

Cardioprotective Activity of Leaves of Cassia Tora on Isoproterenol Induced Myocardial Injury

Nagarathna P. K. M¹, S. Praveen Kumar², Sudheer.M³

Dept. Of Pharmacology, Karnataka College of pharmacy, Bangalore, Karnataka, India.

Available online:, 1st December 2013

ABSTRACT

Cassia tora is an herbal medicinal plant and it is used to cure different disorders. The present study was involved in cardioprotective activity of leaves of Cassia tora on ISO induced myocardial injury. Male wistar rats are divided into five groups of six animals each. All the grouped rats were pre-treated with the extract and standard (propranolol) either s.c. or orally for 1 week. Then, they were given 5.25 and 8.5 mg/kg isoproterenol s.c. on two consecutive days. Early treatment with extract of Cassia tora 100mg/kg, 200 mg/kg showed significant decrease in level of serum marker enzymes aspartate transaminase (AST), lactate dehydrogenase (LDH), alanine transaminase (ALT), alkaline phosphatase (ALP), changes in the oxidative stress markers like lipid peroxidase (LPO), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) caused by ISO (5.25 and 8.5). the defence action of leaves of Cassia tora concluded by observing histopathology and are more consistent at 200mg/kg. Hence, we conclude that pretreatment with extract of leaves of Cassia tora more barrier against the ISO induced myocardial injury.

Key words: cardioprotective, isoproterenol, Cassia tora, antioxidant, myocardial ischemia.

INTRODUCTION

Myocardial infarction is ischemic necrosis of portion of myocardium due to sudden occlusion of branch of coronary artery. It is the one of serial cause of the death in US and other developed countries. Main risk factors for the MI is the atherosclerosis of coronary artery¹, calcium reduction, generation of free radicals, oxidative metabolism of catecholamines, these oxidative products impact on the cardiac myocyte membrane and also depress the cardiac contractile function, prior to which damage in the mitochondria, sarcotubular system and contractile functions². Previous literature has shown that ISO causes the cardiotoxicity by increasing the generation of free radicals via a different oxidative products³. These oxidative free radicals may increase the membrane permeability by promoting the lipid peroxidation, which leads to the cardiac injury⁴. Cassia tora Linn. (Caesalpinaceae) is a small annual herb or under shrub growing as common weed in Asia countries. It is found as a weed throughout India, universally in wild state in Himachalpradesh, Bihar, and Orissa. Constituent an ayurvedic preparation "dahughnavati" which is one of the successful antifungal formulations.^{5,6} It is a well known ayurvedic medicinal plant as a laxative, antiperiodic and is useful for leprosy, ringworm, bronchitis, and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic, haemorrhoids. It was reported that leaves of Cassia tora has antioxidant activity and contain many active substances including chryso phenol, emodin, rhein etc.⁷ Many medicinal properties such as

antimicrobial, antihepatotoxic and antimutagenic activities have been attributed to this plant.

MATERIALS AND METHODS

Acute Toxicity Test: Acute toxicity test of leaves of *Cassia tora* was done by following OPPTS guidelines by up and down method.

Plant material – Cassia tora was collected from Tirupathi located in Chittoor district of Andhra Pradesh.

Preparation of Extracts: Powdered leaves were subjected to successive extraction in a soxhlet extractor with methanol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get a constant weight.

Experimental Animals: In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220g were included for the study. Animals were housed in polypropylene cages on clean paddy husk bedding. Animals were maintained under controlled temperature at 25°C ± 2°C with 12hr light/dark cycle. All animals were a free access to food and water *ad libitum*.

Administration of Drug: The extract was suspended in 1% w/v CMC and the suspension was given orally to the animals once daily for seven days. Two doses were selected depending on the toxicity profile of the extract upon the oral treatment.

Methodology: Male Wistar rats were divided in to 5 groups, each group have six animals.

Group1: Normal control

Group2: Animals were treated with isoproterenol (5.25 and 8.5mg/kg)

Effect of flavonoid of leaves of *Cassia tora* on plasma total cholesterol, TG, LDL, HDL, AST, ALT, ALP, levels in ISO induced myocardial necrosis in rats.

