MS.ID-IJPBS-6256
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (4): 171-181
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

The Investigation of Anti Hypertension and Cardioprotective Properties of Cassia Fistula Linn Leaf Extract in Experimental Animals.

Omkarswamy Maradimath*, Shaista Omer, Ittagi Shanmukha, Sachin Wali and Prashanth K U. Darshan Angadi
Dept. of Pharmacology, Dept. of Pharmaceutics, Dept. of Pharmaceutical Chemistry, SCS College of Pharmacy, Harapanahalli.

Received: 08 Jul 2022/ Accepted: 12 Aug 2022 / Published online: 3 Dec 2022
***Corresponding Author Email:** omkarcm2@gmail.com

Abstract

Objective: This study was designed to determine the Anti-hypertension and Cardioprotective properties of leaves of *Cassia fistula*. Hypertension drives the global burden of cardiovascular disease and its prevalence is estimated to increase by 30% by the year 2025. Nonadherence to chronic medication regimens is common; approximately 43% to 65.5% of patients who fail to adhere to prescribed regimens are hypertensive patients. Nonadherence to medications is a potential contributing factor to the occurrence of concomitant diseases. **Results:** Significant decrease in the elevated level of serum glucose, TG, cholesterol, pulse rate and also there is a decrease in the organ weight such as heart in the anti- hypertension models. The treatment with test extract has reversed all the biochemical parameters in cardiotoxicity to the near normal levels in a dose dependant manner. Histopathological observations confirmed the same. **Conclusion:** From the results it may be concluded that the 70% EELCF possess anti-hypertension and cardioprotective activities. The anti-hypertension and organ protective properties of the plant may be attributed to the alkaloids, terpenoids, steroid, volatile oil, sterols, saponins and polyphenolic compounds like flavonoids and tannins that are present in the plant.

Keywords
leaves of *Cassia fistula*, polyphenol, flavonoid, tannin, anti-hypertension, adrenaline, cardioprotective protective.

*****

INTRODUCTION:

Hypertension is the most common cardiovascular illness and is a major public health issue in developed as well as in developing countries[1]. Human hypertension is probably triggered by environmental influences such as increased salt intake, Obesity and lack of exercise acting on a genetic predisposition[2]. Blood pressure (BP), generally characterized as the pressure exerted by blood against the walls of the arteries and veins, is one of the principal vital signs[3]. Cardiac output, i.e., the volume of blood flow from the heart, Total peripheral vascular resistance is affected by the viscosity of blood, local and circulating substances as well as autonomic nervous systems–sympathetic and parasympathetic[4]

Cardiovascular diseases (CVDs) are the major health problem Due to imbalance of coronary blood supply and myocardial demand[5]. MI is associated with high levels of serum totalcholesterol[6], low density lipoprotein (LDL)[7] and decreased levels of high-density lipoprotein (HDL)[8]. Isoproterenol [1-(30,40-dihydroxyphenyl)-2-isopropylaminoethanolhydrochloride], a synthetic catecholamine and beta- adrenergic agonist, to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle[9,10].

Isoproterenol (ISO) induced myocardial necrosis is a well-known standard model to study the beneficial effect of many drugs on cardiac dysfunction[11]. Isoproterenol [1-(30,40- dihydroxyphenyl)-2-isopropylaminoethanolhydrochloride], a synthetic catecholamine and beta- adrenergic agonist, has been reported to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle[12,13].

Cassia fistula Linn is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin and a tonic and has been reported to treat many other intestinal disorders like healing ulcers. In traditional medicine, Unani, Ayurvedic medicines this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of haematemesis, pruritus, leukoderma and diabetes has been suggested. Traditionally, the plant is also used as an infusion, decoction, or powder. In modern times, and in any controlled clinical trials, commercial preparations have tended to be standardized extracts of the whole plant. The plant has documented to possess analgesic, anti-inflammatory, antioxidant, antidiabetic, as well as hepatoprotective activity[14,15, 16].

The taxonomic position of *Cassia fistula Linn* is as follows[17]

Bengali	: Amultash, sandal, sonali; Cantonese (kakke)
English	: Golden shower, Indian laburnum, pudding pipe tree, purging cassia,
Kannada	: Kakkemara
Gujarati	: Girmala
Hindi	: Bandarlathi, bharva, suvarnaka, amaltas, rajataru, girimalah
Malayalam	: Tengguli, rajah kayu, bereksa
Sanskrit	: Saraphala, survanaka, argwadha, rajtaru
Tamil	: Kavani, konnai, tirukontai, sarakkonne
Telugu	: Kondrakayi, Raelachettu, Aragvadhamu, Koelapenna
Trade name	: Indian laburnum, rajbrikh'

MATERIALS AND METHODS:

Collection and authentication of plants

The leaves of ***cassia fistula*** were collected from the ***kalluru near to Ripponpete***. in the month of june 2019. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli.

Selection of animals

Either sex Wistar albino rats (n=6) of weighing 220-300 g were used for the present study. The animals were procured from animal house, Department of Pharmacology, S C S College of Pharmacy, Harapanahalli India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water ad libitum. All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of S C S College of Pharmacy and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

I. Preliminary Phytochemical Screening:

The preliminary phytochemical screening was carried out on petroleum ether,
chloroform, 70% ethanolic and aqueous extract of ***cassia fistula*** for qualitative identification of type of phytoconstituents present.

