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Evaluation of cardioprotective activity of Ethanolic Extract of *Cordia sebestinato* against myocardial infarction

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A B S T R A C T

Intake of diets rich in antioxidant from medicinal plants have been reported to be associated with reduced risk of cardiovascular diseases. The present study was aimed to appraise the protective role of *Cordia sebestina*to leaf extract in isoproterenol-induced myocardial infarction in rats. Subcutaneous injection of isoproterenol (85 mg/kg) to male albino Wistar rats, exhibited a significant raise in the levels/activities of cardiac marker such as The present study reveals that ISO treatment resulted in marked elevation in the level of cardiac marker enzymes like CK-MB, AST, ALT, LDH and ALP in serum, serum lipid profiles such as HDL, LDL, VLDL,TGA, and Cholesterol are observed such that there is marked decrease in the levels of HDL in ISO treated group was observed. Pretreatment with *Cordia sebestina*to (200 and 400 mg/kg) daily for a period of 31days positively altered the activities of cardiac markers and other biochemical parameters to isoproterenol-induced rats. Thus, the results of our study demonstrate that *Cordia sebestina*to possess cardioprotective role against experimentally induced cardiac toxicity.

Keywords: HDL, LDL, VLDL, TGA, Cholesterol, *Cordia sebestinato*

A R T I C L E I N F O

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Contents

1. Introduction

Cardiovascular disease is escalating in recent years and remains as a leading cause of mortality in developing countries (Susila.R et al., 2013). In 1905, 25% of mortalities in USA were due to coronary heart disease and its complications. Myocardial infarction causes 35% of deaths in men between 35 and 50 age group of peoples.1 The death rate is higher for men than women between the ages for 35 and 55. Myocardial infarction (MI) commonly known as a heart attack, results from the interruption of blood supply to a part of the heart, causing heart cells to die (French et al., 2004). This is most commonly due to occlusion of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (cholesterol and fatty acids) and white blood cells in the wall of an artery.2 Free radicals have been implicated in many conditions, including heart disease,

diabetes, cancer, Alzheimer's disease, Parkinson's disease, cataracts, rheumatoid arthritis, and aging. Isoproterenol (ISO), a synthetic catecholamine and s-adrenergic agonist, has been documented to produce myocardial infarction in large doses3. On auto oxidation, ISO generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids and cause severe damage to the myocardial Membrane.4

Natural herbal preparations are generally well tolerated. Most herbs are nontoxic, with few, if any, harmful side effects. Herbal medicine has a long history and a time tested, valuable place in the treatment of many common health problems.5 Herbal preparations work gently, so they take to act internally.Man has been using herbs and plants products for combating diseases since times immemorial.6 In India, the earliest mention of the use of medicinal plants is to be found in the Rig-Veda which was written between 4500-1600BC (Agarwal, 2007)7. Hence based on the above literature we have selected the ethanolic extract of leaf of Cordia sebestenato evaluate its cardiac depressant activity, initially by investigating its phytochemical constituents and antioxidant activity.

2. Materials and Methods

Collection and identification of plant material

The leaves of *Cordia sebestina* was collected and authenticated from Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chitoor Dt, A.P. The collected leaf material was immediately dried at room temperature for one month, powdered mechanically sieved (10/44) and stored in air tight containers.[8]

Preparation of extract

After collection of the plant, shade dried leaves of *Cordia sebestina* were then blended in to fine powder with a blender and used for the ethanol extract. Ethanol extract was extracted by using soxhlet extractor for 18-20 h. The extract obtained, was concentrated under reduced pressure at controlled temperature (40-50° C) and finally made powdered. (K.R. Khandelwal 1998).[9]

Preliminary phytochemical evaluation: The ethanoilc extract of leaves of *Cordia sebestina* was subjected to different chemical tests separately for the identification of various active constituents.

Experimental animals

Male and female Wistar albino rats (130-160gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22±2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellet was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals.[10]

Experimental Design: The experimental rats were divided into five groups of 6 animals each and treated as follows:**Group 1:** Normal Control Rats treated with vehical,**Group 2:** Rats treated with ISO (85 mg/kg, s.c.),**Group 3:** Rats treated with Propranolol (5mg/kg,p.o)+ISO(85mg/kg rat,s.c.),**Group 4:** Rats treated with EECS (200 mg/kg p.o) + ISO (85 mg/kg rat, s.c.),**Group 5:** Rats pretreated with EECS (400 mg/kg p.o) + ISO (85 mg/kg rat, s.c.).

