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CARDIOPROTECTIVE EFFECT OF SEENTHIL CHOORANAM IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Background: Oxidative stress-mediated myocardial damage is a key factor in the development of cardiovascular diseases. Seenthilchooranam, a classical Siddha formulation, is believed to possess antioxidant and cardioprotective properties. The present study aimed to evaluate the cardioprotective activity of Seenthilchooranam against isoproterenol-induced myocardial toxicity in wistar albino rats.

Aim: To evaluate the cardio protective activity of Seenthilchooranam Against isoproterenol -induced myocardial injury in wistar rats by assessing antioxidant status, heart/body weight ratio, biochemical markers and histopathological changes.

Materials and Methods: Wistar albino rats were allocated into five groups[n=6]: normal, isoproterenol control, vitamin E- treated standard group and two treatment groups receiving low and high doses of SC. Treatments were given orally for 28 days, followed by ISO injections for two consecutive days to induce myocardial injury. Serum cardiac marker enzymes [CK-MB, LDH, AST], heart weight/body weight ratio and cardiac antioxidant parameters such as malondialdehyde [MDA], superoxide dismutase [SOD], catalase [CAT] and reduced glutathione [GSH] were evaluated. Cardiac tissues were further examined histologically. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison tests.

Result: Isoproterenol challenge resulted in significant damage in the myocardium, characterized by increased cardiac enzyme release, elevated lipid peroxidation, and decreased the levels of antioxidant enzymes and structural abnormalities in heart tissue. Treatment with Seenthilchooranam significantly improved these biochemical and tissue parameters in a dose-dependent manner with the higher dose showing effects comparable to the standard drug. Histopathological findings supported the biochemical results showing improved myocardial architecture and reduced cellular degeneration.

Conclusion: Seenthilchooranam demonstrated significant cardioprotective activity against isoproterenol- induced myocardial injury, possibly through antioxidant, membrane-stabilizing and free-radical scavenging mechanisms. These results support the potential role of Seenthilchooranam as a cardioprotective agent in oxidative stress-related cardiac disorders.

Keywords: *Cardioprotective activity, Seenthilchooranam, Isoproterenol, Wistar rats.*

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INTRODUCTION

Cardiovascular diseases are a major cause of death worldwide. Approximately 19.8 million people died from cardiovascular diseases in 2022, accounting for nearly 32% of total deaths worldwide [1]. Myocardial infarction is the most lethal manifestation of cardiovascular diseases [2]. Myocardial damage occurs due to imbalance between oxygen supply and demand, oxidative stress and overproduction of reactive oxygen species, which can lead to membrane destabilization,

cellular injury and leakage of cardiac enzymes. Among various experimental models, the isoproterenol-induced rat model has been used to evaluate several cardiac dysfunctions [3]. Experimental models using β-adrenergic agonist isoproterenol are used to induce myocardial toxicity, as this resembles human myocardial infarction. Oxidative stress is associated with heart failure, in which elevated levels of reactive oxygen species (ROS) are found in myocardium. This stress generally results from imbalance between body's natural antioxidant defenses and amount of ROS produced. It contributes to cellular oxidative damage. Antioxidants help protect cells by interrupting oxidative chain reactions and thereby slowing or preventing the oxidation process [4]. In recent years, growing attention has been given to the potential role of herbal medicines in the prevention and treatment of cardiovascular disorders. The trial drug Seenthilchooranam is a classical Siddha formulation sourced from literature "Agasthiarparipooranam - 400" [5]. The ingredients of this formulation are Seenthil, Karisalai and Earthworm. In previous studies, Seenthil [Tinospora cordifolia] belongs to the Menispermaceae family and has been screened for its lipid-lowering potential, anti-hyperglycemic activity, diuretic activity and cardioprotective activity [6]. Karisalai [Eclipta alba] belongs to Asteraceae family was known to have diuretic, hypolipidemic and anti-diabetic activity [7]. Earthworm [Eudriluseugeniae] extract was known to have anti-thrombotic, antioxidant and hypoglycemic activity in some studies [8].The current study is designed to evaluate the cardioprotective effect of Seenthilchooranam on isoproterenol- induced biochemical and histopathological changes in rats.

