Original Article

Pharmacodynamic interaction of *Tinospora cordifolia* Willd. With *Ocimum sanctum* Linn. in isoproterenol-induced cardiac toxicity

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Abstract

Background: Cardiovascular diseases are the leading causes of deaths despite several advancements in the current medical interventions. Among them, myocardial infarction (MI) is the most alarming disease as about 17.1 million peoples die every year due to MI. **Aim:** The present study was designed to investigate the potential cardioprotective effect of combination of standardized extracts of *Tinospora cordifolia* (SETC) (250 mg/kg and 500 mg/kg) and *Ocimum sanctum* (SEOS) (50 mg/kg) in isoproterenol (ISO)-induced MI. **Materials and methods:** MI was induced in rats by subcutaneous injection of ISO for 2 consecutive days at an interval of 24 h. Rats were pretreated with test drugs for the period of 21 days, and ISO was administered on the 20th and 21st days. At the end of experiment, i.e., on 22nd-day electrocardiograph, a hemodynamic, biochemical, and histopathological study of heart tissues was evaluated from control and experimental groups and statistically analyzed by one-way analysis of variance followed by Tukey's test. **Results:** ISO-administered rats showed significant changes in electrocardiograph, mean arterial blood pressure, heart rate, biochemical markers, antioxidant parameters, and histopathology of heart. The activities of cardiac biomarkers were reduced in serum, and there was an increase in antioxidants in heart tissue of test drug-treated animals. Similarly, electrocardiograph, mean arterial blood pressure, and heart rate were restored to normalcy in all test and standard drug-treated animals. **Conclusion:** The SETC 500 mg/kg in combination with SEOS 50 mg/kg was found to be effective in prevention of myocardial injury induced by ISO.

Keywords: Cardiac toxicity, isoproterenol, *Ocimum sanctum* and *Tinospora cordifolia*

Introduction

Cardiovascular diseases are the leading causes of deaths despite several advancements in the current medical interventions. Among them, myocardial infarction (MI) is the most alarming disease as about 17.1 million people die every year due to MI.[1] The death toll is expected to rise up to 20 million and will be a leading cause of death worldwide by 2025.[2] Risk factors for MI are hypercholesterolemia, increased level of low-density lipoprotein, diabetes, hyperlipoproteinemia, high blood pressure, smoking, obesity, and older age.[3] Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist which increases heart rate and exhausts energy reservoir of cardiac cells leading to cell death. It causes myocardial cell death via multiple modes. It manifests by its stimulation to sarcolemmal adenylate cyclase and Ca_2^+ and Na^+ channels resulting in increased influx of Ca_2^+ and energy consumption that leads to cell death.[4]

Advancement in novel drug discovery technology and search for novel chemical diversity has intensified for exploring lead from Ayurveda.These days drugs from plant origin are used extensively for the treatment of various diseases due to their less toxicity and high efficacy, and the World Health Organization has also recommended the evaluation of the efficacy of plant origin drugs in disease condition where there is a lack of safe modern drugs.[5] Combined use of herbs with drugs may mimic, increase, or decrease the effects of either

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component, resulting in clinically important herb–drug or herb–herb interactions.[6] At present, our understanding of the interactions between drugs and herbs and between herbs, drugs, and food is less. Much more research is still required in herbal therapy to examine individual plant active constituents and to determine how plant constituents interact with drugs and food.[7]

Tinospora cordifolia belonging to the family of Menispermaceae is popularly known as "Giloya." *T. cordifolia* is widely used in Ayurvedic System of Medicine as a tonic, vitalizer, and as a remedy for metabolic disorders and is used in veterinary folk medicine.[8] Previously, it was reported that *T. cordifolia* extract ameliorates cardiac toxicity induced by ischemia-reperfusion,[9] calcium chloride,[10] cadmium,[11] and doxorubicin.[12]. *Ocimum sanctum* (the holy basil) belongs to the family Lamiaceae and is reported to have antioxidant properties. *O. sanctum* is used especially for treating various types of diseases and lowering blood glucose and to treat cold, fever, parasitic infestations, and inflammation of joints.[13] *O.sanctum* also has significant cardioprotective activity in ISO,[14-16] ischemia-reperfusion,[17] and anemic hypoxia-induced cardiac toxicity.[18]

Hence, it is worthwhile to determine the effect of either alone and in combination of two potential cardioprotective herbs, namely of standardized extracts of *T. cordifolia* (SETC) and standardized extracts *O. sanctum* (SEOS) in ISO-induced cardiotoxicity.