Acute oral toxicity study

The acute oral toxicity study was performed as per the Organization for Economic and Cooperation and Development (OECD) 423 guidelines. Six female rats (nulliparous and non pregnant; 130-160 gm bwt) were divided into two groups (3 per group) ie., control and test groups. Control group received 0.5 % carboxy methyl cellulose as vehicle at a dose of 10ml/kg b wt while the test group received an oral dose of 2000mg/kg b wt of ethanolic extract of leaves of *Cordia sebestina* [EECS] (10ml/kg b wt in 0.5% CMC).[11]

Evaluation:

Biochemical analysis: 24hr after the second injection of ISO, the animals were sacrificed by cervical decapitation and blood was collected and the heart was dissected out. The serum was separated immediately by cold centrifugation and used for determination of cardiac biomarkers and lipid profiles like CK-MB,AST, ALT, LDH and ALP in serum, serum lipid profiles such as HDL, LDL, VLDL,TGA, and Cholesterol by using commercial diagnostic kit.[12]

Histopathological studies

For histopathological studies, myocardial tissue obtained from the excised heart was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. Each section was stained with hematoxylin and eosin (H & E stain). The sections were examined under light microscope and photomicrographs were taken. [13] .

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.3). Statistical significance was set accordingly.

3. Results and Discussion

Preliminary phytochemical results of EECS: The result of preliminary phytochemical analysis of ethanolic extract of leaf of *Polyalthia Suberosa* Roth. showed the presence of flavonoids and steroids is shown in **Table No 1**

S.NO	Constiuents	Extract (ethanol)
1.	Alkaloids	+Ve
2.	Carbohydrates	-Ve
3.	Glycosides	+Ve
4.	Saponins	-Ve
5.	Proteins and Amino acids	+Ve
6.	Phytosterols	-Ve
7.	Fixed oils and Fats	-Ve

8.	**Phenolic compounds and flavanoids**	**+Ve**
9.	**Coumarin test**	**+Ve**

Denotes positive test result(-) → Denotes negative test result

Acute toxicity study:

During the study, there had been no treatment related demise or indications of poisoning developed in the control and EECS treated rats. The only behavioral signs of toxicity shown by the animals are kneading of nose and lips on the flooring of the cage and uneasiness. When compared to control group body weight changes are not significant in treated group and there had been no gross pathological abnormalities in both control and treated rats. Hence, as per the reference of Globally Harmonized System of Classification and labeling the chemicals the LD_{50} value of EECS was found to be 2000mg/kg b wt and this presents the consequence for safety hygiene of humans and animals. The observational and body weight changes are depicted in figure 1.

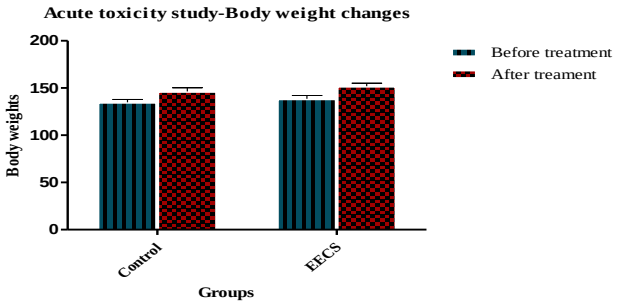


Figure: 1 Body weight changes during acute toxicity study

Cardio protective activity

a) Serum cardiac markers

Effect on CK –MB and LDH: Serum CK-MB levels were estimated by using commercial erba CK-MB kit and the results were shown in **Table no 2.** The result had shown the effect of EECS and standard on serum CKMB and LDH levels in isoproterenol administered experimental groups. There was significant ($p<0.001$) increase in serum CKMB and LDH levels in ISO control group when compared with treated groups.

b. Effect on ALT/SGPT and AST/SGOT:

Serum ALT levels were estimated by using commercial erba ALT kit and the results were shown in **Table no 2**. The result had shown the effect of EECS on serum ALT levels in experimental groups. There was significant ($p<0.001$) increase in serum ALT/SGPT and AST/SGOT levels in ISO control group when compared with normal group. The test-2 group which was receiving EECS (each 400 mg/kg) had shown significant ($p<0.001$) decrease in the serum ALT and AST levels when compared to ISO control group.

C. Effect on Alkaline phosphate (ALP):

Serum ALP levels were estimated by using commercial erba ALP kit and the results were shown in **Table no 2**. There was significant ($p<0.001$) increase in serum ALP levels in ISO control group when compared with normal group. The result had shown the effect of EECS and standardon serum ALP levels in experimental groups.Test-2 group which was receiving EECS (400 mg/kg) had shown more significant decrease ($p<0.001$) in serum ALP levels when compared with test-1 which was receiving EECS (200 mg/kg).

