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INVITRO CHOLESTEROLESTERASE INHIBITORY ACTIVITY AND CARDIOPROTECTIVE ACTIVITY OF LAWSONIA INERMIS LINN FLOWERS AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT
Myocardial Infarction is defined as myocardial cell death due to prolonged ischemia. Clinically, the term Myocardial Infarction is used when there is evidence of myocardial necrosis in a clinical setting, consistent with acute myocardial ischemia and with a rise in cardiac troponin. The chemical constituents isolated from flowers of L. interims are Bi-coumarin, Bioflavonoids, Bi-Quinone compounds. The study was evaluating the myocardial infarction activity of ethanol extract of the flowers of *Lawsonia inermis* Linn in experimental animals. Fresh flowers of *Lawsonia inermis* L. were cleaned and air-dried at room temperature, pulverized; and were used for extraction with ethanol in soxhlet apparatus. A 1500 g of the pulverized sample was extracted in L ethanol. The extract obtained was collected and concentrated by using rotary evaporator and the percentage yield was calculated. Extract was kept in air tight containers in a refrigerator until used. The results of the present study show a considerable in vitro study of cholesterol esterase inhibitory activity (IC50 = 26±30 µg/ml compared with IC50 = 35±3.350 µg/ml [Atrovastatin]) and in vivo method of grade for cardiac damage for different group of rats using L. inermis extract, so that the extract diminished the myocardial, serum lipid levels and total protein levels in rats. Administration of ethanolic extract of *Lawsonia inermis Linn* flowers potently protects the myocardium against isoproterenol induced infarction and suggest that the cardio protective effect.

KEYWORDS: Myocardial Infarction, *Lawsonia inermis Linn*, ethanolic extract, Bioflavonoids.

INTRODUCTION
Acute myocardial infarction is the most severe manifestation of coronary artery disease, which causes more than 2·4 million deaths in the USA, more than 4 million deaths in Europe and northern Asia, one and more than a third of deaths in developed nations annually. Increased use of evidence-based therapies and lifestyle changes have spurred considerable reductions in mortality from coronary heart disease in recent decades.

The heart is a midline, volvuli, muscular pump that is cone shaped and the size of a fist. In adults, it weighs 300 grams and lies in the middle mediastinum of the thorax. The inferior (diaphragmatic) surface sits on the central tendon of the diaphragm, whereas the base faces posterior and lies immediately anterior to the oesophagus and (posterior to that)the descending aorta. The base comprises mainly the left atrium. The left surface (left ventricle) and right surface (right atrium) are each related laterally to a lung and a phrenic nerve in the fibrous pericardium. The contraction mechanism contains numerous steps and "excitation–contraction coupling" is the term used to define the events that translate the depolarization of the cardiac cell membrane to the contraction of the muscle fibres. The contractile filaments actin and myosin in the myocardium are responsible for the contraction. In the cardiac cell, the sarcoplasmic reticulum and the cisternae contains high concentrations of ionized Ca^{++} and depolarisation of the T tubules, which is an extension of the cell membrane, causes the release of Ca^{++} from these structures. In general, an action potential precedes each contraction, and between contractions the Ca^{++} within the cell is very low. The cardiac action potential is unique in that it has plateau phase, which is maintained by the inward Ca^{++} influx (current).

Lawsonia inermis. Linn native to north Africa and south east Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the mediterranean sea. The chemical constituents isolated from flowers of *Lawsonia inermis* are Bicoumarin, Bioflavonoids, Biquinone compounds.

AIM AND OBJECTIVE

The aim of the study was to evaluate the myocardial infarction activity of ethanolic extract of *Lawsonia inermis* in animal model by estimating biochemical and histopathological parameters.

MATERIAL AND METHODS

Lawsonia inermis Linn (Lythraceae) Flowers

It is Aglabrous branched shrub or small tree. Leaves are small, opposite, entire margin elliptical to broadly lanceolate, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, petiole short and glabrous acute or obtuse apex with tapering base. The chemical constituents isolated from flowers of L. inermis are Bicoumarin, Bioflavonoids, Biquinone compounds.

Fig. 1: Flowers of Lawsonia inermis Linn.