Materials and methods

Chemicals

Standardized aqueous extract of aerial parts of *T. cordifolia* and standardized methanolic leaf extract of *O. sanctum* were obtained from SAMI Labs Limited, Bengaluru, Karnataka, India. ISO was purchased from TCI Chemicals (India) Pvt. Ltd. Chennai, Tamil Nadu. Creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and creatine phosphokinase (CPK) standard kits were purchased from local distributors. All chemicals used in the study were of analytical grade.

Experimental animals

Experiments were carried out using male albino Wistar rats weighing 150–200g. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk and renewed every 24 h under a 12:12 h light-dark cycle at an ambient temperature (25°C ± 1°C) and relative humidity (50% ± 10%). The animals had free access to water and food, *ad libitum*. The animals were fed on a standard pellet diet. The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals and approved by the Animal Ethical Committee of SET's College of Pharmacy (Reg. No. 112/PO/ReS/1999/CPCSEA, letter dated February 07, 2017), Dharwad, Karnataka, India.

Dose selection

Based on the literature survey, rat doses 250 and 500 mg/kg for SETC[9] and 50 mg/kg for SEOS were selected.[14] Cardiac toxicity was induced by administration of ISO at the dose 85 mg/kg subcutaneously (s.c.) on the 20th and 21st days at an interval of 24h.[19]

Experimental protocol

The male albino Wistar rats were divided into 7 groups of 6 animals each, i.e. Group I received distilled water (vehicle control) at 1 ml/kg p.o.; Group II ISO control 85 mg/kg s.c.; Groups III and IV received SETC at 250 and 500 mg/kg p.o., respectively; Group V received SEOS extract at 50 mg/kg p.o.; Group VI received SETC 250 mg/kg + SEOS 50 mg/kg p.o.; and Group VII received SETC 500 mg/kg + SEOS 50 mg/kg p.o. For oral treatments, SETC and SEOS were suspended in 0.3% tragacanth and oral application volume for all treatments was 10 ml/kg/day. All the animals were treated orally per day using oral feeding syringe and needle for 21 days.

Isoproterenol-induced myocardial necrosis in rats

After treatment, animals from Groups II–VII were administered with ISO at 85 mg/kg s.c. for 2 consecutive days on the 20th and 21st days[19]. After 24 h, of final subcutaneous injection of ISO, electrocardiograph and mean arterial blood pressure were measured using data acquisition system IWORX model 228S and MRBP rat tail-cuff method blood pressure system by IITC Life Science, USA.

Biochemical estimations

After 24 h of final subcutaneous injection of ISO, blood was collected by retro-orbital puncture under ether anesthesia and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 2500 revolutions per minutes (rpm) at 30°C for 15 min and used for the estimation of marker enzymes, namely CK-MB, AST, LDH, ALT, and CPK. Animals were sacrificed and hearts were dissected out immediately, washed with ice-cold saline, and used to prepare 10% (w/v) homogenates in phosphate buffer (50 mM, pH 7.4). The homogenates were centrifuged at 7000 rpm for 10 min at 4°C and supernatants were used for the assays of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH).