Various pharmacokinetic parameters like Cmax, t_{max} and elimination half-life were determined for pure drug, marketed formulation and optimized formulation and the results were shown in table 13. Relative bioavailability was determined and results obtained were tabulated in table 14 from the results obtained it was observed that the relative bioavailability of optimized formulation was higher than pure drug.

ii. LIPID PROFILES

a) Effect on serum cholesterol and HDL-cholesterol:[14]

Serum cholesterol levels were estimated by using commercial erba cholesterol kit and the results were shown in Table no 2. The result shows the effect of EECS on serum cholesterol levels in normal and experimental groups.

Administration of ISO causes fibrotic changes and accumulation of fat, this can be observed in control group rats because there was significant ($p<0.001$) increase in serum cholesterol and triglycerids levels in ISO control group when compared with normal group. The result had shown the effect of EECS on serum HDL levels in normal and experimental groups. There was significant ($p<0.001$) decrease in serum HDL levels in ISO control group when compared with normal group.

The test-1and test-2 which was receiving EECS 200 and EECS 400 showed decrease in the serum cholesterol levels. The standard group had also shown significant ($p<0.001$) decrease in the serum cholesterol levels when compared to ISO control group.

b) Effect on serum triglycerides (TG):[15]

Serum triglycerides levels were estimated by using commercial erba triglycerides kit and the results were shown inTable no 5.

The result shows the effect of EECS on serum triglycerides levels in normal and experimental groups. ISO causes increase in triglyceride synthesis, so when it is administered in control group it leads to significant ($p<0.001$) increase in serum triglycerides levels.

Flavonoids present in EECS caused reduction of triglycerides. Combination effect had shown more significant ($p<0.001$) decrease in the serum triglycerides levels when compared to ISO control group than the test-1and test-2 which was receiving EECS (200 mg/kg) and EECS (400 mg/kg) individually.

C. Effect on serum LDL and VLDL levels:[16]

Serum LDL levels were estimated by using commercial erba LDL kit and the results were shown in Figure5.7 and Table no 5. The result shows the effect of EECS on serum LDL and VLDL levels in normal and experimental groups.

There was significant ($p<0.001$) increase in serum levels LDL and VLDL in ISO control group when compared with normal group. The test-1and test-2 which was receiving EECS (200 mg/kg) and EECS (400 mg/kg) respectively had shown significant decrease in the serum LDL and VLDL levels.

Table no: 2 Effect of *Cordia sebestina* leaveson Serum biomarkers

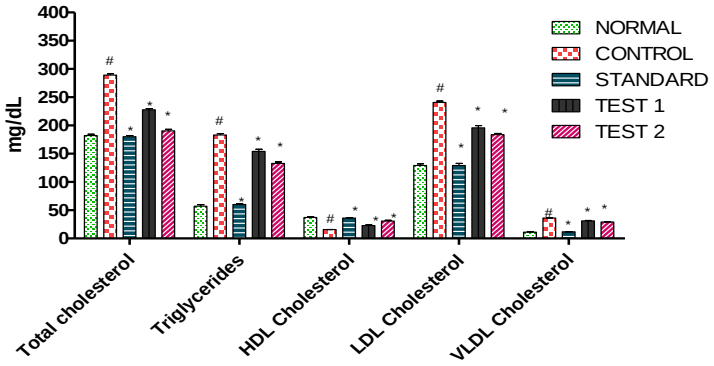
Groups	TREATMENT	On 31st Day				
		CK-MB (IU/L)	LDH (IU/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal	Vehicle	80.12±3.4	358.2±7.47	31.43±1.52	29.33±1.78	52.27±2.04
Control	ISO (85 mg/kg, s.c.)	203.4±3.17###	492.5±9.45###	91.5±2.74###	48.32±2.74###	124.8±2.39###
Standard	Propranolol (5 mg/kg, p.o)+ ISO (85 mg/kg rat, s.c.)	90.34±2.84***	352.3±8.13***	32.93±1.15***	31.52±1.63***	53.3±1.45***
Test-1	EECS (200 mg/kg p.o) + ISO (85 mg/kg rat, s.c.)	145.3±4.11***	321.5±10.52***	60.43±2.24**	35.63±2.31**	84.30±2.13***
Test-2	EECS (400 mg/kg p.o) + ISO (85 mg/kg rat, s.c.)	98.3±2.76***	347.2±9.32***	35.10±1.53***	30.87±2.82***	58.29±1.84***

All values are shown in mean ± SEM and n=6, ###, indicates $p<0.001$, when compared with normal group, *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$, when compared to control group.

