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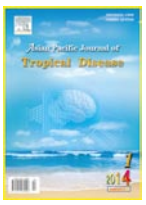

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Cardioprotective effect of hydroalcoholic extract of *Tecoma stans* flowers against isoproterenol induced myocardial infarction in rats

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PEER REVIEW

Peer reviewer

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Comments

The present study made an attempt to investigate the protective effect of 70% ethanolic extract of *T. stans* flowers against experimentally induced myocardial infarction in rats. The aim of the study is interesting and the language of results is quite appropriate and understandable.
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ABSTRACT

Objective: To investigate the cardioprotective effect of 70% ethanolic extract of *Tecoma stans* (*T. stans*) flowers against isoproterenol-induced myocardial infarction in rat myocardium.
Methods: Wister rats were pretreated with 70% ethanolic extract of *T. stans* flowers (250 and 500 mg/kg) orally for 14 d and then intoxicated with isoproterenol [200 mg/(kg · day), *s.c.*] for 2 consecutive d. The biochemical markers for myocardial infarction such as alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatinine kinase, total cholesterol, triglycerides, low density lipoproteins and high density lipoproteins were determined. In addition the antioxidant status on heart tissue is also evaluated by testing the activities of antioxidant enzymes such as lipid peroxidation, superoxide dismutase, reduced glutathione and catalase.
Results: The results indicated that pretreatment with 70% ethanolic extract of *T. stans* flowers prevented fall in antioxidants and retarded elevation of cardiac damage markers in isoproterenol treated rats, significantly. In addition, these findings were evidently supported by the remarkable protection revealed in the histopathological studies, even GC-MS analysis data also substantiated out investigation.
Conclusions: It was concluded that, in addition to poly phenolics, some of the phyto fragments found during GC-MS analysis might also contributed to the cardiac protection offered by the extract.

KEYWORDS

Cardioprotective, GC-MS, Isoproterenol, Myocardial infarction, *Tecoma stans*

1. Introduction

Cardiovascular diseases (CVD) remain the principle cause of death in both developed and developing countries, accounting for roughly 20% of all annual deaths worldwide. Myocardial infarction is the rapid development of myocardial necrosis caused by critical imbalance between oxygen supply and demand of the myocardium. The increased generation of toxic reactive oxygen species (ROS) such as O_2^-, H_2O_2, OH^- *etc*. exerts severe oxidative stress on myocardium predisposing to CVD such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias[1,2].

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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]. Myocardial infarction results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart[2]. CVDs are the secondary cause of deaths in many parts of the world, although modern drugs are effective in preventing the disorders, their use is often limited because of their side effects and adverse reactions. A wide array of plants and their active principles, with minimal side effects, provide an alternate therapy for ischemic heart disease[3].

Isoproterenol (ISO), a synthetic catecholamine and beta-adrenoceptor agonist, has been found to cause severe stress in the myocardium resulting in infarct-like necrosis[4]. It is also well known that ISO generates free radicals leading to lipid peroxidation, which cause irreversible damage to the myocardium[5]. Increase in formation of ROS during ischemia/reperfusion and the adverse effects of oxygen radicals on myocardium have been well established by both direct and indirect parameters. Thus, Isoproterenol causes loss of functional integrity and necrotic lesion in heart muscle[6].

Tecoma stans (*T. stans*) locally available has been used in traditional system of medicine for treating diabetes mellitus, bacterial infections[7-9], arterial hypotension, gastrointestinal tract disorders and various cancers. The plant is an effective remedy for snake and rat bites. It is also used as vermifuge and tonic[10,11]. The literature revealed the presence of triterpenes, hydrocarbons, resins and volatile oils. The flower contains flavonoids, tannins, traces of saponins, alkaloids, tecomine, tecostidine, beta carotene and zeaxanthin[12,13]. Inspite of phyto-antioxidants abundance there is no scientific information available regarding cardioprotective effect, hence in the present study an attempt was made to investigate the protective effect of 70% ethanolic extract of *T. stans* flowers against experimentally induced myocardial infarction in rats.