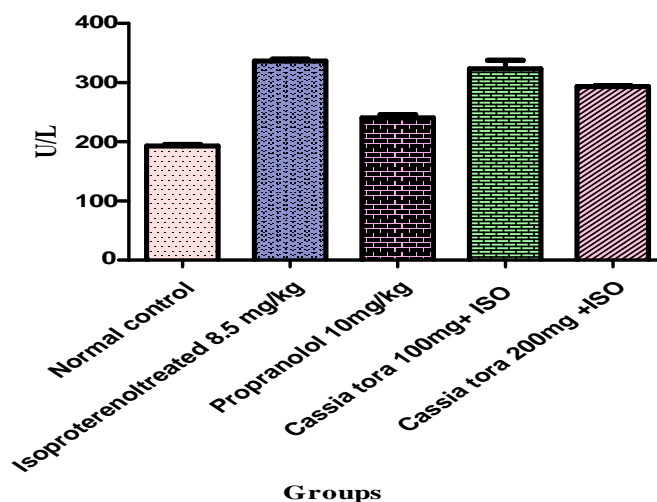
Treatment group	LDH	TG	TC	LDL	HDL	AST	ALT	ALP
Normal control	192.7 ± 2.64***	73.09 ± 0.695**	205.9 ± 1.69***	181.2 ± 1.55***	51.95 ± 0.80**	17.01 ± 1.59***	14.61 ± 1.54***	95.21 ± 1.18**
ISO treated 8.5 mg/kg	336.6 ± 2.95***	120.7 ± 1.18***	264.8 ± 0.95***	237.7 ± 0.63***	23.36 ± 0.00***	51.02 ± 2.05***	53.34 ± 1.22***	333.0 ± 13.26***
Propranolol treated 10 mg/kg	240.5 ± 5.31	77.68 ± 1.17	219.4 ± 2.95	192.1 ± 3.02	45.77 ± 1.67	30.89 ± 1.57	31.76 ± 0.68	166.6 ± 20.50
Cassia tora 100mg/kg	323.2 ± 14.39***	94.99 ± 0.78***	245.8 ± 0.69***	219.6 ± 0.94***	37.16 ± 0.66***	41.16 ± 1.16***	51.50 ± 0.87***	242.2 ± 10.86***
Cassia tora 200 mg/kg	293.1 ± 1.80***	87.02 ± 0.77***	210.8 ± 2.15*	180.7 ± 1.05***	40.75 ± 1.96*	17.63 ± 1.24***	22.07 ± 0.84***	103.0 ± 1.92**

Data was analysed using one way ANOVA followed by Dunnett's *t* test.

*P<0.05, **P<0.01, ***P<0.001, n = 6

LDH:

LDH effect in flavonoid of leaves of cassia tora in ISO induced myocardial necrosis in rats



ISO and pre-treated with flavonoid of leaves of *Cassia tora* group (200mg/kg, b.w. p.o) showed significant reduction in LDH ($P<0.001$) levels when compared to ISO-treated group.

Group3: Animals were treated with standard drug propranolol (10mg/kg)

Group4: Animals were treated with test drug (100mg/kg)

Group5: Animals were treated with test drug (200mg/kg)

All the grouped rats were pre-treated with the extract and standard either s.c. or orally for 1 week. Then, they were given 5.25 and 8.5 mg/kg iso preterenol s.c. on two consecutive days. Symptoms and mortality in each group are recorded and compared with those of rats given isoproterenol alone. Forty-eight hours after the first isoproterenol administration, the rats are sacrificed and autopsied. The hearts are removed and weighed, and frontal sections embedded for histological examination⁸. Biochemical assessment marker enzymes in serum. Twelve hours after the second injection of ISO, the animals were sacrificed by cervical decapitation, blood was collected and the heart was dissected out. The serum was separated immediately by cold centrifugation and used for determination of the myocardial infarction marker

