	0.34±0.02***

All the values are mean±SEM, n=6, ns: p>0.05, *p<0.05, **p<0.01, ***p<0.001 one-way ANOVA followed by Dunette's test compared to positive control. IR: Ischemia reperfusion, GIFE: *Garcinia indica* fruit rind extract, ISO: Isoproterenol, SEM: Standard error mean, IRI: Ischemia reperfusion injury, SOD: Superoxide dismutase, CAT: Catalase, Abs: Absorbance

Table 6: Effect on developed tension and HR in IR induced myocardial injury

Groups	Treatment	Percentage recovery	
		Developed tension	HR
Normal control	Saline	-	-
IRI control	IRI	43.64±7.910	35.85±9.820
Standard	Propranolol 10 mg/kg, p.o	84.64±2.979***	97.95±2.146***
Low dose	GIFE 250 mg/kg, p.o	54.91±5.313*	60.84±7.606***
High dose	GIFE 500 mg/kg, p.o	80.96±3.764***	83.50±2.991***

All the values are in absorbance mean±SEM, % inhibition shown in parentheses, n=6, ns: p>0.05, **p<0.01, ***p<0.001 one-way ANOVA followed by Dunette's test compared to positive control, IR: Ischemia reperfusion, GIFE: *Garcinia indica* fruit extract, SEM: Standard error mean, HR: Heart rate

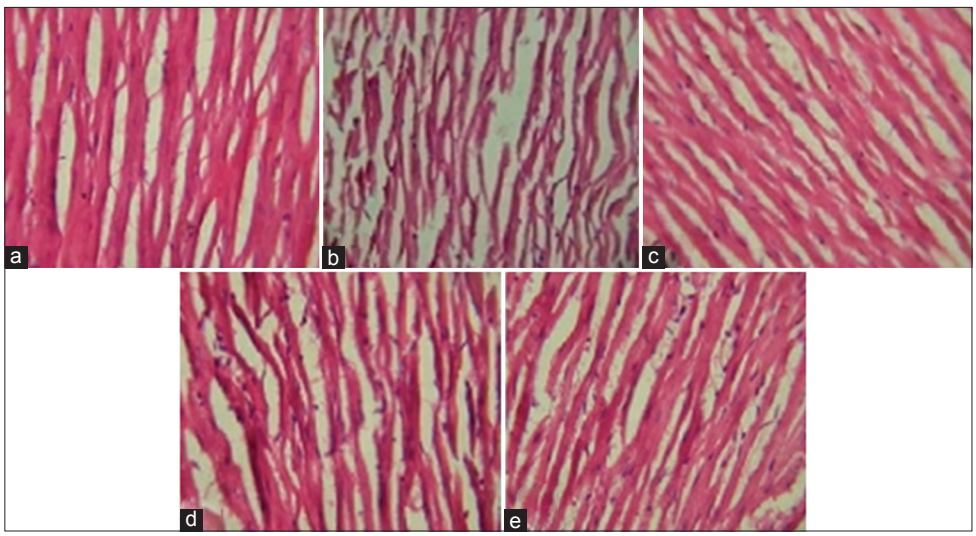

Fig. 1: H and E stained section of heart in isoproterenol (ISO) induced myocardial necrosis, (a) Normal control: Normal texture of heart tissue, (b) positive control (ISO treated): Severe tissue degeneration and necrosis, (c) standard (propranolol): Mild tissue degeneration and necrosis, (d) low dose (*Garcinia indica* fruit extract [GIFE] -250): Moderate heart tissue degeneration and necrosis, (e) high dose (GIFE 500): Mild to moderate tissue degeneration and necrosis