MATERIALS AND METHODS

Study population-30 wistar albino rats
Study design-In-vivo experimental animal study
Study place- Animal bred house, Dept. of Pharmacology, ArulmiguKalasalingam College of pharmacy, Krishnankoil, Srivilliputtur

EXPERIMENTAL ANIMAL

CPCSEA guidelines were followed and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC approval number-AKCP/IAEC/21/25-26). In groups of six, ten-week-old Wistar albino rats (200±25 g) were kept in regular settings (12-hour light/dark cycle, 24 ±2 °C, 35–60% humidity).

EXPERIMENTAL PROCEDURE

Following acclimation, the animals were split into the following groups at random, each with six rats:
Group 1 (Normal control) : The animals received a subcutaneous (S.C.) injection of 0.5% Carboxymethylcellulose (CMC) + saline.
Group 2 (ISO Control) :The animals received 0.5% CMC+ISO (85 mg/kg,b.w.S.C.).
Group 3 (Standard control): The animals received 100 mg/kg of vitamin E orally (p.o.) along with ISO
Group4 SC Low dose:The animals received 200mg/kg,p.o. + ISO.
Group 5 SC High dose : The animalsreceived 400mg/kg,p.o. + ISO.
The cardioprotective effect of SC was assessed using an isoproterenol-induced myocardial toxicity model. Isoproterenolwas freshly dissolved in normal saline and administered within 10 minutes of preparation. Myocardial injury was induced by subcutaneous injection of isoproterenol at a dose of 85 mg/kg body weight for 2 consecutive days at 24-hour intervals, commencing on day 29 of the experimental schedule. Accordingly, total study period comprised 28 days of pretreatment followed by 2 days of isoproterenol administration, resulting in an overall experimental period of 30 days.

Biochemical parameters

Serum biochemical parameters including Lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) and aspartate transaminase (AST) cardiac marker enzymes, were measured using the obtained samples utilizing commercial standard enzyme assay kits.

Heart weight to body weight ratio

In each group, heart weight to body weight ratio was calculated. Body weight was the weight on the day of sacrifice. Heart weight was measured after keeping the heart in cold saline and squeezing out the blood.

Tissue Antioxidant parameters

Lipid peroxidation assay (MDA content)

According to Slater and Sawyer (1971), this assay was used to measure the level of thiobarbituric acidreactive substances (TBARS). After adding 2.0 mL of freshly made 10% w/v trichloroacetic acid (TCA) to 2.0 mL of the tissue homogenate (supernatant), the combination was left in an ice bath for 15 minutes before being centrifuged at 2500 rpm for 15 minutes at 4°C. Two milliliters of freshly made 0.67% w/v TBA was added with two milliliters of clear supernatant solution. The resultant solution was heated in a boiling water bath for ten minutes. After that, it was quickly cooled for five minutes in an ice bath. A UV/VIS spectrophotometer (JASCO-V-530, Japan) was used to evaluate the absorbance of color produced at 532 nm using standard 1,1,3,3,-tetraethoxypropane .

GSH estimation

The GSH assay was conducted using the methodology outlined by Moron et al. (1979). After combining one milliliter of tissue homogenatesupernatant with one milliliter of 20% TCA, the mixture was centrifuged at 2500 rpm for fifteen minutes at 4°C. Two milliliters of 0.6 mM DTNB reagent were added to 0.25 milliliters of supernatant. Phosphate buffer (pH 8.0) was used to make up the volume to 3 mL. The developed yellowcolor was measured at 412 nm against reagent blank. To create a standard curve, different concentrations of reduced glutathione (10–50 µg) ere

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treated as previously described. Reduced glutathione was measured in µg of GSH/mg of protein.