2. Materials and Methods

2.1. Plant material and extraction

Flowers of *T. stans* were collected from Bettada Malleshwara temple, Kumaranahalli Village in Davanagere, and authenticated by Professor K. Prabhu, Department of Pharmacognosy, SCS College of Pharmacy, Harapanahalli. A herbarium specimen SCSCOP.Ph.Col Herb.No. 012/2006-2007 is preserved in the college museum. The dried powder of the flower was defatted with pet and ether, and then extracted with 70% ethanol using Soxhlet apparatus. The extract was concentrated under reduced pressure using rota flash evaporator and stored in airtight container in refrigerator below 10 °C. The same method was used for pharmacological investigations, after subjecting it to preliminary qualitative phytochemical studies.

Chemical ISO was procured from the Sigma-Aldrich Chemicals Ltd, St. Louis, USA, the biochemical kits from Erba Manheim, Germany. All the chemicals used were of analytical grade.

2.2. Animals

Wister 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, Bengaluru. The animals were acclimatized for one 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 *ad asbitsium* was provided.

The husk in the cages was renewed thrice a week to ensure hygiene 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 project work, registration no SCSCOP/665/2008-09 dated 24.11.2008.

2.3. Acute toxicity studies

Acute toxicity study was performed on albino mice (20-25 g) as per OECD guideline[13]. The extract was found to be devoid of mortality at 2 000 mg/kg. Hence, 2 500 mg/kg was considered as LD_{50} cutoff value. As per standard protocol 1/10th (250 mg/kg, *p.o.*) and 1/5th (500mg/kg, *p.o.*) of the doses were selected.

2.4. Induction of myocardial injury

A total of 30 healthy rats of 200-250 g were randomly allotted into 5 groups of 6 animals. In group I animals were treated as negative control and were fed with normal saline for 16 d. The animals of group II, III, IV and V were fed with normal saline, simvastatin (60 mg/kg), 70% ethanolic extract of *T. stans* flowers (250 mg/kg) and 70% ethanolic extract of *T. stans* flowers (500mg/kg) daily, *p.o*, respectively for 16 d. Then, animals of all the groups were

given ISO (200 mg/kg), *s.c.* for two consecutive days at 24 h interval.

At the end of experimental period (after 24 h of second ISO injection or the 16th day of extract/vehicle treatment), all the rats were anaesthetized with urethane (1 g/kg, *i.p.*) and blood was collected from the retro-orbital plexus; the serum was separated and used for the determination of diagnostic marker enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine kinase (CK), total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL) and high density lipoproteins (HDL). These enzymes were assayed in serum using standard kits supplied by Erba mannheim, India.

The heart tissue was excised immediately, washed with chilled isotonic saline, and then tissue homogenates were prepared in ice cold 0.1 mol/L Tris-HCl buffer (pH 7.2), used for the assay of clinical marker enzymes lipid peroxidation, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)[14]. The hearts were also stored in 10% formalin for histological studies to evaluate the details of myocardial architecture in each group microscopically.

2.5. Histopathological studies

Pieces of heart from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50%-100%) alcohol, embedded in paraffin, cut into 4-5 μm thick sections and stained with hematoxylin-eosin[15]. The sections were evaluated for the pathological/rejuvenative changes in the myocardial tissue.

2.6. GC-MS analysis

The GC-MS analysis of hydroalcoholic extract was carried out in a GC-MS model: Thermo GC-trace Ultra ver: 5.0, Thermo MS DSQ II, gas chromatograph fitted with DB 35- MS capillary standard non-polar column (30 Mts, ID: 0.25mm, film: 0.25 μm thickness) or equivalent column. Carrier gas was helium with a flow rate of 1.0 mL/min; column temperature initially was at 70 °C for 2 min, then rose to 3 000 °C at the rate of 8 °C per minute, maintained at 300 °C for 40.52 min; injector temperature was 240 °C, detector temperature 260 °C, volume injected 1 μL with liquid injector of 70% ethanol extract in ethanol (1 g in 5 mL ethanol). The mass spectra operating parameters were as follows: ion source temperature: 250 °C; ionization potential: 70 eV; solvent delay: 3 min; program run time: 31 min; scan range: 30-350 amu; EV voltage: 3 000 V. Finally the structural fragments were identified on the basis of retention time by using Wiley 07, a commercial library software.

