The Journal of Phytopharmacology 2015; 4(3):139-142
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(3):139-142
May- June

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Impact of *Solanum surettense* on membrane bound Na+/K+ ATPase and *in vivo* anti oxidants activity on isoproterenol induced myocardial injury in rats

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Abstract

The present study was designed to evaluate the cardio protective potential of ethanolic extract of Solanum surettense a warm-climate annual herb, on isoproterenol-induced Myocardial Infarction (MI) in rats. Five groups of albino rats, each comprising six animals, were selected for this study. Group I served as a control, Group II rats were given isoproterenol (ISO) (85mg/kg subcutaneously), and Group III rats were treated with propranolol 10mg/kg as standard treatment. Groups IV and V rats were given ESS (200 mg/kg and 400 mg/kg, respectively) along with isoproterenol (85mg/kg). At end of the study cardiac biomarkers like CK-MB and LDH were estimated to accesses cardio protection. The protective effect of heart is also accessed by estimation of membrane bound Na^+/K^+ATPase and tissue antioxidant enzymes like SOD, Catalase and GSH. ESS pre treated animals in various doses significantly decreased the levels of CK-MB and LDH when compared with ISO treated animal. The dose of 400 mg/kg has shown significant protection than 200 mg/kg of ESS i.e. dose dependent cardio protection. The study confirms the cardio protective potential of ethanolic extract of Solanum surettense against isoproterenol-induced biochemical alterations in rats.

Keywords: Myocardial infarction, cardio protection, cardio biomarkers, antioxidants.

Introduction

Myocardial Infarction (MI) 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]. Developing countries like India are struggling to manage the impact of infectious diseases simultaneously with the growing burden on society and health systems caused by non-communicable diseases such as myocardial infarction. In India, myocardial infarction typically occurs 10–15 years earlier than in Western countries. An increasing number of young Indians are succumbing to myocardial infarction [2]. Myocardial Infarction (MI) results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart[3].Free radicals and reactive oxygen species have an impact in various disorders like cardiac diseases and cancer which result due to exposure to chemicals and environmental agents.Experimental and clinical studies have shown that there is increased generation of reactive oxygen species such as superoxide anion ($.O_2^-$) and hydroxyl radicals ($.OH^-$) in heart failure, areinvolved in the formation of lipid peroxides, damage of cell membrane, and destruction of the antioxidative defense system[4].

Isoproterenol (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction.ISO is a β-adrenergic agonist that causes severe stress in myocardium and necrotic lesions in the heart muscles. ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes. The mechanism proposed to explain isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been implicated as one of the causative factors. [5]

Solanum surattense Burm F. (Solanaceae) is a perennial herb and is commonly used in the Indian traditional system for curing various ailments. The plant medicinally used to treat for cough, asthma and rheumatism [6]. Phytochemical investigation of the *Solanum surattense* reported to a have number of alkaloids, sterols, saponins, coumarins and flavonoids and their glycosides and especially it has a high concentration of solasodine, a starting material for the synthesis of cortisone and sex hormones. Pharmacological activities such as antibacterial and antifungal, antinociceptive, antioxidant, hypoglycaemic and larvicidal have been reported in this plant[7].

Experimental evidence for the biochemical role of *Solanum surettense Burm* on isoproterenol induced myocardial infarction is not established yet. The present study was attempted to evaluate the cardio protective activity of ethanolic extract of *Solanum surettense* (ESS)on isoproterenol induced myocardial damage with the reference of heart Na^+/K^+ ATPase, biochemical markers, antioxidants and histopathology.

Material and Methods

Animals

Experimental animals of either sex weighing 170 to 200 g with age of 120 days were obtained from Raghavendra enterprises, Bangalore. The animals were housed in stainless steel cages at a controlled room temperature of 24^0C, under a 12 h light and 12 h dark cycle. After 1week of acclimatization, the experimental animals were divided randomly in to 5 groups (n=6). The experiment was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and it is approved by the Institutional Animal Ethics Committee.

Chemicals

Isoproterenol was purchased from Sigma Aldrich USA. All chemicals of analytical grade were used.

Preparation of plant extract

Plant (*Solanum surettense*) used for experimental purpose was procured from the surroundings of Tirupati. The dried parts of whole plant were cleaned and reduced to powdery form with the help of a mechanical grinder after which 70 gm of powder sample was exhaustively extracted with 140ml of 70% ethanol (7:3 ethanol: water) for 3 days by soxhlet apparatus. The plant material was separated by filtration and the ethanolic extract was concentrated and lyophilized and preserved for further use. The residue obtained from the extract was stored in a refrigerator at 4°C is air dried and percentage yield was calculated.