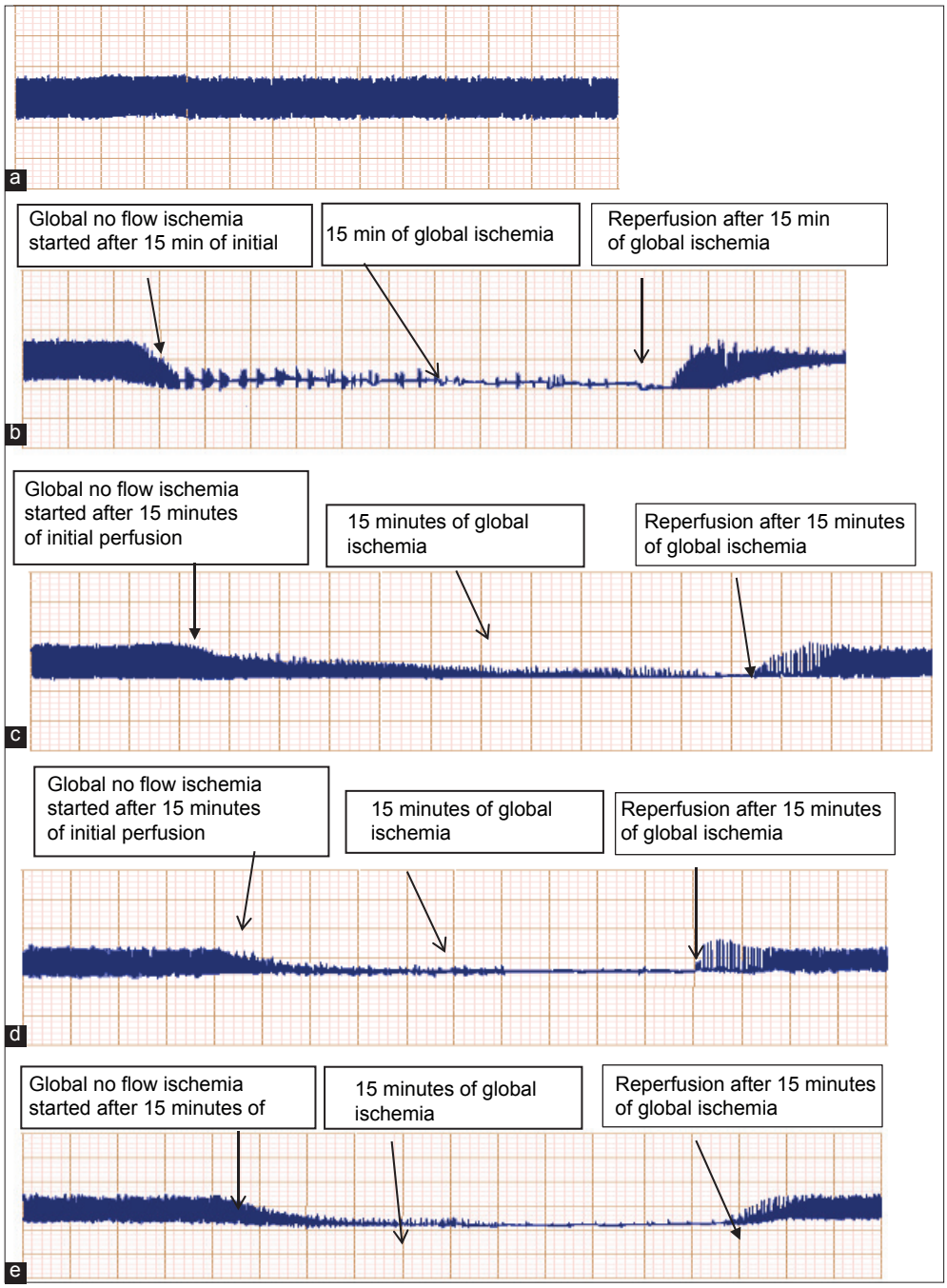

Fig. 2: The effects of drug on ischemia reperfusion on isolated heart of animals, (a) Tracing on polygraph of normal heart, (b) tracing on polygraph of ischemia reperfusion injury control, (c) tracing on polygraph of propranolol (10 mg/kg) treated heart, (d) tracing on polygraph *Garcinia indica* fruit extract (GIFE) - 250 treated heart, (e) tracing on polygraph GIFE-500 treated heart

membranes. Hence, leakage of endogenous biological markers and free radical formation are attributed for myocardial distress in post ISO administration [14]. The activity of GIFE was observed in dose-dependent manners with reference to myocardium protection by scavenging oxidative free radicals and diminishing the permeability of these endogenous biomarkers to surrounding cardiac regions. Propranolol blocks beta-adrenergic receptors and prevents ISO - mediated hyper stimulation of sympathetic system and was used as a standard drug. Excessive activation of sympathetic system by ISO accompanied by vagal hypo activity produces severe myocardial damage [12].