Superoxide dismutase estimation

SOD was assayed by the method of Sun and Zigman in which the activity of SOD was inversely proportional to the concentration of its oxidation product adrenochrome, which was measured spectrophotometrically at 320 nm.[20]

Catalase estimation

CAT was estimated by the method of Clairborne, which is a quantitative spectroscopic method developed for following the breakdown of H_2O_2 at 240 nm in unit time for routine studies of CAT kinetics.[21]

Glutathione estimation

GSH was estimated in the heart homogenate using 5,5'-dithiobis-(2-nitrobenzoic acid) by the method of Ellman. The absorbance was read at 412 nm and the results were expressed as µmol of GSH/g of wet tissue.[22]

Histological studies

At the end of the study, all the rats were sacrificed by cervical decapitation and the hearts were dissected out, washed in ice-cold saline. Then myocardial tissue was immediately fixed in 10% formalin solution. After fixation, tissues were embedded in paraffin and serial sections (4-5 μm thick) were taken and each section is then stained with hematoxylin and eosin. Then the slides were examined under light microscope for histoarchitectural changes.

Statistical analysis

All the experimental results were expressed as mean ± standard error of mean one-way analysis of variance followed by Tukey's test using GraphPad InStat, version 5.0 (GraphPad Software, 2365 Northside Dr. Suite 560 San Diego, California 92108, USA). The inter group difference was considered significant when $P < 0.05$.

Results

Effect of different dose combinations on electrocardiograph pattern and hemodynamic changes

The normal control group animals showed normal patterns of electrocardiograph, whereas the rats treated with ISO exhibited a marked reduction in R-amplitude along with a significant elevation of ST-segment, indicative of MI. Animals treated with drug combination of SETC 500 mg/kg + SEOS 50 mg/kg showed a significant ($P < 0.001$) decrease in ST-segment and a marked ($P < 0.001$) increase in the R-amplitude as compared to electrocardiographs obtained from ISO-alone-treated rats. Whereas, animals treated with SETC 500 mg/kg showed a significant ($P < 0.01$) decrease in ST-segment andsignificant ($P < 0.01$) increase in the R-amplitude. Further, animals treated with SETC 250 mg/kg + SEOS 50 mg/kg showed a significant ($P < 0.05$) decrease in ST-segment and significant ($P < 0.05$) increase in the R-amplitude when compared to the ISO control group [Table 1].

The mean arterial blood pressure was significantly ($P < 0.001$) decreased in the ISO-treated group to 50.17 mmHg compared to the normal control group. The mean arterial blood pressure was increased significantly in animals treated with SETC 500 mg/kg + SEOS 50 mg/kg ($P < 0.001$). Whereas, animals treated with SETC 250 mg/kg + SEOS 50 mg/kg and SETC 500 mg/kg showed a significant ($P < 0.01$) increase in mean arterial blood pressure compared to the ISO-treated group. Animals treated with SETC 250 mg/kg and SEOS 50 mg/kg showed a significant ($P < 0.05$) increase in mean arterial blood pressure. Further, a significant ($P < 0.001$) increase in heart rate was observed in the ISO-treated control group compared to the normal control group. Animals treated with drug combination of SETC 500 mg/kg + SEOS 50 mg/kg showed a significant ($P < 0.001$) decrease in heart rate compared to the ISO-treated control group [Table 1].

The effects of different dose combinations on cardiac biomarker enzymes such as CK-MB, CPK, LDH, AST, and ALT are shown in Table 2. Levels of these enzymes were significantly ($P < 0.001$) increased in the animals treated with ISO compared to the normal control group. Pretreatment with dose combination SETC 500 mg/kg + SEOS 50 mg/kg reduced significantly ($P < 0.001$) the activities of CK-MB, CPK, LDH, AST, and ALT. The administration of SETC 250 mg/kg + SEOS 50 mg/kg combination and SETC 500 mg/kg alone also showed a significant ($P < 0.01$) reduction in activities of CK-MB, CPK, LDH, AST and ALT when compared to the ISO control group.