Table no: 3 Effect of Cordia sebestina on serum

GROUPS	TREATMENT	On 31st Day				
		TG(mg/dL)	CHOLESTROL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Normal	Vehicle (distilled water)	57.16±2.83	182.28±2.17	37.03±1.45	129.1±3.16	11.23±0.57
Control	ISO (85 mg/kg rat, s.c.)	183.00±2.37###	289.23±2.12###	15.72±0.19###	240.8±2.47###	36.00±0.50###
Standard	Propranolol (5 mg/kg, p.o)	60.12±1.42***	180.03±2.24***	36.31±0.68***	129.0±3.70***	11.67±0.20***
Test-1	EECS (200 mg/kg p.o) +ISO (85 mg/kg rat, s.c.)	154.17±3.64***	227.9±2.04***	22.82±1.01*	195.7±4.06***	31.00±0.92***
Test-2	EECS (400 mg/kg p.o) + ISO (85 mg/kg rat, s.c.)	132.94±2.53***	190.36±2.71***	30.83±1.35*	183.9±1.8***	29.00±0.24***

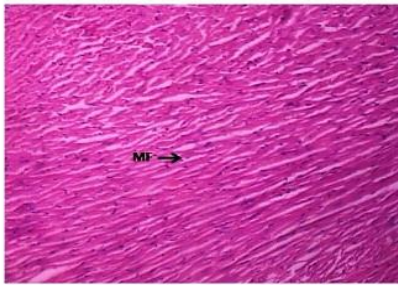
All values are shown in mean ± SEM and n=6, ###, indicates $p<0.001$, when compared with normal group, *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$, when compared to control group.



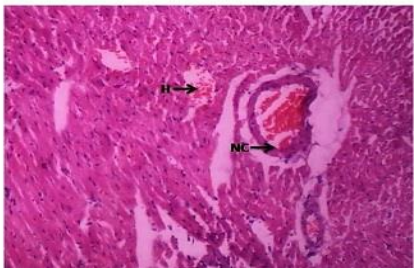
Normal = Vehicle treated; Control = Isoproterenol (85 mg/kg s.c); Standard = Propranolol (5 mg/kg p.o.) + Isoproterenol (85 mg/kg s.c.); Test-1 = EECS (200mg/kg, p.o.) + Isoproterenol (85 mg/kg s.c.); Test-2 = EECS (400mg/kg, p.o.) + Isoproterenol (85 mg/kg s.c.).

Histopathology:

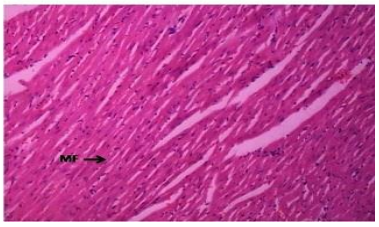
Histopathalogical studies of the normal group of hearts showed normal myocardium structure. In ISO induced group of animals showed the necrotic changes in myocardial tissue along with hemorrhage. In standard group animals showed normal cyto architecture of myocardium. Pre-treatment with EECS (200mg/kg p.o) & EECS (400mg/kg p.o) rats showed regenerative changes in myocardial tissue.[17]



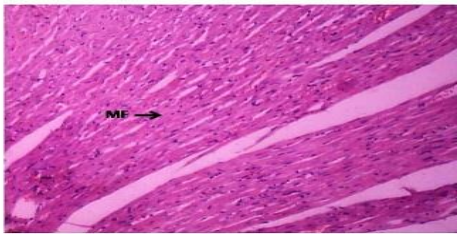
Normal group



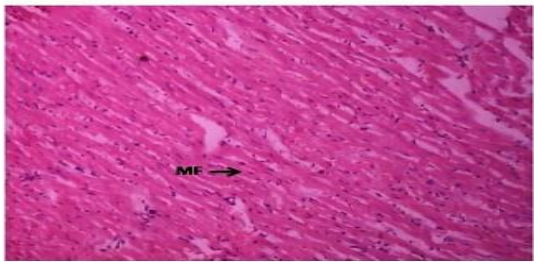
Control group



Standard group



Test-1 group



Test-2 group

4. Conclusion

The present study reveals that ISO treatment resulted inmarked elevation in the level of cardiac marker enzymes like CK-MB,AST, ALT, LDH and ALP in serum, serum lipid profiles such as HDL, LDL, VLDL,TGA, and Cholesterol are observed such that there is marked decrease in the levels of HDL in ISO treated group was observed. Pretreatment with *Cordia sebestina* was normalized the levels of serum biomarkers. Significant increase in the level of lipid peroxidation anda significant decrease in the activity of GSH and CAT were observed after treatment with ISO as compared to control, standard and test groups. There is increase in heart wt. in ISO treated group due accumulation of fluid and infiltration of macrophages which leads to edema and necrosis. The test groups treated shows significant decrease in heart wt. and the difference is less when compared to normal group.

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