In IRI model induction of myocardial injury was done by stopping the flow of carbogenated KH that produce ischemia followed by reperfusion, which further increases the extent of damage. Sudden occlusion of PSS results in immediate biochemical alterations [15]. The increase in intracellular Na^+ serves to drive Ca^{2+} intracellularly via $Na+/Ca^{2+}$ exchange that result in irreversible damage to myocardium at the end of 15 minutes global ischemia [16].

In the present study, it was observed that the oral pre-treatment of GIFE-250 and GIFE-500 decreases the biomarker activities in the perfusate and in the heart tissue homogenate. Myocardial ischemia also resulted in diminished activities of SOD and CAT in heart tissue homogenate that were reverted back close to the normal level with GIFE-500. GIFE-250 pre-treatment resulted as moderately significant percentage recovery in developed tension and GIFE-500 pre-treatment

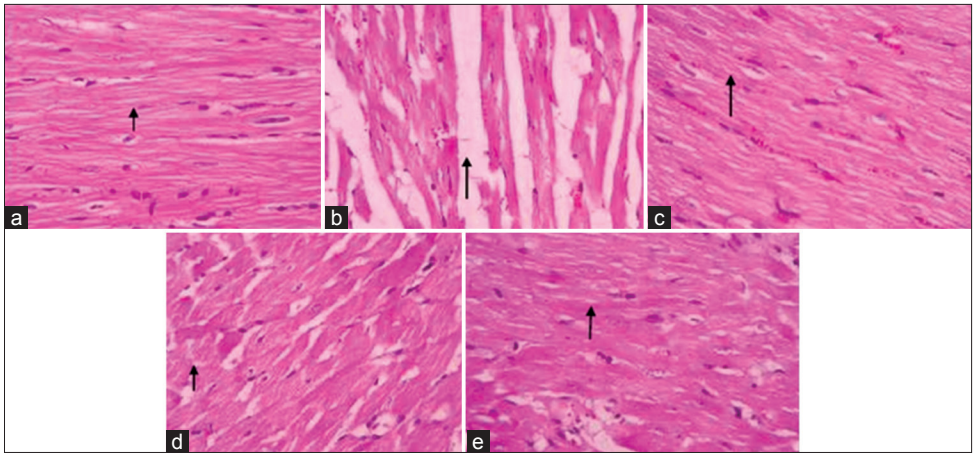

Fig. 3: H and E stained section of heart in ischemia reperfusion injury model (photographed at magnification ×40), (a) Normal control: Normal texture of heart tissue, (b) positive control (ischemia reperfusion injury): Severe tissue degeneration and necrosis, (c) Propranolol: Mild tissue degeneration and necrosis, (d) low dose (*Garcinia indica* fruit extract [GIFE] - 250): Moderate heart tissue degeneration and necrosis, (e) high dose (GIFE-500): Mild to moderate tissue degeneration and necrosis

resulted as extremely significant percentage recovery in developed tension. Propranolol also showed its extremely significant activity by attributing its antioxidant effect [17].

The histopathological studies of the heart tissue evident in myocardial edema and separation of fibers with loss of striation as mark of myocardial injury in IRI control. The myocardial edema and separation of fibers were ameliorated with treatment of GIFE-500. The preliminary phytochemical study indicates the presence of flavonoids, tannins, alkaloids and other poly phenolic compounds in the extract. The previous study showed that the plants, which possessing flavonoids, tannins, etc. are known to exhibit potential cardioprotective activities [5]. Therefore, in the present study the presence of rich source of bioflavonoids and tannins in the extract may attribute to potential cardioprotective effect through its antioxidant properties.

CONCLUSION

GEFE showed cardioprotective effect possibly by reverting back the level of cardiac biomarkers and biochemical parameters and by modulating tissue lipid peroxidation and augmenting endogenous antioxidant (CAT, SOD) defense mechanisms. There is direct correlation between *in-vivo* antioxidant and cardioprotective activity.

Histopathological observation revealed that treatment with GEFE has reversed the cardiac damage more effectively in ISO induced damaged and reasonably in IR induced damaged. The exact mechanism for the cardioprotective activity of GEFE is still not clear and further studies are needed to isolate, characterize the active principles and to find out the mechanism responsible for its cardioprotective activity.

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