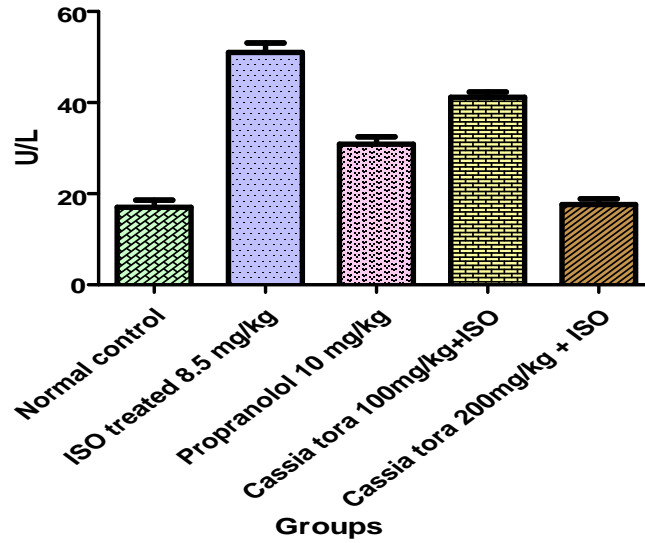
enzymes LDH, AST, ALT, and ALP along with serum total cholesterol, triglycerides, LDL, and HDL⁹. Statistical analysis can be carried out by one way ANOVA method.

RESULTS AND DISCUSSION

Section studied from the myocardium shows intact architecture comprising of cardiac muscle fibers with intervening vascular spaces and interstitium [Fig.1, arrow]. The cardiac muscle fibers show integrity of myocardial cell membrane consisting of myofibrillar structure with striations and continuity with adjacent myofibrils [Fig.2, arrow]. The interstitial space appears within normal limits. Section studied from the myocardium shows haphazard arrangement of the cardiac muscle fibers. The cardiac muscle fibers show necrosis consisting of loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils [Fig.2, arrow]. The interstitial space appears increased [Fig.1, Arrow]. There are seen scattered

AST:

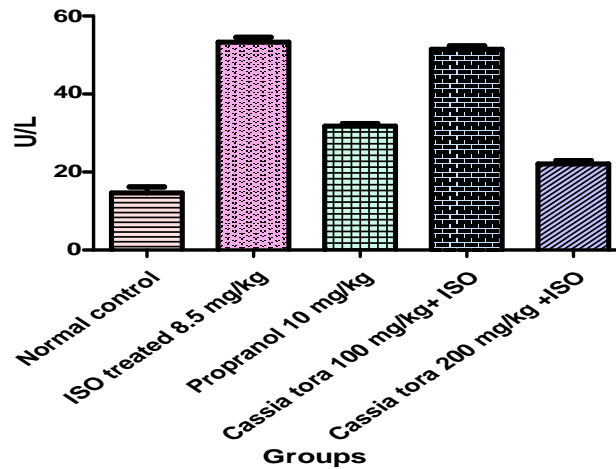
AST effect in flavonoid of leaves of cassia tora in ISO induced myocardial necrosis rats



ISO and pre-treated with flavonoid of leaves of Cassia tora group (200mg/kg, b.w. p.o) showed significant reduction in AST ($P<0.001$) activities when compared to ISO-treated group.

ALT:

ALT effect in flavonoid of leaves of cassia tora in ISO induced myocardial necrosis in rats



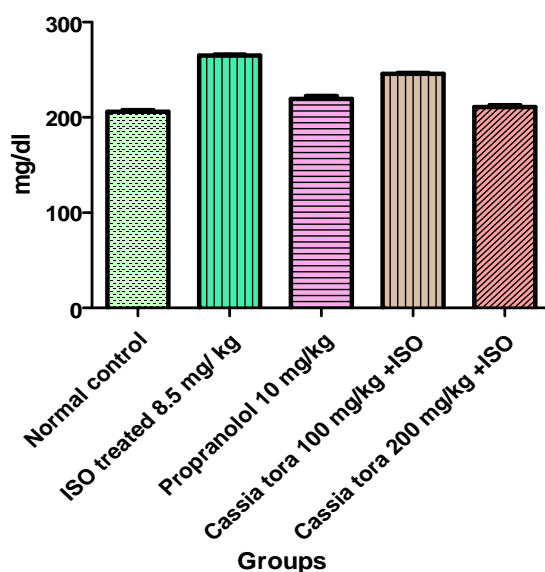
ISO and pre-treated with flavonoid of leaves of Cassia tora group (200mg/kg, b.w. p.o) showed significant reduction in ALT ($P<0.001$) activities when compared to ISO-treated group.