The effect of different extract combinations on antioxidant parameters such as SOD, CAT, and GSH are depicted in Table 3. ISO-alone-treated animals had shown a significant ($P < 0.001$) reduction in the antioxidants compared to normal control animals. Pretreatment with SETC 500 mg/kg + SEOS 50 mg/kg had significantly ($P < 0.001$) restored the antioxidants. Whereas, animals treated with SETC 250 mg/kg + SEOS 50 mg/kg combination and SETC 500 mg/kg alone showed a significant ($P < 0.01$) increase in antioxidants. While animals treated with SETC 250 mg/kg and SEOS 50 mg/kg showed a significant ($P < 0.05$) increase in antioxidants when compared to the ISO control group.

Histopathological examination

The histopathological results of heart tissue are shown in Figure 1. The normal control [Figure 1a] group revealed normal cardiac fiber arranged uniformly with clear striations and no

Table 1: Effect of different dose combinations on electrocardiographic changes, mean arterial blood pressure, and heart rate in normal and isoproterenol-induced myocardial infarcted rats

Treatment	Mean arterial blood pressure (mmHg)	R-amplitude	ST-segment	Heart rate
Distilled water 1 ml/kg	92.33±2.418	0.7850±0.1384	0.1800±0.0416	346.7±3.018
ISO	50.17±2.688$^{###}$	0.4167±0.0477$^{###}$	0.3317±0.0119$^{###}$	414.3±3.556$^{###}$
SETC 250 mg/kg	63.50±2.141*	0.7167±0.0307*	0.2567±0.0055*	399.3±3.343*
SETC 500 mg/kg	66.17±2.626**	0.7667±0.0494**	0.2383±0.0065**	395.7±2.716**
SEOS 50 mg/kg	64.42±4.251*	0.7246±0.0274*	0.2486±0.0048*	397.3±2.358*
SETC 250 mg/kg + SEOS 50 mg/kg	64.83±2.960**	0.7333±0.0494*	0.2517±0.0047*	397.2±3.745**
SETC 500 mg/kg + SEOS 50 mg/kg	68.50±2.262***	0.7333±0.0333***	0.2400±0.0073***	383.5±3.274***

*P<0.05, **P<0.01, ***P<0.001 as compared to ISO-treated group, $^{###}P$<0.001 values compared to control groups. The values are expressed as mean±SEM (n=6). SEM: Standard error of mean, ISO: Isoproterenol, SETC: Standardized extracts of *Tinospora cordifolia*, SEOS: Standardized extracts of *Ocimum sanctum*

Table 2: Effect of different dose combinations on cardiac biomarker enzymes

Treatment	CK-MB (IU/L)	CPK (IU/L)	LDH (IU/L)	AST (IU/L)	ALT (IU/L)
Distilled water 1 ml/kg	407.4±12.01	176.2±2.853	377.4±3.881	39.60±2.015	86.00±3.633
ISO	862.2±9.967###	494.4±4.106###	703.8±6.184###	80.40±1.600###	195.8±2.728###
SETC 250 mg/kg	821±5.236*	486±3.254*	675.4±4.658*	71.69±1.266*	169.7±4.425*
SETC 500 mg/kg	815±6.125**	472±2.354**	635.9±3.265**	65.45±2.369**	161.2±5.326**
SEOS 50 mg/kg	823.4±10.40*	481.5±11.22*	670.7±9.769*	70.58±1.600*	168.4±3.033*
SETC 250 mg/kg + SEOS 50 mg/kg	818.8±4.091**	475.2±4.684*	677.2±4.432*	69.80±1.068*	158.6±18.74**
SETC 500 mg/kg + SEOS 50 mg/kg	751.2±10.20***	421.8±3.426***	532.8±6.591***	59.80±2.746***	147.6±2.400***

*P<0.05, **P<0.01, ***P<0.001 as compared to ISO-treated group, ###P<0.001 values compared to control groups. The values are expressed as mean±SEM (n=6). SEM: Standard error of mean, ISO: Isoproterenol, SETC: Standardized extracts of *Tinospora cordifolia*, SEOS: Standardized extracts of *Ocimum sanctum*, CK-MB: Creatine kinase, CPK: Creatine phosphokinase, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 3: Effect of different dose combinations on antioxidant parameters