Investigation of anti-hypertension activity against adrenaline induced hypertension model[18]:

In the present study, adult male albino rats of 150 – 200 g were divided into six groups of six animals each.

Rats will be randomly divided into six groups of 6 rats each and will be treated as follows;

Group I:- 1% normal saline (2ml/kg/day, p.o) for 5days.
Group II:- Adrenaline (0.5 mg/kg) i.p for 5 days)
Group III:- Propranolol (10 mg/kg, i.p. for 7days)
Group IV:- Low dose of EELCF (250mg/kg/day, p.o) for 7 days.
Group V:- Medium dose of EELCF (333.33mg/kg/day, p.o) for 7 days
Group VI:- High dose of EELCF (500mg/kg/day, p.o) for 7 days

Grouping and treatment of animals was done as shown above. *Cassia fistula* leaves extract and commercial drug Propranolol were administered through p.o and intraperitoneal (i.p) route for one week at their respective doses in every morning till the completion investigation. Before treatment different biochemical parameters such as heart weight, serum triglyceride level, serum cholesterol level, blood glucose level and body weight of group I and group II rats were measured. The rats were sacrificed to collect blood sample and heart from each rat and investigated. Collected blood samples were analyzed for the determination of blood glucose level by auto-analyzer. Then the data were compared with the standard value collected blood samples about 1-2 ml was centrifuged at 4000 rpm for 10 minutes to separate the serum to determine STL, SCL by measuring absorbance using UV spectrophotometer (Shimidzu UV-1200, Tokyo,

Category	Systolic (mmHg)	Diastolic (mmHg)
Normal	<130	<85
High normal	130-139	85-89
Hypertension		
Stage 1 (mild)	140-159	90-95
Stage 2 (moderate)	160-179	100-109
Stage 3 (severe)	180-209	110-119
Stage 4 (very severe)	≥210	≥120

CARDIO PROTECTIVE ACTIVITY:

1. Isoproterenol (ISO) induced cardio toxicity model[19]:

In the present study, adult albino rats of 150-250g will be divided into five groups of six animals each as below.

Group 1: Normal saline (2ml/kg/day, p.o for 16days).
Group 2: Normal saline (2ml/kg/day, p.o) for 14 days + Isoproterenol (200mg/kg/day s.c. on 14th and 15th day).
Group 3: Standard (Atorvastatin 20mg/kg, S.C.) + Isoproterenol (200mg/kg/day s.c. on 14th and 15th day).
Group 4: Lower dose of extract of 14 days + Isoproterenol (200mg/kg/day s.c. on 14th and 15th day).
Group 5: Medium dose of extract of 14 days + Isoproterenol (200mg/kg/day s.c. on 14th and 15th day).
Group 6: Higher dose of extract of 14 days + Isoproterenol (200mg/kg/day s.c. on 14th and 15th day).

At the end of experimental period (after 24h of second isoproterenol injection or 16th day of extract/vehicle treatment) all the rats will be anaesthetized with light anesthetic ether and blood

will be collected from the retro-orbital plexus, the serum was separated and used for the determination of diagnostic marker enzymes like AST, ACT, LDH, CK, TG, TC, HDL, and VLDL. It is also planned to estimate LPO, GSH and SOD. The histopathology of heart will also be studied. Japan), using reagent diagnostic kits (Erba) according to manufacturer's protocol.

RESULTS:

Effect of 70% EELCF on biochemical markers in adrenaline induced hypertension

Groups	T.C mg/ml	T.G mg/ml	Blood glucose mg/ml	LDH IU/l	CPK IU/l	Animal body Wt(gm)	Wt of heart (gm)	Pulse rate (bpm)
-ve control	113.3± 14.91	1.456± 0.1769	88.7± 9.712	7.592± 3.231	14.58± 2.846	159.0 ± 5.859	0.603± 0.034	251.2± 10.65
+ve control Adrenaline (0.5mg/kg/100µl)	187.5± 9.539	0.071± 0.007	156.3± 19.27	60.78± 5.405	107.5± 3.720	244.2± 4.902	0.926± 0.021	379.3± 16.47
Std Propranolol (10mg/kg)	108.2± 6.803***	1.129± 0.243**	66.15± 3.357***	12.72± 0.707***	40.68± 0.754***	155.0± 2.236***	0.616± 0.018***	257.0± 5.26***
70% EELCF (250mg/kg)	144.7± 12.09*	0.739± 0.115NS	96.10± 12.12**	24.56 ± 2.623***	59.51± 5.712***	205.0± 6.191***	0.798± 0.029*	329.7± 17.71*
70% EELCF (333.33mg/kg)	136.6± 5.418**	1.060± 0.307**	62.69± 4.271***	21.03± 2.734***	54.87± 5.068***	180.0± 2.236***	0.736± 0.032**	285.2± 12.45***
70% EELCF (500 mg/kg)	107.5± 8.984***	1.122± 0.233**	67.56± 12.18***	15.14± 1.387***	42.70± 10.76***	167.5± 5.284***	0.678± 0.047***	272.2± 9.02***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non-significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to Adrenaline alone treated rats. One-way ANOVA followed by Dunnett's comparison test.