2.7. Statistical analysis

Results were expressed as mean±SEM, (n=6). Statistical analyses were performed with One-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test by using Graph Pad Instat Software. 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 70% ethanolic extract of T. stans flower on biochemical markers

Pretreatment ISO alone (positive control group) developed significant heart injury as an evidence by a significant elevation in the biochemical markers like ALT, AST, LDH, CK, TC, TG, LDL and depletion of HDL levels were observed when compared with negative control (group I). Oral administration of the test extract exhibited dose dependent significant reduction in the ISO induced increase in the biochemical levels and prevented the fall of HDL levels. Obviously the simvastatin 60 mg/kg has restored all the biochemical parameter levels significantly to near normal levels. All the results were statistically significant. The results are summarized in Table 1.

Table 1

Effect of 70% ethanolic extract of *T. stans* on biochemical markers in isoproterenol induced cardiotoxicity.

Group	ALT IU/L	AST IU/L	CK IU/L	LDH IU/L	LDL mg/dL	HDL mg/dL	TC mg/dL	TG mg/dL
Normal saline (2 mL/kg)	30.670±1.229	34.00 ±1.390	73.64±3.648	34.830±3.361	140.200±3.868	156.500±4.639	161.200±4.636	79.00±4.824
Isoproterenol (200 mg/kg)	71.830±2.007***	72.67±1.453***	164.3±5.880***	57.830±3.270**	180.700±3.169***	82.670±4.112***	211.000±7.243***	175.3±3.783***
Simvastatin (60 mg/kg)	37.330±1.202***	33.50±1.118***	95.00±5.756***	46.830±3.628**	146.200±2.880***	112.800±2.915**	166.700±3.972***	84.33±4.828***
ethanolic extract 250 mg/kg	50.5000±0.7638***	49.85±2.366***	123.7±4.856***	55.830±3.219^{ns}	166.000±4.712*	85.170±2.372^{ns}	177.700±4.566***	145.3±7.575**
ethanolic extract 500 mg/kg	44.830±1.740***	48.17±2.120***	110.7±6.556***	50.500±2.202*	156.300±3.792***	101.000±4.640*	173.600±3.370***	120.0±4.389***

Values are mean ± SEM of six rats/ treatment. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared to positive control; ns; non significant.

3.2. Effect of 70% ethanolic extract of *T. stans* flowers on tissue GSH, lipid peroxidation SOD and CAT

There was a marked depletion of GSH, SOD and CAT levels in ISO treated group. Treatment with 70% ethanolic extract of *T. stans* flowers prevented fall in GSH, SOD and CAT levels in a dose dependant manner to a near normal level. The test extract was found to be statistically significant at both lower and higher doses in normalizing tissue GSH, SOD and CAT levels. Treatment with 60 mg/kg simvastain, the standard drug, prevented the depletion of GSH, SOD and CAT.

The levels of lipid peroxidation were restored to near normal levels by pretreatment with 70% ethanolic extract of *T. stans* flowers as compared to positive control group in a dose dependent manner. All the results were statistically significant ($P<0.05$). The results are summarized in Table 2.

The plant extract during GC-MS observation confirms the peak of quercetin and catechol like compounds with the range of 296-310 amu and 106 to 130 amu respectively were identified from hydroalcoholic fraction of ethanolic extract of *T. stans* flowers (Figure 1).

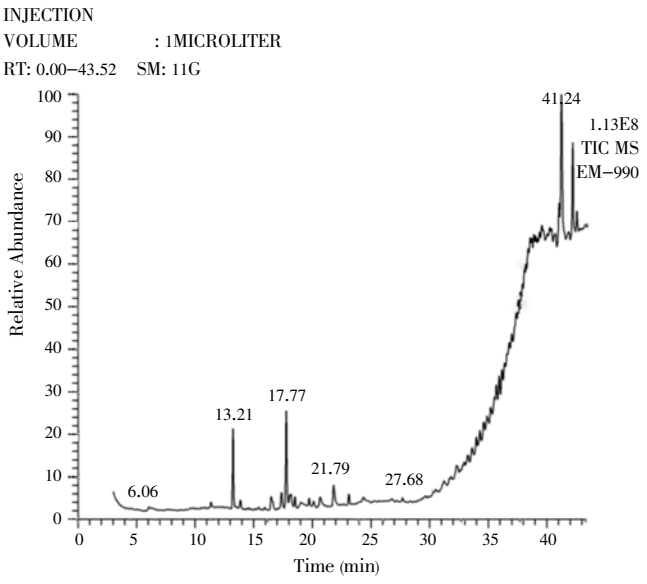

Figure 1. GC-MS report of 70% ethanolic extract of *T. stans* flowers.