inflammatory infiltrations and some damaged vascular spaces amidst these cardiac muscle fibers. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers. These cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils [Fig.1, arrow]. The interstitial space appears mildly increased [Fig.2, Arrow] at focal areas. There are seen mild inflammatory infiltrations and thrombosed vascular spaces amidst these cardiac muscle fibers. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers. These cardiac muscle fibers show necrosis consisting of loss of integrity

of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils [Fig.2, Short-Arrow]. The interstitial space appears moderately increased [Fig.2, Long-Arrow] at focal areas. There are seen some proliferating vascular spaces amidst these cardiac muscle fibers [Fig.1, Arrow]. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers. These cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils [Fig.1, arrow]. The interstitial space appears mildly increased [Fig.2, Arrow] at focal areas. There are seen congested vascular spaces amidst these cardiac muscle fibers.

Cholesterol:

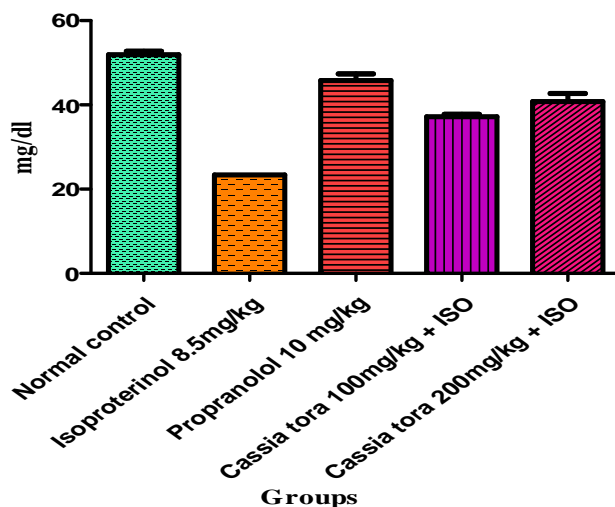
Cholesterol in effect of flavonoid of leaves of cassia tora in ISO induced myocardial induced necrosis in rats



ISO and pre-treated with flavonoid of leaves of Cassia tora group (200mg/kg, b.w. p.o) showed significant reduction in cholesterol ($P < 0.001$) activities when compared to ISO-treated group.

HDL- Cholesterol:

HDL effect in flavonoid of leaves of cassia tora in ISO induced myocardial necrosis in rats



ISO and pre-treated with flavonoid of leaves of Cassia tora group (200mg/kg, b.w. p.o) showed significant increase in HDL- cholesterol ($P < 0.001$) activities when compared to ISO-treated group.

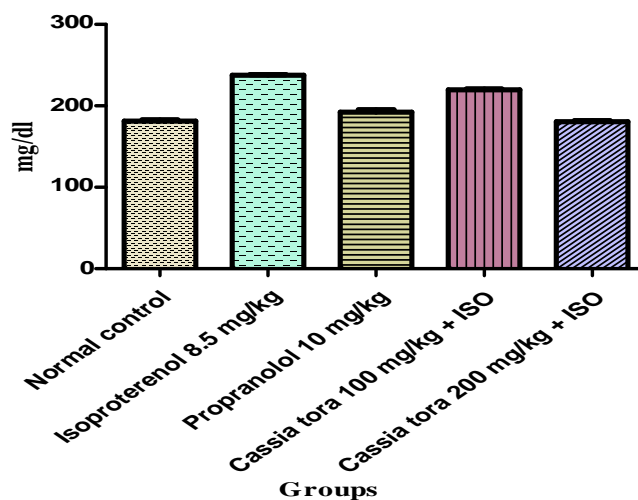
DISCUSSION

Reactive oxygen species (ROS) are formed at an accelerated rate in ISO-treated myocardium. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis. 'Infarct-like' lesions are produced in the myocardium when injected with ISO. myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemmal membrane, followed by stimulation of adenylate cyclase, activation of Ca^{2+} and

Na^+ channels, exaggerated calcium inflow and excess of excitation-contraction coupling mechanism leading to energy consumption and cellular death. Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. The metabolic damage of myocardium results in increase in the concentration of the marker enzymes like LDH, AST, and ALP. The CAT and SOD were increases