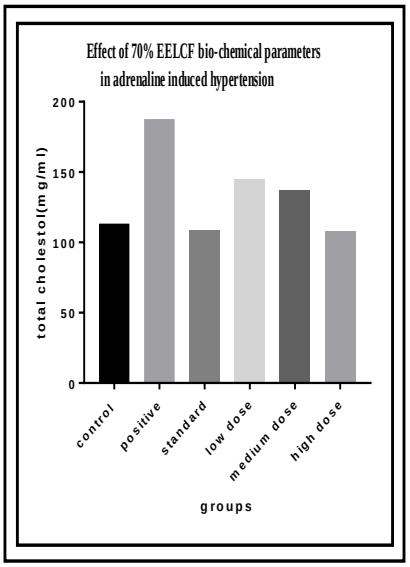


Fig. No- 01

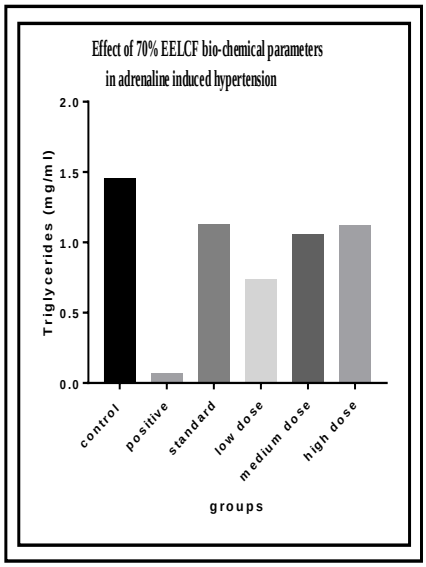


Fig. No- 02

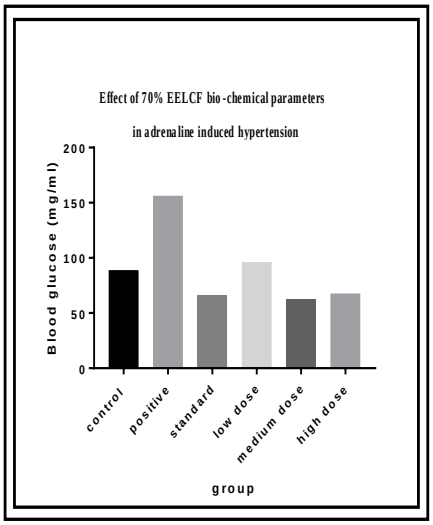


Fig. No- 03

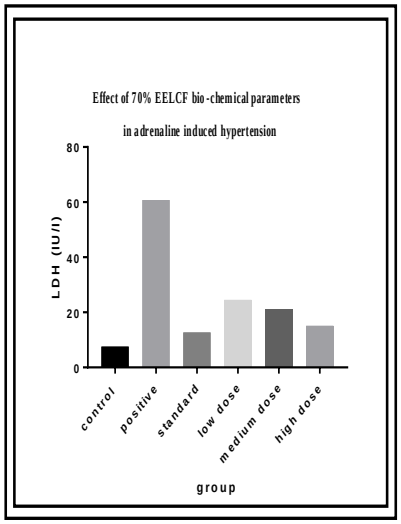


Fig. No- 04

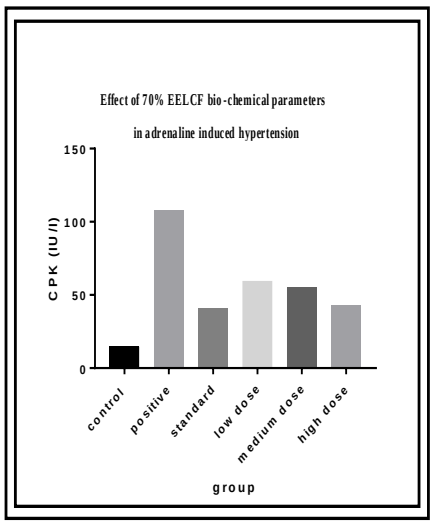


Fig. No- 05

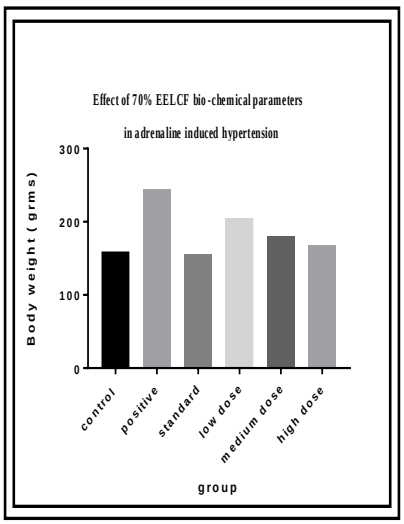


Fig. No- 06

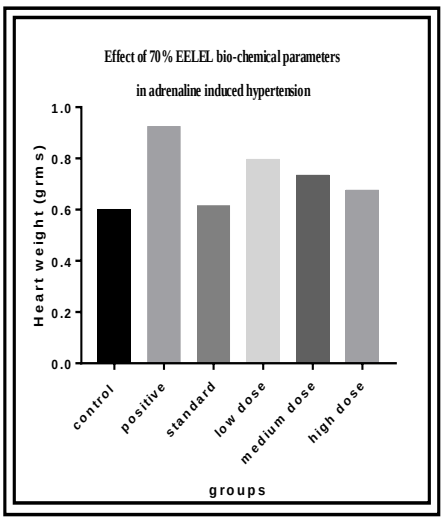


Fig. No- 07

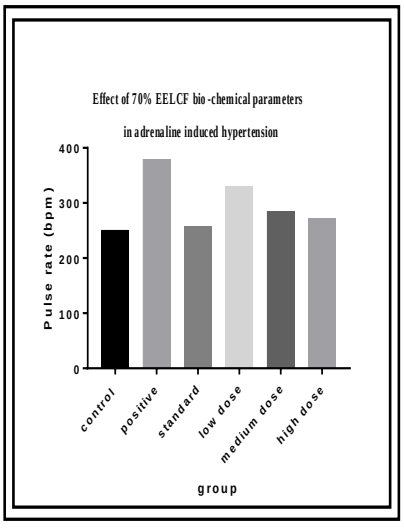


Fig. No- 08

Histopathological Studies in Adrenaline induced Hypertension

RESULT:

In the case of normal control (-ve control), Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers [Fig.1, Arrow]. These cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils [Fig.2, Arrow]. The interstitial space appears intact. The vascular spaces amidst these cardiac muscle fibers appear unremarkable.

Normal control

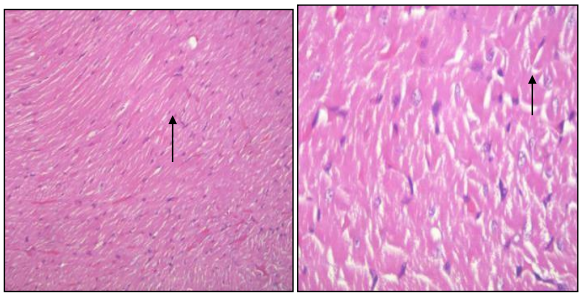


Fig. No. 01(a) Fig. No. 01(b)

Positive control

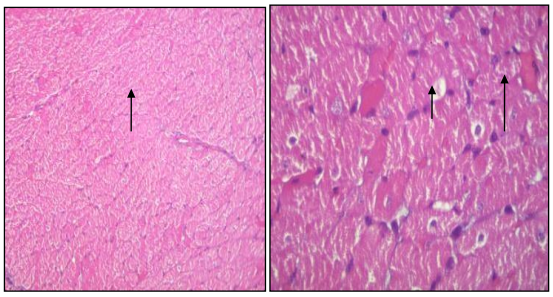


Fig. No. 02(a) Fig. No. 02(b)

Standard group

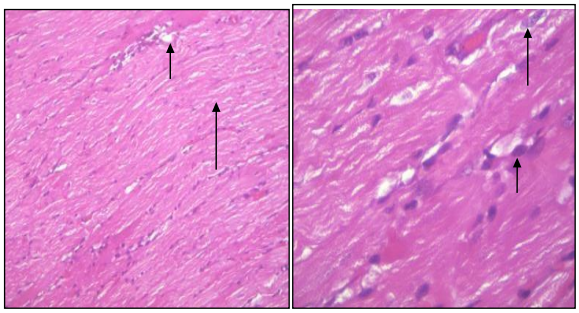


Fig. No. 03(a) Fig. No. 03(b)

Adrenaline+ 250mg/kg of 70% EELCF

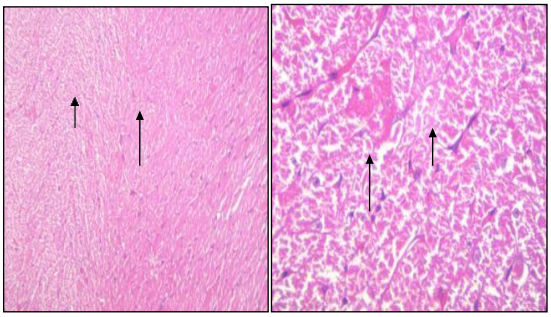


Fig. No. 04(a) Fig. No. 04(b)

Adrenaline+ 333.33mg/kg of 70% EELCF

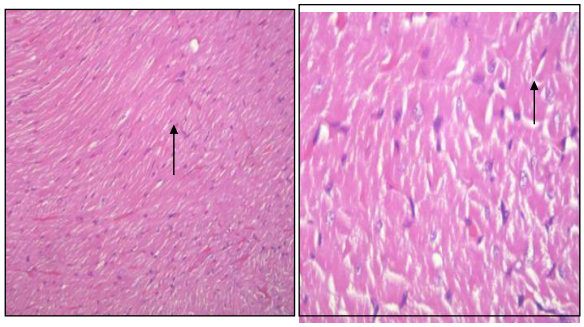


Fig. No. 05(a) Fig. No. 05(b)

