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Cardioprotective effect of *Spathodea campanulata* bark on isoproterenol-induced myocardial infarction in rats

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ABSTRACT

Objective: To evaluate the cardioprotective potential of 70% ethanolic extract of bark of *Spathodea campanulata* P. Beauv (EEBSC). **Methods:** Wister strain rats were pretreated with EEBSC in two different doses (250 and 500mg/kg) orally for 14 d and then intoxicated with isoproterenol (200 mg/kg, *s.c.* for 2 consecutive days at an interval of 24 h on 14th and 15th day of treatment protocol) to induce myocardial injury. **Results:** Cardioprotection was assessed by estimating serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine phosphokinase, triglycerides, total cholesterol, low density lipoproteins and high density lipoproteins. The levels of thiobarbituric acid reactive substances and reduced glutathione were analyzed in heart homogenate. In isoproterenol-treated group, shrinkage of cardiac markers in serum and elevated lipid peroxidation accompanied by decreased content of reduced glutathione in heart. The prior administration of EEBSC significantly ($P<0.05$) prevented the isoproterenol-induced alterations and restored the cardiac markers. The observed results were further confirmed by histopathological findings. **Conclusions:** These findings indicate that EEBSC exerts cardioprotective effect against isoproterenol induced myocardial infarction due to its free radical scavenging effects, which maintains the tissue defence system against myocardial damage.

1. Introduction

Ischemic heart disease is the leading cause of morbidity and mortality worldwide, and according to the world health organization, it will be the major cause of death in the world by the year 2020[1,2]. Myocardial infarction results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart[3]. Isoproterenol is a beta-adrenergic agonist and synthetic catecholamine, which has been reported to cause severe stress in the myocardium and necrotic lesions in the heart muscles[4,5]. There is a substantial evidence that ischemic tissue generates oxygen derived free radicals (oxygen radicals), *i.e.*, oxygen molecules containing an odd number of electrons, making them chemically reactive and often leading to chain reactions which contributes to cell death[6]. Myocardial infarction, the most dreaded sequel among ischemic heart disease, is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycemia and hyperlipidaemia, leading to qualitative and quantitative alterations of myocardium[7]. Herbal medicines possessing antioxidant and free radical scavenging activities may therefore have a protective role in cardiovascular diseases and provide viable alternatives. There is growing trend towards use of herbal medicines worldwide to treat a wide range of pathological conditions including cardiovascular diseases. This may be due to their relative safety and lack of significant side effects[8].

Spathodea campanulata P. Beauv (S. *campanulata*) of family Bignoniaceae, commonly known as African tulip tree is planted in gardens and avenues, is reported to be useful in the treatment as diuretic, anti-inflammatory, kidney diseases, antidote, enemas, herpes, stomach ache, antisecretolytic, antiparasitic, urethra inflammations, fungal skin diseases, diarrohea, anti-HIV, anti-malarial and

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hypoglycemic activity[9,10]. The major chemical constituents of the plant are steroids, cardiac glycosides, flavonoids, tannins and polyphenols[11]. However, there is lack of information regarding the effect of *S. campanulata* on the cardiac changes associated with isoproterenol induced myocardial infarction. Hence, the present study was designed to investigate the modulation of ethanol extract of *S. campanulata* bark in isoproterenol induced biochemical indicators, histopathological changes and *in vivo* antioxidant potential.

2. Materials and methods

2.1. Plant materials and extraction

S. campanulata bark was collected along the roadsides of Davanagere, Karnataka, India and the plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S College of pharmacy, Harapanahalli, India. The dried powder of the bark was defatted with pet ether and then extracted with 70% (v/v) ethanol using a Soxhlet apparatus. The extracts were concentrated under reduced pressure using a rota flash evaporator and stored in an airtight container in a refrigerator below 10 °C.

2.2. Chemicals

Isoproterenol hydrochloride was purchased from Sigma Chemical Co., (St Louis, MO, USA). The biochemical kits was purchased from Erba Mannheim (Mannheim, Germany) and all other chemicals used were of analytical grade.