SOD activity estimation

The Misra and Fridovich (1972) approach was used to calculate the SOD activity. A vortex mixer was used to thoroughly mix 0.5 mL of cardiac homogenate, 0.5 mL of ice-cold distilled water, 0.25 mL of ice-cold ethanol, and 0.15 mL of ice-cold chloroform. The mixture was then centrifuged at 2500 rpm for 15 minutes at 4°C. 1.5 mL of carbonate buffer (pH 10.2) and 0.5 mL of 0.4 mM ethyl enediaminetetraacetic acid (EDTA) solutions were added to 0.5 mL of the supernatant. Absorbance/minute was measured at 480 nm in comparison to the reaction blank after the reaction was started. The injection of 0.4 mL of epinephrine bitartrate (3 mM) and the shift in optical density units per mg protein were used to measure SOD activity. The enzyme unit was determined by measuring the change in absorbance per minute at 50% auto-oxidation of epinephrine to adrenochrome. 10–125 units of SOD were used to create the calibration curve.

Histopathology

Following sacrifice, the heart was quickly removed, cleaned with saline, and preserved in 10% buffered formalin. The fixed tissues were paraffin-embedded and sectioned at 5 µm. Hematoxylin and eosin (H&E) was used to stain each section. The sections were analyzed for histopathological alterations using a light microscopeand the photomicrographs were captured. The pathologist conducting the histological assessment was blinded to the various treatment groups.

STATISTICAL ANALYSIS

All the data were statistically evaluated with ANOVA and the differences among groups were determined by Dunnett's multiple comparison tests using Graph pad prism 5.0. Values were considered to be significant when $P < 0.05$. All the results were presented as mean ± SEM

Table 1: Effect of SC on Biochemical parameter in Rat

PARAMETER	NORMAL CONTROL	ISO CONTROL	STANDARD (VIT-E+ISO)	SC (LOW DOSE)	SC (HIGH DOSE)
CK-MB (IU/L)	**84.5** ±34.5	**224.5** ±91.7	**147.5** ±60.2	**177.5** ±72.5	**157.5** ±64.3
LDH (IU/L)	**181** ±73.9	**551.3** ±225.1	**299.8** ±122.3	**365.2** ±149.2	**314.2** ±128.3
AST(IU/L)	**46.0** ±18.8	**107.3** ±43.8	**70.0** ±28.6	**82.5** ±33.7	**74.0** ±30.2

RESULT

All values were expressed as mean ± SEM, n = 6, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test.

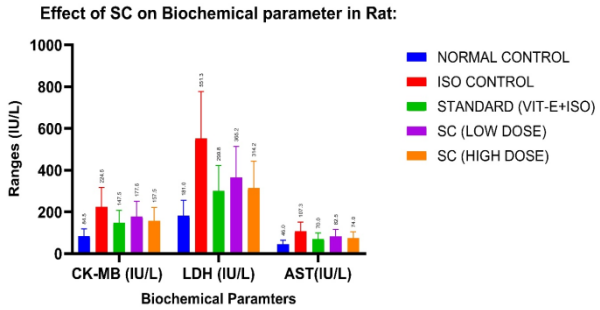

Fig 1: Effect of SC on biochemical parameter

Table 2: Effect of SC ON Heart Weight / Body Weight Ratio in Rat

GROUP	Heart weight / Body weight ratio ($\times 10^{-3}$)
NORMAL CONTROL	**2.75 ± 0.09**
ISO CONTROL	**4.10 ± 0.18**
STANDARD (VIT-E + ISO)	**2.98 ± 0.11**

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SC (LOW DOSE)	**3.25 ± 0.13**
SC (HIGH DOSE)	**2.90 ± 0.10**

All values were expressed as mean ± SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test