LDL- Cholesterol:

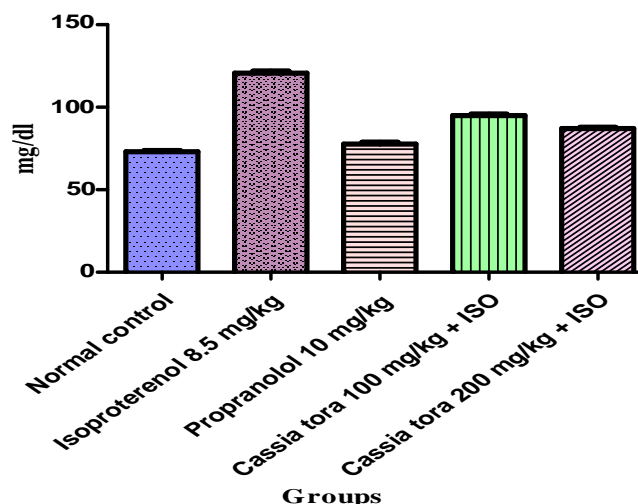
LDL effect in flavonoid of leaves of *Cassia tora* in ISO induced myocardial necrosis in rats



ISO and pre-treated with flavonoid of leaves of *Cassia tora* group (200mg/kg, b.w. p.o) showed significant reduction in LDL-cholesterol ($P < 0.001$) activities when compared to ISO-treated group.

Triglycerides:

TG in effect of flavonoid of leaves of *Cassia tora* in ISO induced myocardial necrosis in rats



ISO and pretreated with flavonoid of leaves of *Cassia tora* group (200mg/kg, b.w. p.o) showed significant reduction in triglycerides ($P < 0.001$) activities when compared to ISO-treated group.

while LPO increased in the myocardial homogenate of ISO administered rats indicating oxidative stress.

Cassia tora (flavonoid) 200 mg/kg prevented the alterations in marker enzymes of myocardial infarction, and oxidative stress. Myofilament alterations such as myocytosis and myofibrillar degeneration are reported in ISO-treated rats. Cardiac sections of the ISO-treated animals showed infiltration of inflammatory cells and continuity in the muscle fiber was lacking suggesting an irreversible cell injury. Rats pre-treated with *Cassia tora* showed normal myofibrillar structures with striations and revealed a marked protection by the extract against myocardial necrotic damage. Administration of ISO raised LDL cholesterol and decreased HDL cholesterol level in the serum. An increase in concentration of total cholesterol

and LDL cholesterol, and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction. High level of circulating cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage. *Cassia tora* elevated HDL level and decreased LDL cholesterol level. There is a growing body of evidence from epidemiologic, clinical, and laboratory data indicating that elevated triglyceride levels are an independent risk factor for cardiovascular disease. 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. Hypertriglyceridemia observed in ISO- treated rats is clinically reported in ischemic heart disease. Pre-treatment

HISTOPATHOLOGY:

Normal control:

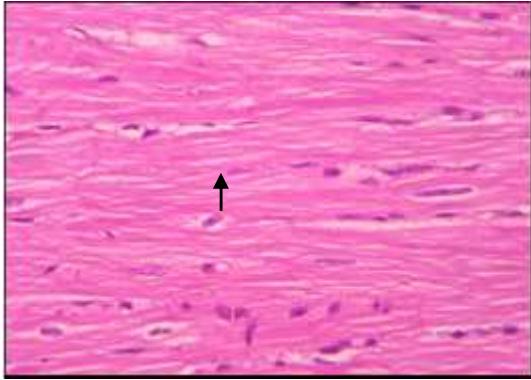


Fig.1 [H&E, x400]

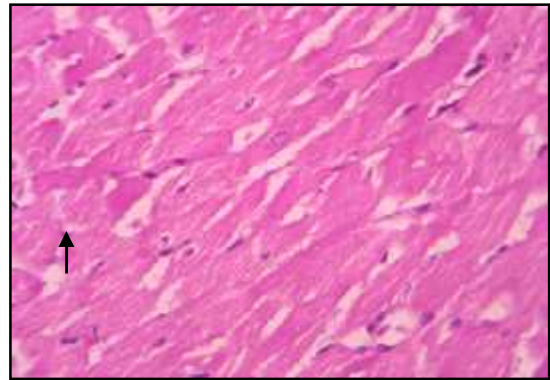


Fig.2 [H&E, x400]