2.3. Rats

Wistar albino rats weighing 150-250 g and albino mice weighing 20-25 g of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Bangalore, India. The animals were acclimatized for 1 week under laboratory conditions. They were housed in polypropylene cages and maintained at (27±2) °C under 12 h dark/light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water was provided *ad libitum*. The husk in the cages was renewed thrice a week to ensure hygeinity and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animal Ethical Committee prior to the beginning of the research work, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Animal Welfare Division, Government of India, New Delhi, India.

2.4. Acute toxicity study

Acute toxicity study was conducted using albino mice (20-25 g) for ethanolic extract of bark of *S. campanulata* (EEBSC) in accordance with OECD guideline No. 420 given by CPCSEA[12] which was adopted for toxicity studies. The extract was found to be devoid of mortality at 2000 mg/kg. Hence, 2500 mg/kg was considered as LD_{50} cutoff value. Accordingly, 1/10 (250 mg/kg, p.o.) and 1/5 (500 mg/kg, p.o.) of the dose were selected for the screening of cardioprotective activity.

2.5. Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected subcutaneously to rats (200 mg/kg) daily for 2 consecutive days twice at an interval of 24 h (*i.e.*, on 14th and 15th day of extract treatment) to induce experimental myocardial infarction.

2.6. Experimental design

In the dose response experiment, albino rats were randomly assigned into four groups of six individuals each[13,14]. Group 1: Normal control rats treated with 10 g/L normal saline at a dose of 2 mL/(kg·d) (p.o) for 16 d. Group 2: Rats treated with 10 g/L normal saline at a dose of 2 mL/(kg·d) (p.o) for 14 d and then isoproterenol at a dose of 200 mg/(kg·d) (s.c) for 2 d twice at an interval of 24 h for 2 consecutive days. Group 3: Rats pretreated with EEBSC at a dose of 250 mg/(kg·d) (p.o) for 14 d and then isoproterenol at a dose of 200 mg/(kg·d) (s.c) for 2 d twice at an interval of 24 h for 2 consecutive days. Group 4: Rats pretreated with EEBSC at a dose of 500 mg/(kg·d) (p.o) for 14 d and then isoproterenol at a dose of 200 mg/(kg·d) (s.c) for 2 d twice at an interval of 24 h for 2 consecutive days.

2.7. Biochemical analysis

At the end of experimental period (after 24 h of second isoproterenol injection or 16th day of extract/vehicle treatment), all the rats were anaesthetized with light anaesthetic ether and blood was collected from the retro-orbital plexus. The serum was separated and used for the determination of diagnostic marker enzymes like AST (aspartate aminotransferase), ALT (alanine aminotransferase), LDH (lactate dehydrogenase), CPK (creatine phosphokinase), TG (triglycerides), TC (total cholesterol), LDL (low density lipoproteins) and HDL (high density lipoproteins). The animals were sacrificed and heart was dissected out. The samples of heart tissue were analysed for tissue GSH[15] and lipid peroxidation for measurement of thiobarbituric acid reactive substances according to the method of Ohkawa *et al*[16].

2.8. Histopathological studies

At the end of the study, myocardial tissues from all the groups were subjected to histopathological studies[17]. The small pieces of heart tissues were collected in 10% (v/v) formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6 μm in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope and analyzed for any altered architecture of the heart tissue due to isoproterenol challenge or improved heart architecture due to pretreatment with test extracts[17].

2.9. Statistical analysis

Results were expressed as mean ± SEM (n = 6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey's Kramer comparison test by using Graph Pad Prism Instat Software (version 4.0, USA). P value less than 0.05 was considered to be statistically significant. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$, when compared with control and toxicant group as applicable.

3. Results

3.1. Effect of EEBSC on serum marker enzymes and in vivo antioxidant properties

Rats subjected to the isoproterenol challenge alone (positive control) developed obvious heart injury as evident from significant ($P<0.05$) elevation in the biochemical indicators like AST, ALT, LDH, CPK, TG, TC, LDL and depletion of HDL levels were observed when compared to negative control group (Group 1). There were marked depletion of tissue GSH levels and increase of lipid peroxidation levels when compared with control. Oral administration of the test extract exhibited dose dependent significant ($P<0.05$) reduction in the isoproterenol induced increase of the biochemical levels. However, an increase in the level of HDL was found with the administration of the test extract. The levels of endogenous antioxidants (lipid peroxidation and tissue GSH) were dose dependently restored to near normal levels by pretreatment with EEBSC as compared to positive control group. All the results were statistically significant ($P<0.05$). The results are summarized in Table 1 and 2.