3.3. Histopathological Studies

In Figure 2, section studied from the myocardium shows intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils. The interstitial space appeared intact. The vascular spaces amidst these cardiac muscle fibers appeared unremarkable.

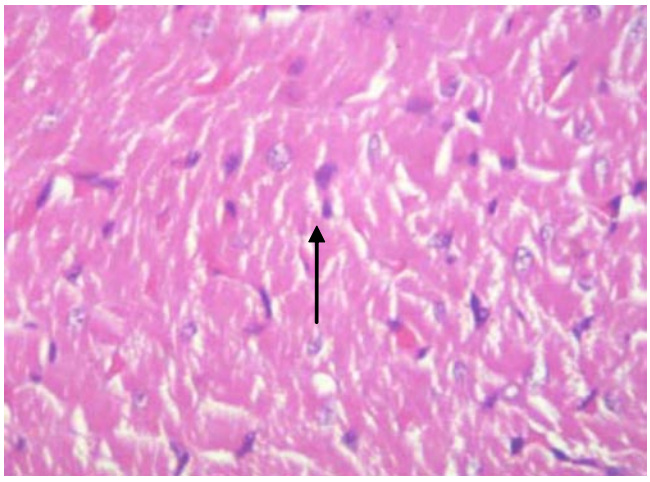

Figure 2. Normal control.
Arrow shows intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils. The interstitial space appeared intact.

In Figure 3 some of the cardiac muscle fibers show loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils (short-arrow). The interstitial space at few areas appeared increased (long-arrow). The vascular spaces appeared unremarkable amidst these cardiac muscle fibers.

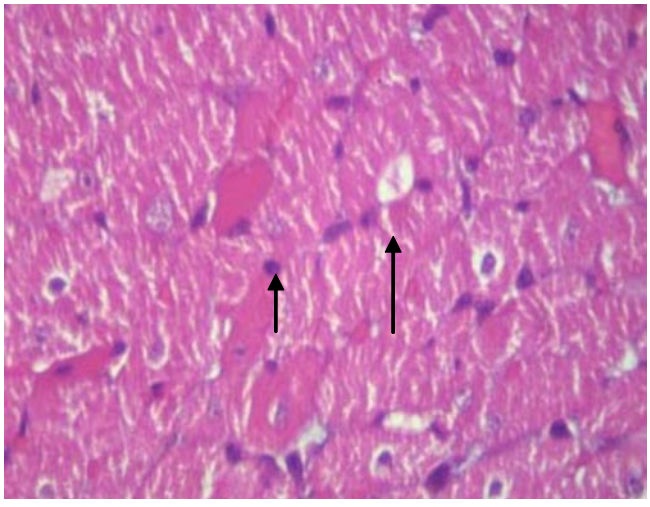

Figure 3. Positive control (ISO 85 mg/kg).
Short-arrow show loss of integrity of myocardial cell membrane, myofibrillar structure with lossof striations; Long-arrow shows interstitial space at few areas appears increased.

Table 2
Effect of 70% ethanolic extract of *T. stans* flowers on tissue GSH, lipid peroxidation, SOD and CAT.