Preliminary Phytochemical Screening

Ethanolic extract of *Solanum surettense (ESS)* was subjected for the qualitative preliminary phytochemical identifications by the standard methods. Various chemical tests were carried out for the detection of Alkaloids, Carbohydrates, Glycosides, Saponins, Phytosterols, Tannins, Flavonoids, Proteins, coumarins and Fixed oils [8].

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines. ESS was administered as the dose of 50mg/kg, 100, 500, 1000 and 2000 mg/kg body weight to groups of animals (n=6). During the 1hr of administration rats were observed for gross behavioral changes as described by Irvin scale, and the mortality rate was observed for 48 hr and LD_{50} value was calculated.

Experimental design

Isoproterenol was dissolved in normal saline and injected subcutaneously in to rats (85 mg/kg) daily for last two consecutive days to induce myocardial infarction[4].The experimental animals were randomly divided into 5 groups (n=6) and treated for duration of 28 days as per the treatment schedule. Group I animals receives normal saline and serve as normal control. Group II animals receive isoproterenol (85mg/kg) last two consecutive days and serve as disease control. Group III animals pretreated with propranolol as standard drug (10mg/kg) and the subcutaneous injection with ISO (85mg/kg) serve as a standard treatment control. Group III animals pretreated with ESS (200mg/kg) and the subcutaneous injection with ISO (85mg/kg) serve as test control at low dose. Group IV animals pretreated with ESS (400mg/kg) and the subcutaneous injection with ISO (85mg/kg) serve as test control at high dose. At the end of experiment blood was collected from retro orbital puncture and serum separated by centrifugation. Serum was used for various biochemical estimations. Hearts were excised and used for Na^+/K^+ATPase estimation. Rest of the hearts was stored in 10% formaldehyde solution for histopathological studies.

Biomarkers estimation

After administration of ISO the animals were sacrificed by cervical decapitation, blood was collected and the heart was dissected out. The serum was separated immediately by cold centrifugation and used for determination of cardiac biomarkers markers LDH, CK-MB and total protein by using commercial diagnostic kits (Agappe Pvt. Ltd, Kerala, India).

Na^+ /K^+ ATPase activity of myocardial membrane

Na^+ /K^+ ATPase activity of myocardial membrane was determined according procedure [9].

Estimation of tissue antioxidants

At the end of the study hearts were excised and homogenate in 0.1M Tris buffer and the homogenate were used for estimation of tissue antioxidants like SOD [10], GSH [11], CATALASE [12] and LPO [13] as per the prescribed procedures.

Histological examinations

The hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin. The hearts stored in 10% buffered formalin were embedded in paraffin, sections cut at 5 μm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical analysis

Descriptive statistics such as mean and standard deviation has been calculated for each and every variable for each group. One-way analysis of variance (ANOVA) has been applied for statistical analysis with Turkey as post metric test and a value of $p < 0.05$ has been considered as statistical significance level.

Results

In the acute toxicity study, none of the dose of ESS was shown mortality even at 2000 mg/kg. Therefore, $1/10^{th}$ and $1/5^{th}$ of the dose were selected for the study as low and high test doses.

There was significant ($p < 0.001$) decrease in Na^+ /K^+ ATPase activity in ischemic control when compared with normal control. There was significant ($p<0.001$) increase in serum levels of Na^+ /K^+ ATPase activity in groups treated with test drugs at doses of 200 and 400 mg/kg when compared with an ischemic control group (Table: I).

There was a significant elevation in cardiac markers like LDH and CK-MB profiles in isoproterenol treated animals when it compared to the controls (Table: 1). For the animals pretreated with ESS in IV and V groups was observed a significant reduction ($p<0.001$) in the cardiac markers like LDH and CK-MB level when compared with the isoproterenol treated rats (Group II). The decrement of cardiac markers is almost similar to the standard treatment with propranolol 10mg/kg i.e. Group II animals (Table: I).

The tissue antioxidant markers like GSH, CAT, and SOD were significantly increased while malondialdehyde (MDA) which is a measure of LPO was significantly reduced in animals treated with isoproterenol (Group II) when it is compared with normal animals (Group I). By the prophylactic treatment with ESSat 200 and 400

mg/kg body weight observed a significant increase (P<0.001) of tissue antioxidant markers like GSH, CAT, and SOD and significant decrease of LPO levels when compared to isoproterenol (Group II) treated rats (Table: II)

Histopathological examination of myocardial tissue of normal animals shows a clear integrity of myocardial cell membrane (Fig: 1a). Endocardium and pericardium were seen within the normal limits. No inflammatory cells infiltration was observed in normal rat heart. Isoproterenol treated animals' shows a focal myonecrosis with myophagocytosis and lymphocytic infiltration (myocarditis) was observed (Fig: 1b). The animals were pre-treated with ESS at 200 and 400mg/kg doses were found less damaged and low fatty infiltration was observed when compared with an ischemic control group (Fig: 1d&1e).