Table 1
Effect of ethanolic extract of *S. campanulata* bark on biochemical indicators in rats with isoproterenol induced myocardial infarction.

Group	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	CPK (IU/L)	TG (mg/dL)	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
1	78.76±1.20	164.96±4.94	80.37±4.54	34.67±1.50	157.25±8.24	148.83±13.07	80.31±1.004	42.21±1.12
2	171.85±3.74	205.48±5.82	136.07±8.41	93.58±7.25	172.30±2.45	195.43±5.15	119.60±5.53	28.97±5.05
3	148.08±5.86*	147.22±13.92**	76.16±1.37***	61.83±5.79**	142.00±3.00*	181.00±4.50*	100.83±3.74*	51.53±1.98**
4	93.95±8.70***	110.26±7.12***	56.86±4.37***	39.91±5.58***	132.33±9.26**	175.33±3.68*	96.86±2.68*	54.66±1.45**

Values are mean ± SEM of six rats. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to positive control. AST: aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; TG, triglycerides; TC, total cholesterol; LDL, low density lipoproteins; HDL, high density lipoproteins. Group 1 was normal control rats. Group 2 was positive control rats with induced myocardial injury. Group 3 was rats pretreated with EEBSC at a dose of 250 mg/(kg·d) and then isoproterenol. Group 4 was rats pretreated with EEBSC at a dose of 500 mg/(kg·d) and then isoproterenol.

Table 2
Effect of ethanolic extract of *S. campanulata* bark on tissue reduced glutathione and lipid peroxidation in rats with induced myocardial infarction.

Group	Tissue reduced glutathione	Lipid peroxide
1	0.270±0.032	0.103±0.003
2	0.126±0.010	0.153±0.002
3	0.214±0.006***	0.109±0.001***
4	0.248±0.008***	0.101±0.001***

Values of absorbance are mean ± SEM of six rats. $^{***}P<0.001$ as compared to positive control. Group 1 was normal control rats. Group 2 was positive control rats with induced myocardial injury. Group 3 was rats pretreated with the extract at a dose of 250 mg/(kg·d) and then isoproterenol. Group 4 was rats pretreated with the extract at a dose of 500 mg/(kg·d) and then isoproterenol.

3.2. Histopathological findings

Figure 1A represents the light micrograph of control heart showing normal myofibrillar structure with striations and branched appearance. The isoproterenol-induced rat heart showed the extensive myofibrillar degeneration which is related to infiltration with neutrophil granulocytes, and hyaline necrosis and interstitial edema were observed (Figure 1B). Pretreatment with EEBSC at a dose of 250 or 500 mg/kg demonstrated marked improvement in isoproterenol induced alterations such as vacuolar changes, capillary dilatation and leukocyte infiltration (Figures 1C and 1D) as compared to isoproterenol administered group.

4. Discussion

Isoproterenol is a well known cardiotoxic agent due to its ability to destruct myocardial cells. As a result of this, cytosolic enzymes such as LDH, transaminases (ALT, AST) and CPK are released into blood stream and serve as the diagnostic markers of myocardial tissue damage[18-20]. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability. In the present study, isoproterenol treated rats