Extraction

Fresh flowers of Lawsonia inermis L. were cleaned and air-dried at room temperature, pulverized; and the powdered sample stored in airtight container for extraction. The coarsely powdered Lawsonia inermis flowers were used for extraction with ethanol in soxhlet apparatus.

Experimental Animals

Male Wistar rats weighing 150-200 g were used for isoproterenol induced myocardial infarction study. In vivo cardio-protective activity Forty two male Wistar rats would be divided into 6 groups (n=6 rats in each group except control it has 6 animals). Group I will serve as control, and will receive 10 mg/kg of 0.6% saline p.o without the induction by isoproterenol. Group II serves as disease (negative) control, and will receive 10 mg/kg of 0.6% saline. Group III will receive EELI low dose (100 mg /kg; p.o). Group IV would receive EELI high dose (200 mg/ kg; p.o). Group V receives EELI alone at the dose of 200 mg/kg; p.o without the induction by ISO. Group VI serves as standard and receives α- tocopherol (60 mg/kg; p.o). Drug treatment will be given once daily for 30 days. Animals in the group I-IVand the standard group VI will be administered with ISO at dose of 8.5 mg/kg b.w.; s.c. on 28th and 29th day of drug treatment at an interval of 24 hours.

RESULT

Cholesterol Esterase Inhibitory Activity

The ethanol EELI flower was studied for cholesterol esterase inhibitory activity at various concentrations ranging from 7.25, 12.5, 25, 50, 100, 200, and 400 µg/ml. The absorbance of the mixture was measured at 405nm. It was observed that there is a dose dependent increase in the percentage inhibition from the concentration 7.25µg/ml to 400 µg/ml and the values are shown in Table-2. IC50 values of the extract was calculated and compared with the standard Atrovastatin. For the EELI, IC50 value was calculated and was found to be 26±3.30µg/ml. Atrovastatin was used as a reference standard with an IC50 value of 35±3.35µg/ml. The result was given in Table 1.

Table 1: Cholesterol esterase inhibitory activity of EELI standard.

Concentrations (µg/ml)	Percentage Inhibition		Ic50 (µg/ml)	
	Ic50 (µg/ml)	Atorvastatin	Ic50 (µg/ml)	Atorvastatin
7.25	41.63±0.81	41.12±0.96	26±3.30	35±3.35
12.5	50.52±7.15	45.74±4.05		
25	52.17±2.29	51.15±3.25		
50	60.00±0.71	54.45±0.43		
100	69.12±0.81	65.16±0.46		
200	70.06±1.50	70.56±0.77		
400	75.09±0.05	73.50±0.19		

EELI – Ethanolic extract of Lawsonia inermis Linn. All determinations were carried out in triplicate manner and values are expressed as the Mean ± SEM (n= 3)

The Group I show no lesions in heart so the grade was given as 0. Group II show capillary dilations, more necrotized portion, and diffuse heart and scar formation therefore grade 4 was given. The Groups III (EELI 100 mg/kg) showed little redness, inflammation and oedema (Grade-2) when compared with ISO group. The Groups IV (EELI 200 mg/kg) exhibited a grade-1 with mild inflammation and redness when compared with Group-II (negative control). The group-VI pretreated with EELI alone resembled control group (Grade-0). The Group V (α- Tocopherol - 60 mg/kg) showed protection from oedema, scar formation, redness with little capillary dilation (Grade-1) when compared with ISO group. The Gross Examination of Heart tissue performed. Grade 0 = No Lesion, Grade 1 = Inflammation, redness, capillary dilations, Grade 2 = Edema, yellowish ventricle portion,

Grade 3 = Atrium & ventricle turns yellow, scar formation, Grade 4 = Diffuse heart, absolute scar formation, increased necrosis portion. It was shown in figure 2.