Diseased Control

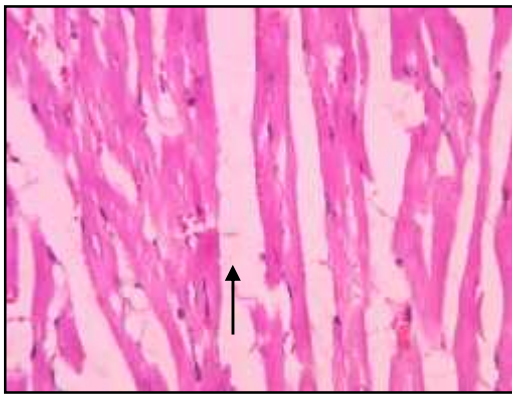


Fig.1 [H&E, x400]

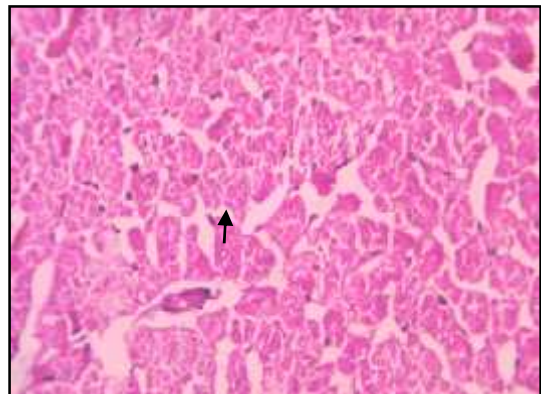


Fig.2 [H&E, x400]

Standard:

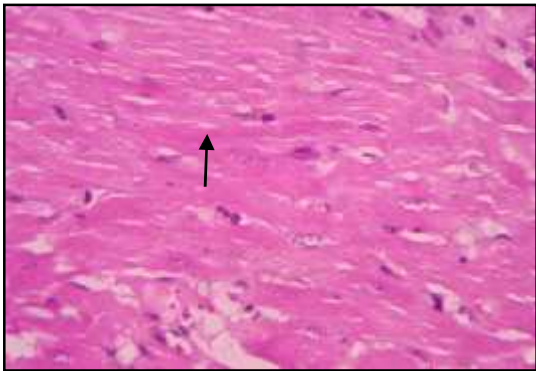


Fig.1 [H&E, x400]

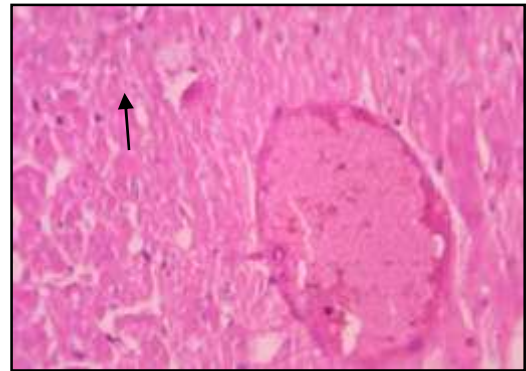


Fig.2 [H&E, x400]

CASSIA TORA 100MG/KG

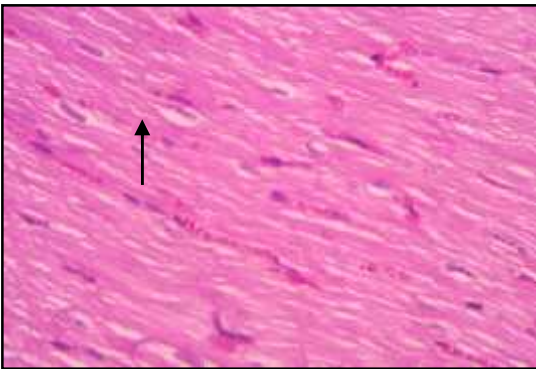


Fig.1 [H&E, x400]