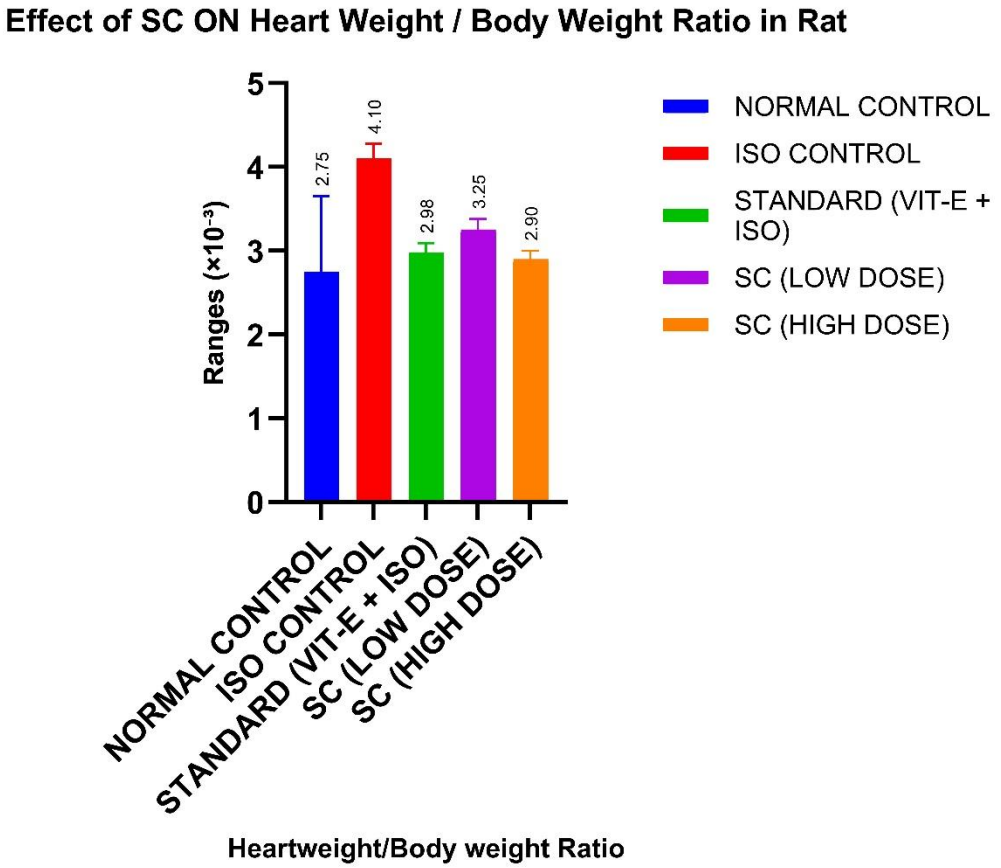

Fig 2: Effect of SC on heart weight /body weight ratio

Table 3: Effect of SC on Cardiac Tissue Parameters

Parameters	NORMAL CONTROL	ISO CONTROL	STANDARD (VIT-E + ISO)	SC (LOW DOSE)	SC (HIGH DOSE)
Lipid peroxidation (nmol MDA/mg protein)	1.35 ± 0.07	4.05 ± 0.18	2.05 ± 0.11	2.45 ± 0.14	1.90 ± 0.10
SOD (U/mg protein)	6.45 ± 0.28	2.60 ± 0.16	5.85 ± 0.24	5.05 ± 0.22	5.70 ± 0.25
CAT (µmol H_2O_2/min/mg protein)	60.2 ± 2.3	30.5 ± 1.7	55.8 ± 2.0	49.6 ± 1.8	53.9 ± 2.1
GSH (µg/mg protein)	7.45 ± 0.32	2.95 ± 0.19	6.75 ± 0.29	5.95 ± 0.26	7.10 ± 0.30