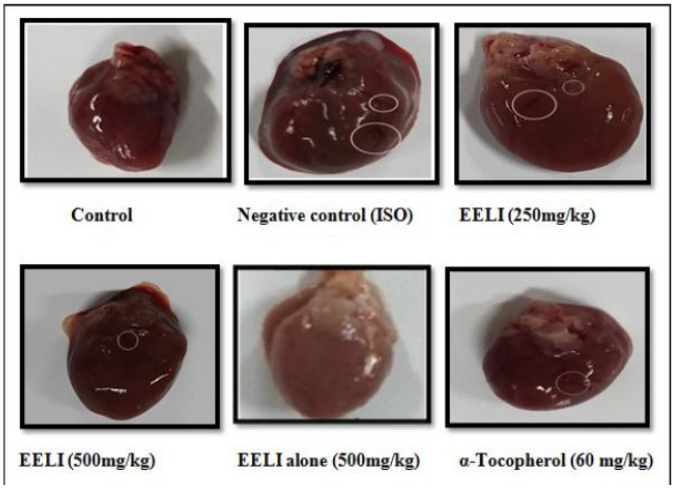

Fig. 2: Photographs of excised heart from Group I to Group VI.

Histopathological Studies

At the end of study, the heart was excised and washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and five micrometer thick sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope; photomicrograph was taken and examined the histopathological changes. The result are given in table 2.

Table 2: Grade of cardiac damage for different groups of rats.

Groups	Treatment	Dose	Grading of cardiac damage
Group I	Control (0.5% CMC)	2 mL/kg/day	Grade 0
Group II	Negative control (0.5% CMC)	2 mL/kg/day	Grade 4
Group III	EELI(Low dose)	100 mg/kg	Grade 2
Group IV	EELI(High dose)	200 mg/kg	Grade 1
Group VI	Standard α-tocopherol	60mg/kg	Grade 1
Group V	EELI alone(High dose)	200mg/kg	Grade 0

The Histopathological study results indicates (A) Normal Control - Normal myocardium with myocytes. (B) Normal Control - Normal myocardial fibers. (C) Negative Control – ISO - Blood vessel congestion. (D) Negative Control – ISO – Congested blood vessels with mild inflammatory infiltrates. (E) EELI- 100 mg/kg – Myocardial fibers (F) EELI- 100 mg/kg - Focal mild inflammatory infiltrates (G) EELI– 200 mg/kg - Mild edema. (H) EELI alone - 200 mg/kg - Myocardium with normal myocytes. (I) α-TOCOPHEROL - 60mg/kg, STD - Normal myocardium with myocardial fibres. The result was shown in figure 3.

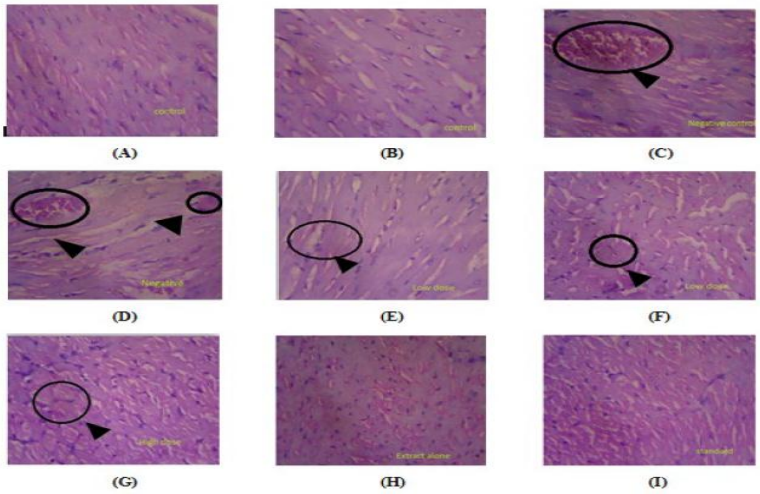

Fig. 3: Histopathological study results.

CONCLUSION

The ethanolic extract of Lawsonia inermis flowers treated with different concentrations of extract with animal groups shows mild inflammatory infiltrates, mild oedema and treated with L.inermis extract alone it shows myocardium with normal mycocytes and there is no evidence for loss of architecture and in treated with α-tocopherol it shows normal myocardium with myocardial fibres was maintained. Based on the improvement in cardiac marker enzyme level and histopathological studies, it is concluded that the ethanolic extract of Lawsonia inermis flowers possess cardio protective activity.

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