Treatment	LPO		GSH		SOD		CAT	
	Mean±SEM	%inhibition	Mean±SEM	%increase	Mean±SEM	%increase	Mean±SEM	%increase
Normal Saline (2 mL/kg)	0.0875±0.0043	–	0.3335±0.0163	–	0.6080±0.0263	–	0.6370±0.0631	–
Isoproterenol (200 mg/kg)	0.1573±0.0073	–	0.1640±0.0049	–	0.2808±0.0062	–	0.1790±0.0173	–
Simvastatin	0.0980±0.0041***	12.00%	0.3105±0.0057***	68.10%	0.5510±0.0239***	93.00%	0.3703±0.0112***	41.80%
ethanolic extract (250 mg/kg)	0.1295±0.0059**	48.00%	0.2390±0.0189**	28.00%	0.3845±0.0258*	36.70%	0.2398±0.0148***	28.90%
ethanolic extract (500 mg/kg)	0.1108±0.0043***	26.00%	0.2628±0.0109***	39.00%	0.4153±0.0317**	41.90%	0.2803±0.0220***	35.90%

Values are the mean±SEM of six rats /treatment. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared to positive control.
LPO=Lipid peroxidation.

Simvastatin 60 mg/kg (Figure 4) section studied from the myocardium shows integrity of myocardial cell membrane, intact myofibrillar structure with striations and continuity with adjacent myofibrils (short-arrow), and scattered inflammatory infiltration. The 70% ethanolic extract of *T. stans* flowers (250 mg/kg) (Figure 5) section studied from some of the cardiac muscle fibers show loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils (long-arrow). The interstitial space at focal areas appeared increas (short-arrow). Whereas in 70% ethanolic extract of *T. stans* flowers (500 mg/kg) treated group (Figure 6) intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils (long-arrow) was shown, the interstitial space appeared intact and scattered inflammatory infiltration (short-arrow) amidst these cardiac muscle fibers.

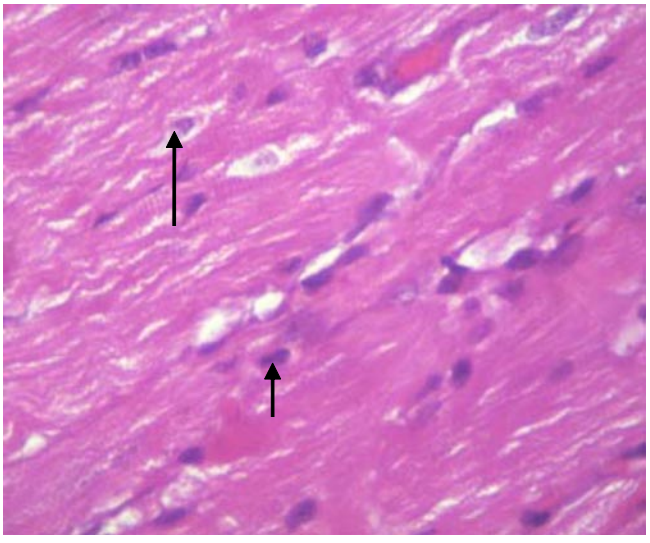

Figure 4. Standard (Simvastatin 60 mg/kg).
Long-arrow shows focal loss of arrangement of the cardiac muscle fibers, along with scattered inflammatory infiltration (short-arrow) amidst these cardiac muscle fibers.

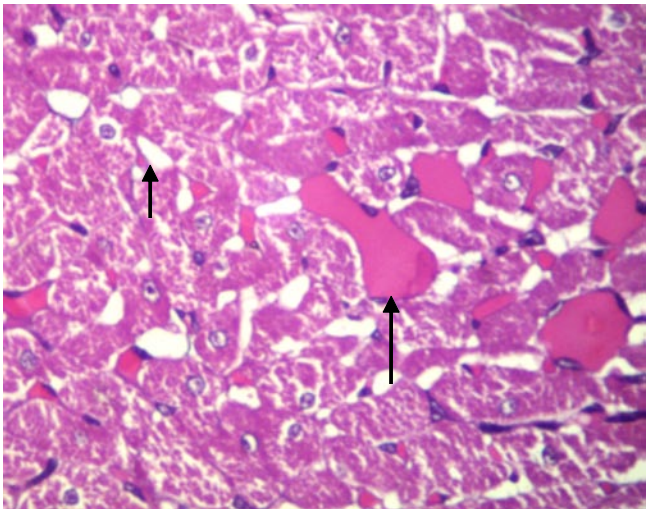

Figure 5. The 70% ethanolic extract of *T. stans* flowers (250 mg/kg).
Long-arrow shows loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils. The interstitial space at focal areas appeared increase (short-arrow).