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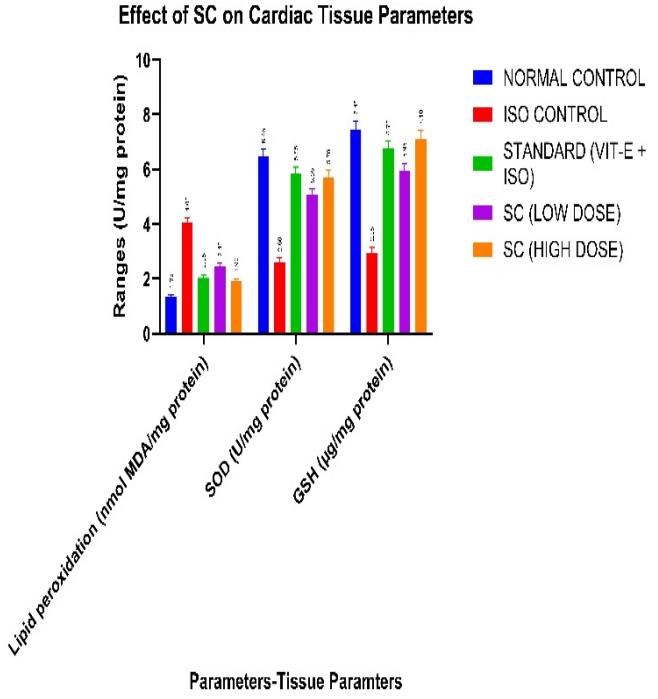