Adrenaline+ 500mg/kg of 70% EELCF

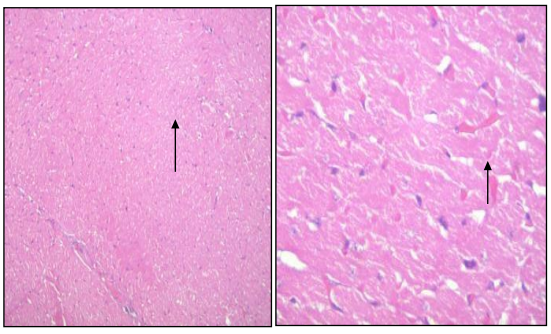


Fig. No. 06(a) Fig. No. 06(b)

Effect of 70%EELCF on biochemical markers in isoproterenol induced cardiotoxicity

groups	Heart wt (gm)	AST IU/l	ALT IU/l	CK IU/l	LDH IU/l	LDL Mg/ml	HDL Mg/ml	TC Mg/dl	TG mg/dl
-ve control	0.796± 0.024	44.87± 1.695	71.47± 2.576	97.84±2.972	76.48±4.657	74.39± 2.888	40.30± 1.364	113.4± 4.715	93.16±2.232
+ve control Isoproternol (85mg/kg)	0.548± 0.030	122.9± 5.587	157.1± 4.448	133.6± 3.049	135.8±2.462	115.5±6.119	28.63±1.374	186.0±4.507	139.9±3.715
Std Atrovastatin (60mg/kg)	0.716± 0.023**	90.09± 3.513 ***	101.0± 2.492 ***	95.28± 1.220 ***	77.93± 3.071 ***	49.87± 2.012 ***	51.57± 2.308 ***	111.4± 3.848 ***	117.1± 4.826 ***
EELCF 250 mg/kg	0.525± 0.030ns	105.5± 3.078 **	142.5± 4.068 *	122.1± 2.592 **	120.0± 2.477 **	97.19± 2.024**	31.08± 1.851 *	167.4± 3.168 *	123.9± 3.028 *
EELCF 500 mg/kg	0.636± 0.040 *	99.03± 2.475 ***	119.7± 4.973 ***	111.4 1.653 ***	95.28± 1.651 ***	92.32± 7.440 **	36.44± 2.026**	143.0± 4.296 **	120.0± 3.425**

Values are mean ± SEM of six rats/ treatment. * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to positive control

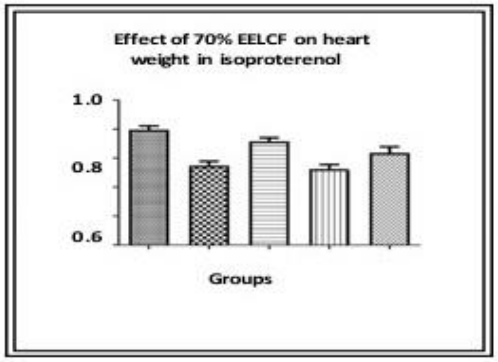


Fig. No. 02 (Heart rate)

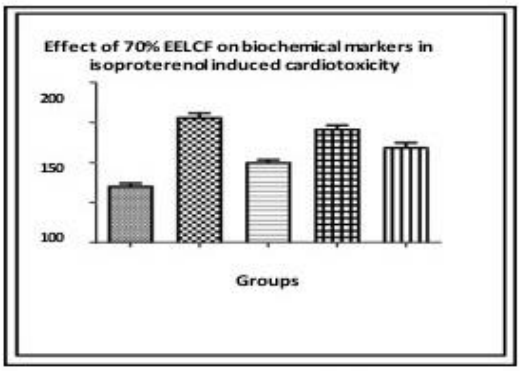


Fig. No. 02 (ALT)

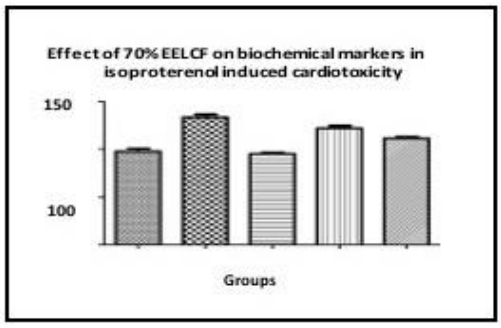


Fig. No. 03 (CK)

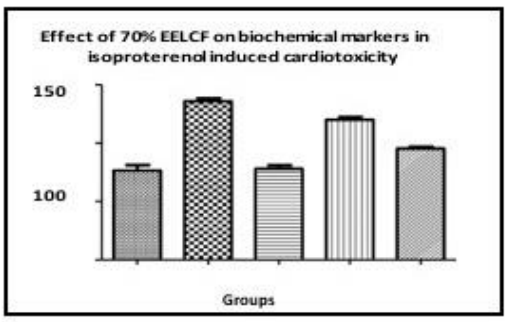


Fig. No. 04 (LDH)