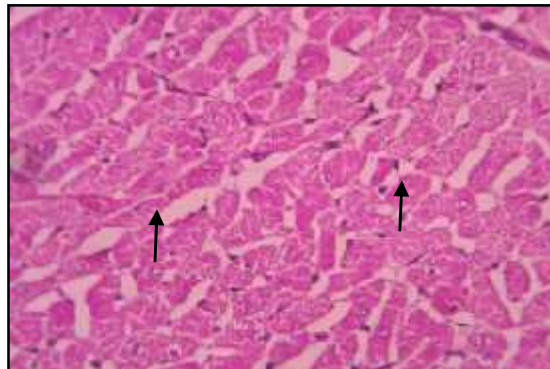


Fig.2 [H&E, x400]

CASSIA TORA 200 MG/KG:

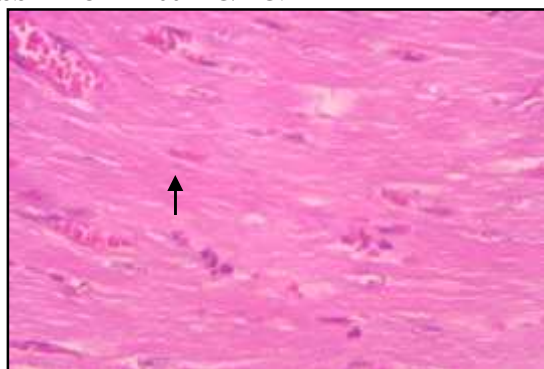


Fig.1 [H&E, x400]

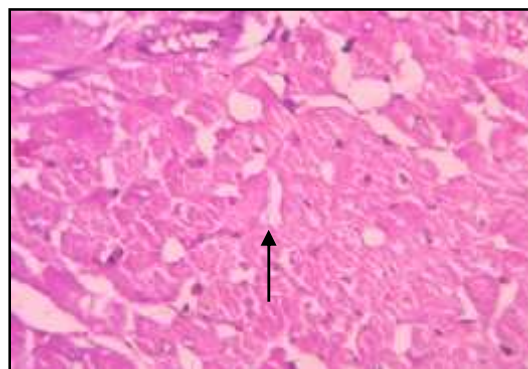
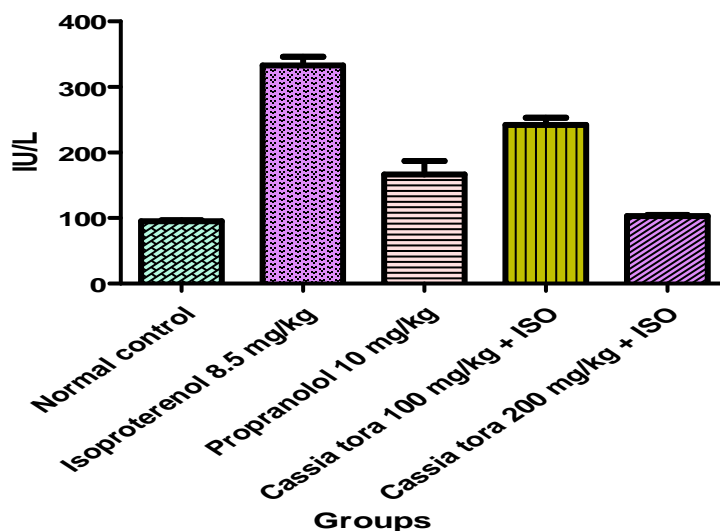


Fig.2 [H&E, x400]

ALP:

ALP effect in flavonoid of leaves of cassia tora in ISO induced myocardial necrosis in rats



ISO and pre-treated with flavonoid of leaves of *Cassia tora* group (200mg/kg, b.w. p.o) showed significant reduction in ALP ($P < 0.001$) activities when compared to ISO-treated group.

with *Cassia tora* prevented the elevation of triglycerides cholesterol and LDL in serum, signifying that the myocardial membrane is intact and not damaged.

As *Cassia tora* is rich in phytochemical constituents like flavonoids, polyphenols which are said to act as antioxidants. Based on these assumptions leaves of *Cassia tora* was used to study the cardioprotective activity.