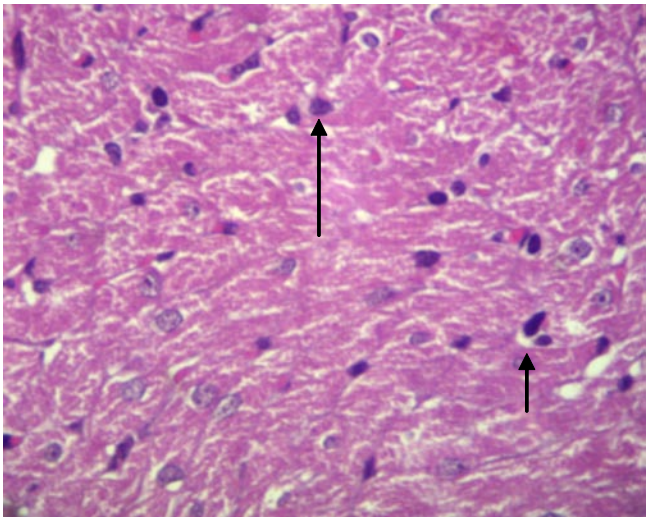

Figure 6. The 70% ethanolic extract of *T. stans* flowers (500 mg/kg).
Long-arrow shows intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils. Short-arrow shows the interstitial space appears intact and amidst these.

4. Discussion

ISO produces relative infarction or hypoxia due to myocardial hyperactivity and coronary hypotension, and induces myocardial ischemia due to cytosolic Ca^{2+} overload ISO, a synthetic β-adrenergic agonist, by its positive inotropic and chronotropic actions, increasing the myocardial oxygen demand that leads to ischemic necrosis of myocardium in rats similar to that seen in human myocardial infarction. A number of pathophysiologic mechanisms have been outlined to explain the ISO-induced myocardial damage, *viz*. altered membrane permeability, increased turnover of nor-epinephrine and generation of cytotoxic free radicals. In addition, ISO administration reduces blood pressure that triggers reflex tachycardia, thereby increasing myocardial oxygen demand[16,17]. Myocardium possesses high abundance of enzymes like CK, LDH, ALT and AST. These enzymes serve as sensitive index to assess the severity of myocardial infarction[18,19]. ISO induces hyperlipidemia and myocardial infarction characterized by the increased TG, TC, LDL, very LDL and lipid peroxides such as malanoldehyde generated by the auto oxidation of ISO to semiquinone which reacts with oxygen produces superoxide anions and H_2O_2. Subsequently, endogenous antioxidants such as SOD and GSH were also depleted[20,21].

The prior administration of extracts showed significant reduction in elevated serum marker enzymes of myocardial infarction. This reduction in the enzyme level confirms that plant extract is responsible for protection of normal structural and architectural integrity of cardiac myocytes. In this study, ISO treated rats show significant elevation in the levels of serum transaminases (ALT, AST,

LDL, CK, TG, TC, LDL, serum glucose and decrease in HDL).

There was a marked depletion of GSH, SOD and CAT levels in ISO treated group. Treatment with 70% ethanolic extract of *T. stans* flowers has prevented the declination of GSH, SOD and CAT levels in a dose dependant manner. There was also dose dependent inhibition of *in vivo* lipid peroxidation by both the doses (250 mg/kg and 500 mg/kg) of 70% alcoholic extract.

Plants extract administration showed a protective effect against ISO induced altered biochemical parameters and eliminated the acute fatal complication by protecting the cell membrane damage.

In our investigation, it was observed that the flower possess polyphenolic compounds (flavonoids and tannins) and these constituents are reported to have antioxidant and organ protective properties. The data of the present study clearly showed that plant extract modulated most of the biochemical and histopathological parameters to near normal status in ISO treated rats, assuring its cardioprotection role.