Treatment	SOD (unit/mg protein)	CAT (unit/mg protein)	GSH (μmol/g wet tissue)
Distilled water 1 ml/kg	10.16±0.4149	20.21±0.3983	5.450±0.3133
ISO	4.804±0.3888###	8.348±0.3185###	2.344±0.2194###
SETC 250 mg/kg	5.325±0.0236*	9.236±0.4526*	3.021±0.02543*
SETC 500 mg/kg	5.725±0.05326**	9.624±0.1254**	3.125±0.05269**
SEOS 50 mg/kg	5.428±0.2718*	9.200±0.3298*	3.029±0.06591*
SETC 250 mg/kg+SEOS 50 mg/kg	5.992±0.07262**	9.796±0.1864**	3.364±0.06447**
SETC 500 mg/kg+SEOS 50 mg/kg	6.318±0.06807***	12.75±0.1755***	3.722±0.08339***

*P<0.05, **P<0.01, ***P<0.001 as compared to ISO-treated group, ###P<0.001 values compared to control groups. The values are expressed as mean±SEM (n=6). SEM: Standard error of mean, ISO: Isoproterenol, SETC: Standardized extracts of *Tinospora cordifolia*, SEOS: Standardized extracts of *Ocimum sanctum*, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione

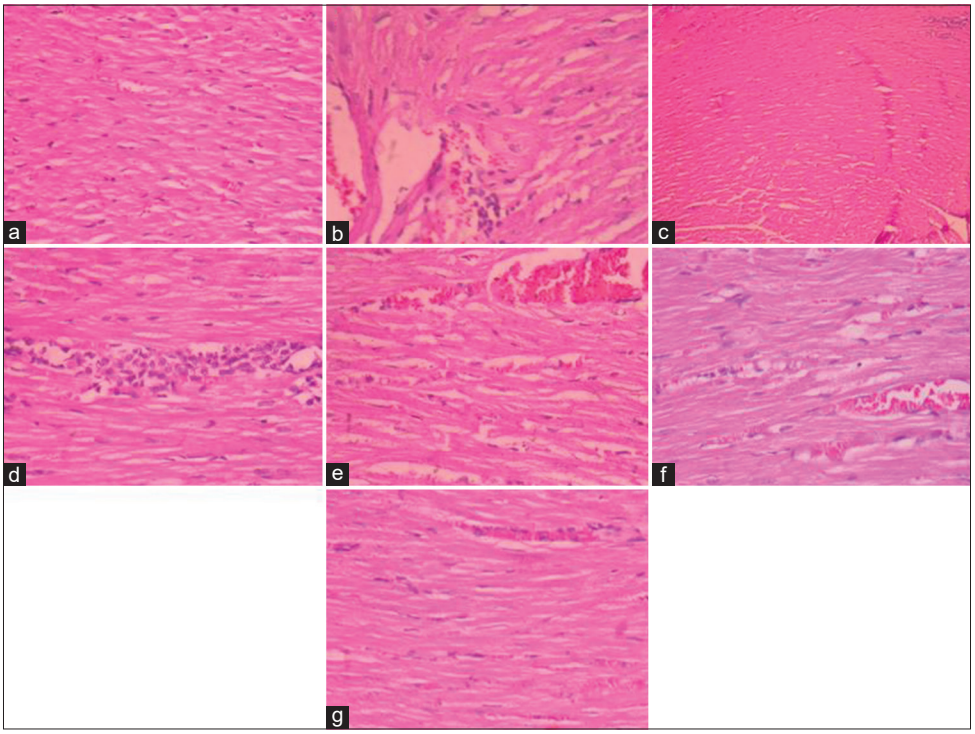

Figure 1: Photomicrographs of sections of heart. (a) Normal control group, (b) isoproterenol control group, (c) standardized extracts of *Tinospora cordifolia* 250 mg/kg, (d) standardized extracts of *Tinospora cordifolia* 500 mg/kg, (e) standardized extracts of *Ocimum sanctum* 50 mg/kg, (f) standardized extracts of *Tinospora cordifolia* 250 mg/kg + standardized extracts of *Ocimum sanctum* 50 mg/kg, (g) standardized extracts of *Tinospora cordifolia* 500 mg/kg + standardized extracts of *Ocimum sanctum* 50 mg/kg

apparent degeneration or necrosis. ISO alone [Figure 1b] showed widespread, hypertrophy, subendocardial necrosis and abundant fibroblastic hyperplasia along with elevated edematous intramuscular gap.