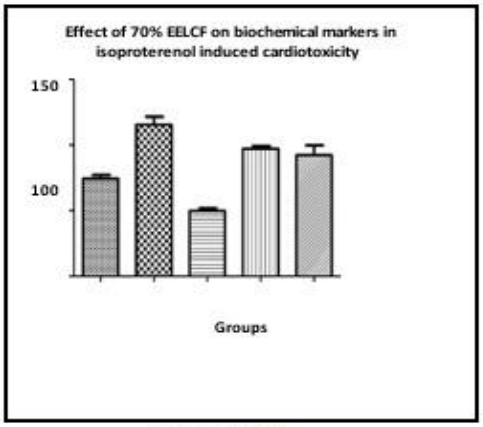


Fig. No. 05 (LDL)

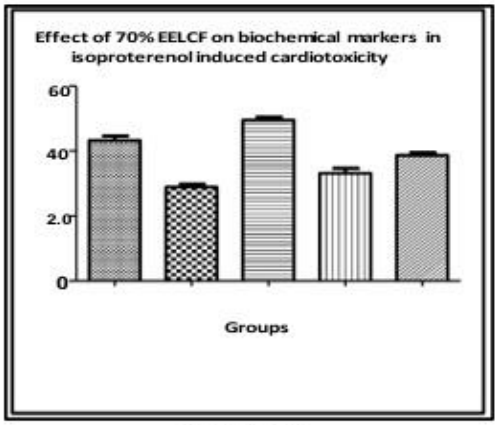


Fig. No. 06 (HDL)

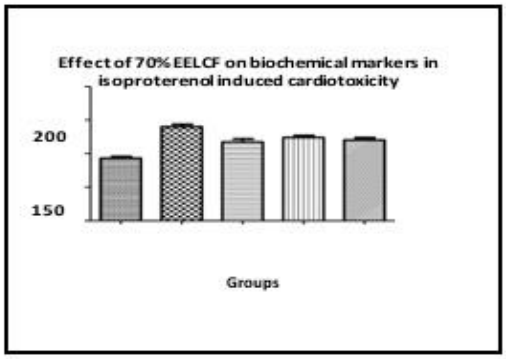


Fig. No. 07 (TG)

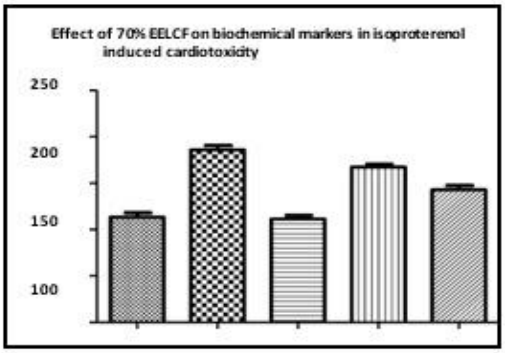


Fig. No. 08 (TC)

Histopathological Studies in isoproterenol induced cardiotoxicity:

RESULT:

Group A: In the case of normal control (-ve control), Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers [Fig.1, Arrow]. These cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils [Fig.2, Arrow].

Fig. No. 01(a) Fig. No. 01(b) Fig. No. 02(a) Fig. No. 02(b)

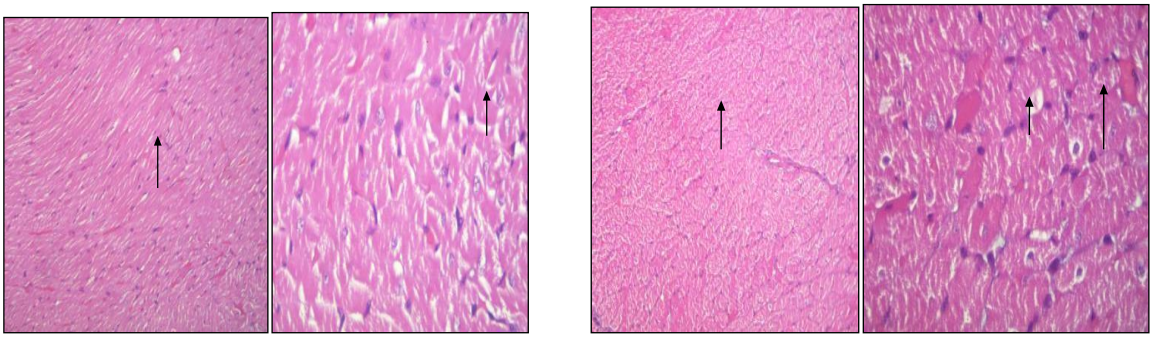


Fig. No. 03(a) Fig. No. 03(b)

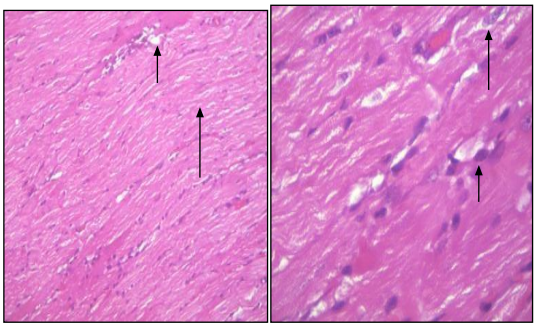


Fig. No. 04(a) Fig. No. 04(b)