LDH is a cytosolic enzyme, which is essentially present in all the tissues involved in glycolysis. From the damaged tissue it is released into the blood streams which become a definitive diagnostic and prognostic criterion. In ISO-treated rats compared to control rats showed significant increase LDH, TG, TC, LDL, AST, ALT, ALP (336.6 ± 2.925 , 120.7 ± 1.18 , 264.8 ± 0.95 , 237.7 ± 0.683 , 51.02 ± 2.05 , 53.34 ± 1.52 , 333.0 ± 13.26) and significant decrease in HDL (23.36 ± 0.00). When ISO-treated rats compared to ISO and propranolol (240.5 ± 5.391 , $77.68 \pm 1,107$, 219.4 ± 2.95 , 192.1 ± 3.02 , 30.89 ± 1.57 , 31.76 ± 0.68 , 166.6 ± 20.50) treated group showed significant decrease in LDH. ISO and pre-treatment of and flavonoids of leaves

of *Cassia tora* (100mg/kg, b.w. p.o) (323.2 ± 14.39 , 94.99 ± 0.768 , 245.8 ± 0.69 , 219.6 ± 0.984 , 41.16 ± 1.16 , 51.50 ± 0.87 , 242.2 ± 10.86), HDL (37.16 ± 0.67) and 200mg/kg (293.1 ± 1.870 , 87.20 ± 0.757 , 210.8 ± 2.15 , 180.7 ± 1.05 , 17.63 ± 1.24 , 22.01 ± 0.84 , 103.0 ± 1.92), HDL (40.75 ± 1.96), showed significant reduction in the activity of serum LDH and significant increase in HDL levels compared to ISO-treated rats. This could be due to the protective effect of flavonoids of leaves of *Cassia tora* in myocardium thereby preventing the leakage of LDH.

CONCLUSION

From the experimental studies, flavonoid of leaves of *Cassia tora* administered at two different doses (100mg/kg and 200mg/kg) showed dose dependent cardioprotective activity. The higher dose 200mg/kg showed significant protection compared to lower dose 100 mg/kg.

ACKNOWLEDGEMENT

Authors are thanks full the department of pharmacology, Karnataka college of Pharmacy, Bangalore, Karnataka, India.

REFERENCES

1. Pantely GA, Bristow JD. Ischemic cardiomyopathy. *Prog Cardiovasc Dis.* 1984; 27: 95–114.
2. Rona G. Catecholamine cardiotoxicity. *J Mol Cell Cardiol.* 1985; 17: 291–306.
3. Singal PK, Yates JC, Beamish RE, et al. Influence of reducing agents on adrenochrome induced changes in the heart. *Arch Pathol Lab Med.* 1981; 105: 664–669.
4. Noronha-Dutra AA, Steen-Dutra EM, Woolf N. Epinephrine-induced cytotoxicity of rat plasma. Its effects on isolated cardiac myocytes. *Lab Invest.* 1988;59: 817–823.
5. Acharya TK chatterjee IB. Isolation of chrysophanic acid-9-anthrone, the major antifungal principle of *cassia tora*, *Lloydia*, 38, 1975; 218-220.
6. Hatano T, Uebayashi h, Ito H, Shiota S, Suchiya T, Yoshida T. Phenolic constituents of *cassia tora* seeds and antibacterial effects of some naphtholenes and anthraquinone on methicillin-resistant staphylococcus aureus, *chemical and pharmaceutical bulletin (tokyo)*, 47,1999; 121-1127.
7. Huang KC. Anti hypercholesterolemic herbs. In: the pharmacology of Chinese herbs. CRC press, boca raton, FL. 1993; p. 103.
8. Bhargava AS, Preus M, Khater AR, Günzel P (1990) Effect of iloprost on serum creatine kinase and lactate dehydrogenase isoenzymes after isoprenaline-induced cardiac damage in rats. *Arzneim Forsch/Drug Res.* 40: 248–252.
9. Raju k, Balaraman R, Hariprasad, Vinoth kumar M, Ali A. Cardioprotective effect of *momordica cymbalaria* fenzi in rats with isoproterenol-induced myocardial injury. *Journal of clinical and diagnostic research* [serial online] 2008 february [cited: 2008 february 4]; 2: 699-705.