In GC-MS analysis compounds were identified from hydroalcoholic fraction, which are already used in food, cosmetic and pharmaceutical industries, and some of them are reported to possess antioxidant property *viz*. 2-methyl-1-pentene, which reacts strongly with oxidizers[22]; N-methyl-2-pyrrolidine which is used as an antioxidant in cosmetics and veterinary preparations (LD_{50}-7 g/kg); propanidionic acid which is a precursor for synthesis of vitamin B1, B6, B12 and also used to prevent free radical mediated resorption of bones in broiler chicks (LD_{50}-4 g/kg); 3-ethoxy-2-butanone (Banana oil) which is used as rust preventer, anti-freezing, antioxidant (LD_{50}-16.6 g/kg)[23] *etc*.

The results of present study propose that the test extract successfully quenched oxidative stress induced by ISO, Further, some of the fragments found in GC-MS analysis also are reported to possess antioxidant property and contributed to cardioprotection. However, further investigations are required to elucidate its exact mechanism of action and to establish possible basis for its clinical utility.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the financial support by Rajiv Gandhi University of Health Sciences Karnataka, Bangalore, India. The authors are also thankful to the President and Secretary, TMAE Society through the Principal, SCS College of Pharmacy, Harapanahalli, Karnataka for providing necessary facilities to carry out this work.

Comments

Background

CVD remain the principle cause of death in both developed and developing countries, accounting for roughly 20% of all annual deaths worldwide. Myocardial infarction is the rapid development of myocardial necrosis caused by critical imbalance between oxygen supply and demand of the myocardium.

Research frontiers

The present study is an attempt to characterize the 70% ethanolic extract of *T. stans* flowers by GC-MS analysis and to investigate the cardio-protective effect of 70% ethanolic extract of *T. stans* flowers against ISO induced myocardial infarction in rats by measuring the biochemical and histo-pathological studies.

Related reports

It was reported that ISO has been found to cause severe stress in the myocardium resulting in infarct-like necrosis. It is also well known that ISO generates free radicals leading to lipid peroxidation, which cause irreversible damage to the myocardium.

Innovations & breakthroughs

The data of the study showed that flowers extract modulated most of the biochemical and histopathological parameters to near normal status in ISO induced myocardial infracted rats, assuring its cardioprotection role.

Applications

It may be significant to know that *T. stans* flowers has been scientifically studied in order to use the flowers as drug in the management of ischemic heart diseases.

Peer review

The present study made an attempt to investigate the protective effect of 70% ethanolic extract of *T. stans* flowers against experimentally induced myocardial infarction in rats. The aim of the study is interesting and the language of results is quite appropriate and understandable.

References

[1] laLakshmi SV, Padmaja G, Kuppusamy P, Kutala VK. Oxidative stress in cardiovascular disease. *Indian J Biochem Biophys* 2009; **46**: 421-440.

[2] Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. *Pharmacol Ther* 2001; **89**: 187-206.

[3] Thippeswamy BS, Thakker SP, Tubachi S, Kalyani GA, Netra MK, Patil U, et al. Cardioprotective effect of *Cucumis trigonus* Roxb on isoproterenol-induced myocardial infarction in rat. *Am J Pharmacol Toxicol* 2009; **4**(2): 29-37.

[4] Sharmila ST, Rajadurai M. Preventive effect of bio-aq on cardiac markers, lipids, and membrane bound enzymes in isoproterenol-induced myocardial infarction in rats. *Asian J Pharm Clin Res* 2012; **5**(2): 107-113.

[5] Mudagal PM, Karia S, Goli D. Preventive effect of *Rhododendron arboreum* on cardiac markers, lipid peroxides and antioxidants in normal and isoproterenol-induced myocardial necrosis in rats. *Afr J Pharm Pharmacol* 2011; **5**(6): 755-763.

[6] Wells SM, Sleeper M. Cardiac troponins. *J of Vet Emerg Criti Care* 2008; **18**(3): 235-245.

[7] Costantino L, Raimondi L, Pirisino R, Brunetti T, Pessotto P, Giannessi F, et al. Isolation and pharmacological activities of the *Tecoma stans* alkaloids. *Farmaco* 2003; **58**: 781-785.

[8] Hernandez-Galicia E, Aguilar-Contreras A, Aguilar-Santamaria L, Roman-Ramos R, Chavez-Miranda AA, Garcia-Vega LM, et al. Studies on hypoglycemic activity of Mexican medicinal plants. *Proc West Pharmacol Soc* 2002; **45**: 118-124.