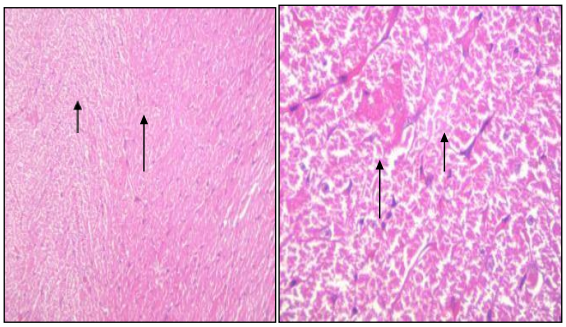
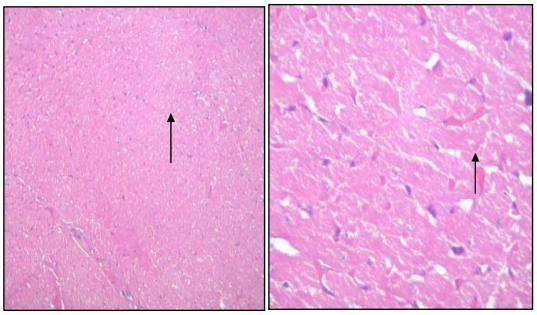


Fig. No. 04(a) Fig. No. 04(b)



DISCUSSION:

Hypertension is a common causing serious illness among peoples in both developed and developing countries. The disease continues to be a leading cause of morbidity and mortality from the coronary artery disease and stroke.

The spectroscopic determination of total alkaloid, flavonoid and tannin contents in the leaves, it was found the 70% EELCF possess 12.96mg/100g, 12.2mg/g and 45mg/g of the respective constituents from 70% EELCF. This 70% EELCF contains higher concentration of total alkaloid, flavonoid and tannin and hence this is selected for further studies like anti-hypertension and Cardioprotective activity Based upon previous literature on the leaves of the plant, three doses have been selected for the study which is as follows. A).250mg/kg B).333.33mg/kg C).500mg/kg

Standard values of different biochemical parameters were investigated. Except serum triglyceride level, the standard values of body weight, heart weight, blood glucose level and serum cholesterol level were high in adrenaline induced hypertensive rats when compared to control rats. Because of metabolic effects of adrenaline, serum triglyceride level was low in hypertensive rats. This study was performed to analyze the differential effects of 70% EELCF and Propanolol on heart weight, blood glucose level serum triglyceride level, serum cholesterol level, pluse rate and body weight of hypertensive rats and compared with those of control rats.

Reduced the pulse rate of the rats after treatment for one week and this was dose dependent. There was significant ($P<0.001$) increase in the pulse rate of AIHR when compared with the control. The increase in pulse rate of animals or humans could be due to heart diseases or increased metabolic activity. Adrenaline increases metabolic activity as well as pulse rate. Treatment with *Cassia fistula* and Propanolol significantly ($P<0.001$) decreased the pulse rate of hypertensive rate. This decrease was dose dependant. The reduction in pulse rate of the hypertensive rats confirms the hypotensive effect of *Cassia fistula* leaf extract.

Increased the metabolic activity of adrenaline used to induce hypertension. LDH is found in the cells of almost all body tissues. It catalyzes the inter conversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. Cellular injury in tissues containing LDH can result in its release into the blood stream.

The effect of *Cassia fistula* extract and Propanolol on blood glucose, serum triglyceride and cholesterol level of the experimental rats are as presented in table 6. There was significant decrease in blood glucose level of the experimental rats when compared with the control group for doses of the extract. Significant ($P<0.01$) decreases in serum triglyceride and cholesterol levels were observed in animals treated with the extract and Propanolol.

Isoproterenol is well known cardiotoxic agent due to its ability it will destruct myocardial cells. As a result

of this, cytosolic enzymes such as Lactate Dehydrogenase (LDH), transaminases (ALT, AST) and Creatine kinase (CK) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage.

In the present study, ISO treated rats showed significant elevation in the levels of these diagnostic marker enzymes (AST, ALT, LDH, CK, triglycerides, total cholesterol, LDL and decrease in HDL). The tissues GSH, SOD were reduced and enhanced the lipid peroxidation.

The prior administration of EELCF (250 and500 mg/ kg) showed significant reduction in ISO induced elevated serum marker enzymes.

Acute β-adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also depresses total cellular antioxidant capacity, down regulates copper-zinc superoxide dismutase enzyme activity we found that EELCF protected myocardium from isoproterenol-induced myocardial functional and structural injury via normalization levels of diagnostic marker enzymes[20].

In the present study, it was observed that the leaves possess polyphenolic compounds (flavonoids and tannins) and these constituents are reported to have antioxidant and organ protective properties. Hence the anti-oxidant and organ protective properties may be attributed to the polyphenolic constituents that are present in the Leaves of *Cassia fistula.*

CONCLUSION:

The present investigation revealed that the leaves of *Cassia fisula* contain significant amount of phenols, flavonoids and tannins. This constituent may play pivotal role as Anti-hypertension and Cardioprotective properties. Thus, our studies support the folklore use of the title plant in Hypertension and Cardiac disorders.