Animals treated with SETC 250 mg/kg, SETC 500 mg/kg, SEOS 50 mg/kg, and SETC 250 mg/kg + SEOS 50 mg/kg showed mild multifocal myocytenecrosis with mild diffuse lymphocytic infiltration along the endocardium [Figure 1c-f]. Whereas, animals treated with SETC 500 mg/kg + SEOS 50 mg/kg [Figure 1g] combination showed significant protection against ISO-induced myocardial damage.

Discussion

ISO, a potent synthetic catecholamine when administered to the animals in high doses, produces "infarct-like" lesions in the heart, which are similar to those found in acute MI and sudden death in man.[23] The pathogenesis of MI has not yet been understood fully, but several studies are carried out on ISO-induced cardiotoxicity. It was observed that 85 mg/kg dose of ISO significantly altered various biochemical parameters in rats.[19] Therefore, the cardioprotective activity of different drug combinations was evaluated against this dose.

The electrocardiograph is considered the most important clinical test for diagnosis of MI. The ST-segment elevation reflects the potential difference in the boundary between ischemic and nonischemic zones and the consequent loss of cell membrane function, whereas the decreased R-amplitude might be due to the onset of myocardial edema following ISO administration.[24] Significant protective effect is seen in the drug combination SETC 500 mg/kg + SEOS 50 mg/kg, which showed improvement in the electrocardiograph pattern. Similarly, the mean arterial blood pressure and heart rate were significantly normalized by the treatment with SETC 500 mg/kg + SEOS 50 mg/kg drug combination. Whereas, the mean arterial blood pressure and heart rate were increased in the ISO-treated animals.

Myocardial cells contain an abundant amount of diagnostic biomarker enzymes of MI and once metabolically damaged, it releases its contents into the extracellular fluid.[25] Hence after the treatment with ISO, animals showed increased activities of CK-MB, CPK, LDH, AST, and ALT which indicates MI. Oral treatment with SETC 500 mg/kg + SEOS 50 mg/kg attenuates raised activities of CK-MB, CPK, LDH, AST, and ALT in serum indicates cardioprotective effect.

Reduced GSH is one of the most abundant nonenzymatic antioxidant biomolecules present in the body. Together with CAT and SOD, it efficiently scavenges free radical species such as hydrogen peroxide, superoxide anions, and alkoxy radicals.[26] In ISO-treated animals, there was a marked increase in levels of SOD, CAT, and GSH which are mutually supportive systems of the first-line cellular defense against oxidative injury, decomposing oxygen and hydrogen peroxide before their interaction to form the more harmful hydroxyl (OH*) radical.[27] Pretreatment with SETC 500 mg/kg + SEOS 50 mg/kg combination that significantly restored the levels of SOD, CAT, and GSH shows the antioxidant property. Due to the oxidative stress, the concentration of free radical is increased in the animals administered with ISO, which in turn causes necrosis, fibroblastic hyperplasia andhypertrophy, etc., in the myocardial cells, but SETC 500 mg/kg + SEOS 50 mg/kg combination significantly protected myocardial cells.

Conclusion

From the present study, it may be concluded that the herb–herb combination of standardized extract of *Tinospora cordifolia* 500 mg/kg and standardized extract of *Ocimum sanctum* 50 mg/kg has shown increased cardioprotective activity than they were used alone. However, further mechanism studies need to be performed for better understanding.

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Conflicts of interest

There are no conflicts of interest.

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