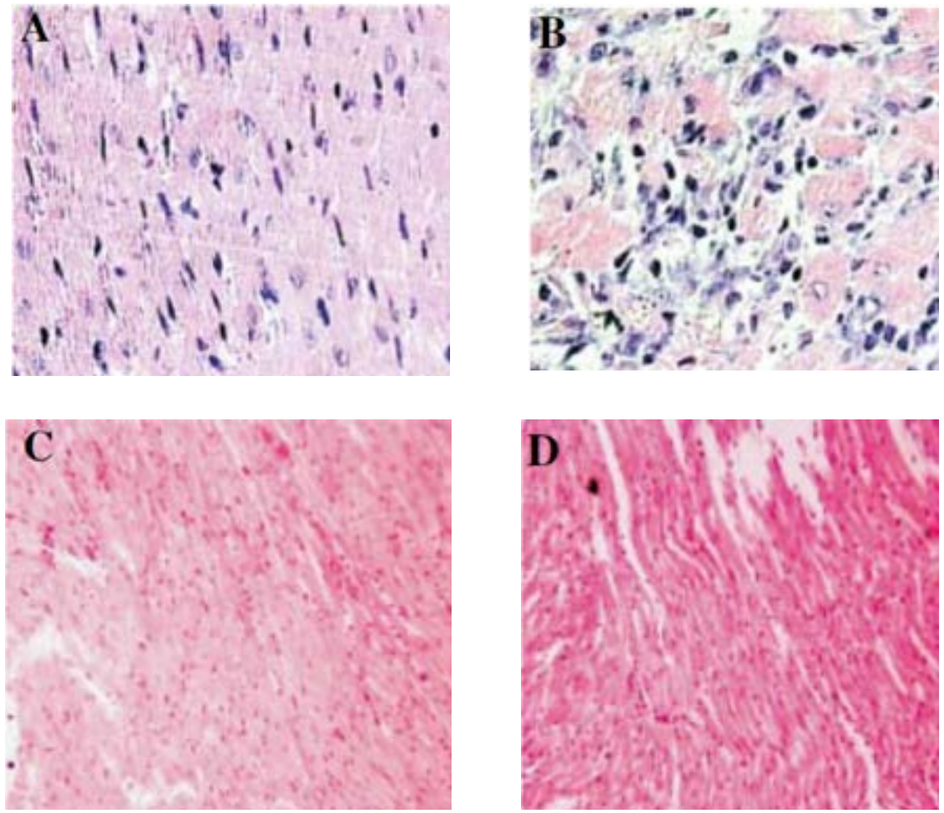

Figure 1. Histological examination of heart in experimental animals.
A: Heart architecture of normal control. B: Heart architecture of isoproterenol treatment. C: Heart architecture of isoproterenol treatment and ethanolic extract of *S. campanulata* bark (250 mg/kg). D: Heart architecture of isoproterenol treatment and ethanolic extract of *S. campanulata* bark (500 mg/kg).

showed significant elevation in the levels of these diagnostic marker enzymes including AST, ALT, LDH, CPK, TG, TC and LDL and a decrease in HDL. The tissues GSH levels were reduced and there was enhanced lipid peroxidation. Moreover, elevated levels of these enzymes are an indicator of the severity of isoproterenol induced myocardial membrane necrosis[18]. Free radicals are implicated in an increasing large number of diseases and are reported to have a deleterious effect on heart function[21,22]. An increased level of lipid peroxides in the heart following isoproterenol administration indicates enhanced lipid peroxidation by free radicals[23]. Due to this increased lipid peroxidation, glutathione levels are lowered[21]. The prior administration of EEBSC (250 and 500 mg/kg) showed significant reduction in isoproterenol induced elevated serum marker enzymes. This reduction in enzyme levels could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes. It also significantly ($P<0.05$) prevented the depletion of glutathione and reduced lipid peroxidation in a dose dependent manner. In the present study, we found that EEBSC protected myocardium from isoproterenol induced myocardial functional and structural injury via normalization levels of diagnostic marker enzymes and by restoring the glutathione and lipid peroxidation levels. The cardioprotective property of the extract was further confirmed by remarkable improvement of the cardiac architecture by reversing the focal lesions, fragmentation of muscle fibres and retrogressive lesions over the isoproterenol treated groups. The data of the present study clearly showed EEBSC modulated most of the biochemical and histopathological indicators which were maintained to normal status in isoproterenol rats, suggesting the beneficial action of EEBSC as a cardioprotective agent.

In conclusion, the results of the present investigation illustrated that EEBSC has remarkable protection over isoproterenol-induced alterations in marker enzyme activity and cellular damage. Combined effect of active principles present in the ethanolic extract of *S. campanulata* might offer protection against myocardium damage rendered by isoproterenol in rats. Thus, EEBSC exhibits remarkable protection against isoproterenol induced myocardial damage in rats by virtue of its antioxidative potential. However, further studies are needed to further isolate the active components and to investigate the exact mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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