FURTHER SCOPE OF STUDY:

Since, our study has indicated only the usefulness of *Cassia fistula* leaves in treating Hypertension and Cardiac disorders, there is room for further study to identify, isolate, characterize and evaluate the active principles responsible for the Antihypertension and cardioprotective activities of the plant. In addition, toxicological aspects of the plant are not studied in this project work. Hence, a study may be undertaken from the toxicological point of view. Even formulation and evaluation of this herb may also be studied.

BIBLIOGRAPHY:

1. Umang Joshi H, Tejas Ganatra H, Tusharbindu Desai R, Pravin Tirgar R. Evaluation of Antihypertensive Activity of *Evolvulus Alsinoides* in Adrenaline Induce Hypertensive Rats. Int J Pharm Pharm Sci 2012; 4(4): 194-198.
2. Enyioma Obineche N. Management of hypertension. Bulletin of the Kuwait Institute for Medical Specialization 2003; 2:73-82.
3. Doggrell SA, Brown L. Rat models of hypertension, cardiac hypertrophy and failure. Cardiovascular Research 1998; 39: 89 –105.
4. Klabunde R. E. Cardiovascular physiology concepts. 2nd ed., Philadelphia:Lippincott Williams Wilkins 2011.
5. Bernatova I, Pechanova O, Pelouch V, Simko F. Regression of chronic L-NAME treatment-induced left ventricular hypertrophy: effect of captopril. J Mol Cell Cardiol 2000; 32:177-185.
6. Moncada A, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43:109-42.
7. Loscalzo J, Welch G. NO and its role in the cardiovascular system. Prog Cardiovasc Dis1995;38:87-104.
8. Suzanne Oparil, Amin Zaman M, David A Calhoun. Pathogenesis of Hypertension. Ann Intern Med. 2003 ;139:761-776.
9. Naznin ara, hasan nur. in vitro antioxidant activity of methanolic leaves and flowers extracts of *Lippia Alba*. Research Journal of Medicine and Medical Sciences. 2009; 4(1): 107-110.
10. Zuraini zakaria, Rais aziz, Yoga latha lachimanan, Sasidharan Sreenivasan, Xavier rathinam. Antioxidant Activity of *Coleus blumei, Orthosiphon stamineus, Ocimumbasilicum* and *Mentha arvensis* from Lamiaceae Family. International Journal of Natural and Engineering Sciences. 2008; 2(1): 93-95.
11. Wexler, BC., 1978. Myocardial infarction in young vs old male rats: Pathophysiological changes. Am. Heart J., 96: 70-80. DOI: 10.1016/0002- 8703(78)90128-X
12. Chagoya de Sanchez V, HernandezeMunoz R, LopezeBarrera F, Yanez L, Vidrio S, Suarez J, et al. Sequential changes of energy metabolism and mitochondrial function in myocardialinfarction induced by isoproterenol in rats; a long-term and integrative study. Can J Physiol Pharmacol 1997; 75:1300-11.
13. Ithayarasi AP, Devi CSS. Effect of alpha tocopherol on lipid peroxidation in isoproterenolinduced myocardial infarction in rats. Indian J Physiol Pharmacol 1997; 41:369-76.
14. Sathish Mohan Botsa*, Seetharam P, I. Manga Raju, Suresh P, G. Satyanarayana, Sangaraju Sambasivam, Susmitha Uppugalla, Tejeswararao D, Nanohybrid material of Co-TiO2 and optical performance on methylene blue dye under visible light illumination. Hybrid Advances, 1:100008 (2022).
15. Susmitha Uppugalla, Kavitha Rajesh, Amareswarapu V Surendra, Kiran Kumar Y, Mohammed Gayasuddin, Sriram N, Prasad P Nadedkar, Effect of Pisonia Alba Root Extract on Cafeteria Diet-Induced Obesity in Rats. Journal of Pharmaceutical Negative Results, 13, 3732-3739, 2022.

16. N Sriram, Susmitha Uppugalla, Kavitha Rajesh, S. Someshwaran, B Senthil Kumar, Prasad P Nadedkar, Shanta K Adiki, Cognitive Enhancing and Antioxidant Activity of Ethyl Acetate Soluble Fraction of The Methanol Extract of Pisonia Alba Leaves in Scopolamine-Induced Amnesia. Journal of Pharmaceutical Negative Results,13,3740-3749,2022.
17. Wexler, BC., 1978. Myocardial infarction in young vs old male rats: Pathophysiological changes. Am. Heart J., 96: 70-80. DOI: 10.1016/0002- 8703(78)90128-X
18. https://en.wikipedia.org/wiki/Cassia_fistula
19. Urve P, Vallo M, Ain R. Total tannin content in distinct *Quercus robur* L. Galls Journal of Medicinal Plants Research. 2010; 4(8): 702-5.
20. Roy S P, Niranjan CM, Jyothi TM Shankrayya MM, Vishawanath KM, Prabhu K, Gouda VA, Setty RS. Antiulcer and Anti-inflammatory Activity of Aerial Parts *Enicostemma littorale* Blume. Journal of young pharmacists. 2010; 2:369-37