Fig 3: Effect of SC onlipid peroxidation, SOD, GSH

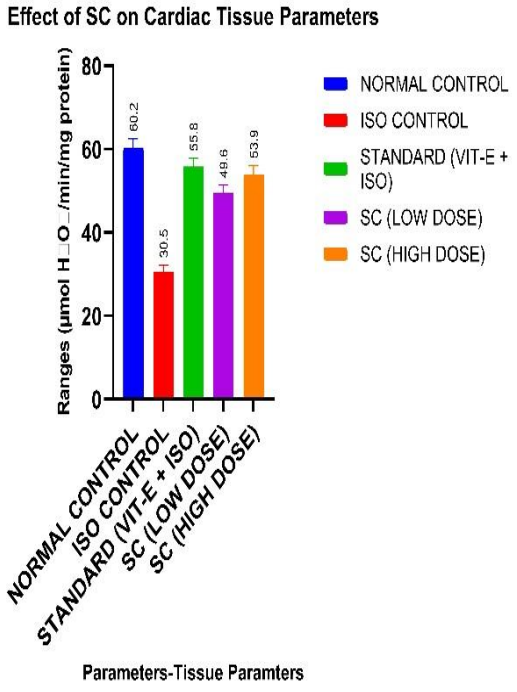

Fig 4: Effect of SC on CAT

Histopathology

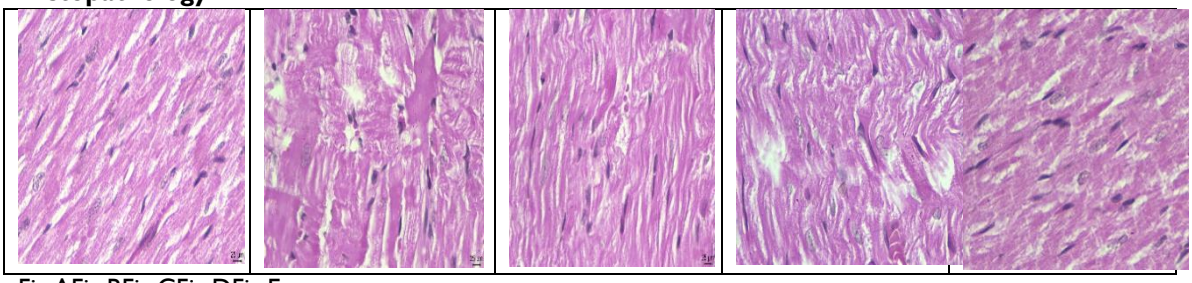

Fig:AFig:BFig:CFig:DFig:E

Histopathological analysis of H&E stainedheart sections from several experimental groups revealed marked differences corresponding to myocardial injury caused by isoproterenol (ISO) and the preventive effects of the test chemical and standard treatment. The myocardium was within normal histological architecture in the normal control group (0.5% CMC + saline, s.c.), which displayed normal myocardial architecture with intact cardiac myocytes and no signs of degeneration, vacuolation, or inflammatory alterations. On the other hand, the ISO control group (0.5% CMC + ISO, 85 mg/kg, s.c., twice at a 48-hour interval) exhibited myocardial damage characterised by mild to moderate multifocal intracytoplasmic vacuolation of cardiac myocytes, indicating ISO-induced cellular degeneration.The standard control group treated with vitamin E (100 mg/kg/day, p.o. + ISO) showing marked improvement in myocardial architecture with only minimal focal vacuolation, indicating significant cardioprotection. The SC low-dose group (p.o. + ISO) showed moderatevacuolar degeneration of myocytes, suggesting a partial protective effect.Significantly, compared to the ISO control and SC low-dose groups, the SC high-dose group (p.o. + ISO) showed nearly normal myocardial histological features with minimal focal vacuolation and better preservation ofstructural integrity, indicating a dose-dependent cardioprotective effect of the SC treatment.

DISCUSSION

The present study demonstrates the cardioprotective potential of Seenthilchooranam against isoproterenol-induced myocardial injury in Wistar albino rats. Isoproterenol is a well-established experimental agent used to induce myocardial infarction-like lesions

through excessive β-adrenergic stimulation, leading to oxidative stress, calcium overload, lipid peroxidation and subsequent myocardial necrosis. In the ISO control group, serum cardiac marker enzymes such as CK-MB, LDH and AST were significantly increased indicating severe myocardial damage. Pretreatment with seenthilchooranam reduced these enzyme levels in a dose-dependent manner. Fig 1. Shows that the high-dose seenthilchooranam group showed values close to the standard vitamin E group, suggesting effective protection of cardiac cell membranes and prevention of enzyme leakage.The heart weight to body weight ratio was significantly increased in ISO-treated rats, indicating myocardial edema and hypertrophy. Fig 2. Shows that treatment with seenthilchooranam significantly reduced this ratio, especially at the higher dose, showing its ability to reduce cardiac swelling and abnormal tissue changes. Oxidative stress plays a major role in ISO-induced cardiac injury. The ISO control group showed increased lipid peroxidation [MDA levels] and decreased antioxidant enzymes such as SOD, CAT and GSH. Fig 3 and 4 shows that the Seenthilchooranam treatment significantly reduced lipid peroxidation and restored antioxidant enzyme levels in a dose-dependent manner. These findings suggest that seenthilchooranam has strong antioxidant activity and helps protect the heart from oxidative damage. Histopathological examination supported the biochemical results. The ISO control group showed myocardial degeneration with intracytoplasmic vacuolation, whereas the vitamin E-treated group showed near-normal myocardial structure. The seenthilchooranam high-dose group also showed almost normal cardiac architecture, indicating effective protection against ISO-induced myocardial damage.

CONCLUSION

This study shows that Seenthilchooranam has a protective effect against isoproterenol-induced myocardial damage in rats. Isoproterenol caused severe heart injury, shown by increased cardiac enzymes, heart weight/body weight ratio, oxidative stress and tissue damage. Treatment with seenthilchooranam reduced cardiac enzyme levels, improved antioxidant status, decreased lipid peroxidation and preserved normal heart structure. The high dose of seenthilchooranam showed better protection, similar to vitamin E. Overall, seenthilchooranamcan protect the heart mainly due to its antioxidant and membrane- stabilizing properties and may be useful in preventing heart damage caused by oxidative stress.

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SPONSORSHIP AND CONFLICT OF INTEREST

Nil

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ETHICAL APPROVAL

Approved

Abbreviation:

SC- Seenthil Chooranam

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