[9] Munuswamy H, Thirunavukkarasu T, Rajamani S, Elumalai EK, David E. A review on antimicrobial efficacy of some traditional medicinal plants in Tamilnadu. *J Acute Dis* 2013: 99-105.

[10] Giri RK, Kanungo SK, Tripathi NK. Lipid lowering activity of the hydro-alcoholic extract of *Tecoma stans* L. flowers in hyperlipidemic models of wistar albino rats. *Der Pharmacia Lettre* 2012; **4**(5): 1386-1389.

[11] Das C, Dash S, Sahoo DC. Macroscopical and microscopical study of *Tecoma stans* Linn. leaf. *Res J Parm Biol Chem Sci* 2012; **3**(2): 100-104.

[12] Das V, Dash S, Sahoo DC, Mohanty A. Evaluation of methanolic bark extract of *Tecoma stans* Linn, for wound healing in albino rats. *Int J Pharm Technol* 2010; **2**: 735-742.

[13] Rajamurugan R, Thirunavukkarasu C, Sakthivel V, Sivashanmugam M, Raghavan CM. Phytochemical screening, antioxidant and antimicrobial activities of ethanolic extract of *Tecoma stans* flowers. *Int J Pharm Bio Sci* 2013; **4**(2): 124-130.

[14] Ramadoss S, Kannan K, Balamurugan K, Jeganathan NS, Manavalan R. Cardioprotective effect of *Cyathula prostrata* Linn on isoproterenol induced myocardial infarction in rat. *Int J Res Pharm Biomed Sci* 2012; **3**(2): 551-555.

[15] Palani S, Raja S, Kumar RP, Jayakumar S, Kumar BS. Therapeutic efficacy of *Pimpinella tirupatiensis* (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. *Int J Pharm Tech Res* 2009; **1**(3): 925-934.

[16] Zanwar AA, Hegde MV, Bodhankar SL, Cardioprotective activity of flax lignin concentrate extracted from seeds of *Linum usitatissimum* in isoprenaline induced myocardial necrosis in rats. *Interdiscip Toxicol* 2011; **4**(2): 90-97.

[17] Ramadoss S, Kannan K, Balamurugan K, Jeganathan NS, Manavalan R. Efficacy of cardioprotective effects in ethanolic extract of *Sida Rhombifolia* Linn. on isoproterenol-induced myocardial infarction in albino rats. *Res J Pharm Biol Chem Sci* 2012; **3**: 488-493.

[18] Gunjal MA, Shah AS, Wakade AS, Juvekar AR. Protective effect of *Moringa olifera* Lam stem bark on serum lipids, marker enzymes and heart antioxidant parameters in isoproterenol induced cardiotoxicity in wistar rats. *Indian J Nat Prod Resour* 2010; **1**(4): 485-492.

[19] Mastan SK, Chaitanya G, Latha BT, Srikanth A, Sumalatha G, Kumar KE. Cardioprotective effect of methanolic extract of *Syzygium cumini* seeds on isoproterenol-induced myocardial infarction in rats. *Der Pharmacia Lettre* 2009; **1**(1): 143-149.

[20] Upaganlawar A, Patel V, Balaraman R. Tomato lycopene attenuates myocardial infarction induced by isoproterenol: electrocardiographic, biochemical and anti-apoptotic study. *Asian Pac J Trop Biomed* 2012; **2**: 345-351.

[21] Rahmathulla SB. Effect of *Tribulus terrestris* fruit aqueous extract on hyperlipidaemia and maintenance of liver architecture in isoproterenol-induced myocardial infarction in rats. *Int J Pharm Bio Sci* 2013; **4**(1): 983 - 992.

[22] Kałużna-Czaplińska J. GC-MS analysis of biologically active compounds in cosmopolitan grasses. *Acta Chromatographica* 2007; **19**: 279-282.

[23] Murthy RL, Nataraj HN, Ramachandra SS. Nephroprotective activity of *Cyanotis fasciculata* var. against cisplatin induced nephrotoxicity. *Int Res J Pharm* 2011; **2**(9): 137-142.