Table 1: Effect of ethanolic extra of *Solanum surettense* on serum cardiac manners like CK-MB, LDH and Na^+/K^+ ATPase levels

S.NO	GROUPS	CK.MB (IU/L)	LDH (IU/L)	Na^+/K^+ ATPase (nM of IP liberated/min/mg protein)
1	NORMAL	22.67 ± 0.49	228.5 ± 2.64	1.07 ± 0.05
2	CONTROL	53.67 ± 0.42[a]	441.5 ± 1.77[a]	0.21 ± 0.008[a]
3	STANDARD	25.50 ±0.56[b]	248 ± 2.65[b]	0.84 ± 0.02[b]
4	Test-1 (ESS 200mg/kg)	32.50 ± 0.71[b]	284.2 ± 4.36[b]	0.61 ± 0.02[b]
5	Test-2(ESS 400mg/kg)	26.83 ± 0.47[b]	260.5 ± 2.43[b]	0.73 ± 0.01[b]

Values were expressed as mean ± S.E.M of 6 observations. **a** Indicates p < 0.001 when compared to respective normal group. **b** Indicates p < 0.001 when compared to respective disease control group.

Table 2: Effect of ethanolic extra of *Solanum surettense* on tissue antioxidant enzyme.

Group	Treatment	CATALASE (µM H_2O_2 consumed/mg protein)	GSH (µM of GSH/mg protein)	SOD (U/mg protein)	LPO (nM of MDA/mg protein)
Group-I	NORMAL	9.16 ± 0.47	9.01± 0.21	19.87 ± 0.47	3.86± 0.11
Group-II	CONTROL	3.66 ± 0.33[a]	4.01± 0.29[a]	6.66 ± 0.66[a]	9.33± 0.26[a]
Group-III	STANDARD	8.16 ± 0.30[b]	7.96± 0.15[b]	16.83 ± 0.47[b]	4.98± 0.10[b]
Group-IV	Test-1(ESS200mg/kg)	6.16 ± 0.30[b]	6.25± 0.07[b]	14.00 ± 0.57[b]	5.78± 0.31[b]
Group-IV	Test-2(ESS 400mg/kg)	7.33 ± 0.33[b]	7.01± 0.20[b]	15.67 ± 0.49[b]	5.10± 0.26[b]

Values were expressed as mean ± S.E.M of 6 observations. **a** Indicates p < 0.001 when compared to respective normal group. **b** Indicates p < 0.001 when compared to respective disease control group.

Discussion

The present study was aimed to evaluate the cardio protective activity of ethanolic extract of *Solanum surettense* on isoproterenol induced myocardial infarction in albino rats. Isoproterenol is well known cardio toxic agent due to its ability to destruct myocardial cells. As a consequence, cytosolic enzymes such as LDH, ALT, AST and CK-MB were released into the blood stream and serve as the diagnostic markers of myocardial tissue damage.[14] The amount of these cellular enzymes present in blood reflects the alterations in the plasma membrane integrity and/or permeability. In our study, isoproterenol treated rats showed a significant elevation in the levels of these cardiac diagnostic marker enzymes. Moreover, elevated levels of these enzymes are an indicator of the severity of isoproterenol-induced myocardial membrane necrosis. It is well known that isoproterenol-induced myocardial injury is mediated primarily via the β_1-adrenergic receptor. Acute β_1-adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also depresses total cellular antioxidant capacity, down regulates copper-zinc superoxide dismutase enzyme activity, protein and mRNA and reduces glutathione level, leading to the loss of membrane integrity and inducing heart contractile dysfunction and myocytes toxicity finally producing myocardial necrosis [14]. A Number of studies strongly suggest that free radicals play an important role in catecholamine-induced cardio toxicity by causing peroxidation of membrane phospholipids, which can result in permeability changes in the membrane as well as intracellular calcium overload [15].

The preliminary Phytochemical screening of ethanolic extract of *Solanum surettense* shows thepresence of high level of secondary metabolites like coumarins, flavonoids, phenolic compounds and phytosterols. Number of investigations suggested the presences flavonoids, coumarins and phenolic compounds have shown an important in the reduction of oxygen free radicals.

Alteration in LDH and CK-MB has been considered as one of the most important cardiac marker of myocardial infarction. Wexler and Kittinger *et al.*, demonstrated that there was a dramatic rise and fall in serum CK-MB and LDH following isoproterenol induced MI in rats, and the degree of rise and fall in serum enzyme activities were commensurate with the extent of the myocardium infracted[14].

In the present study, ISO treated rats showed a significant elevation in the levels of these diagnostic marker enzymes (LDH and CK-MB). Moreover, elevated levels of these enzymes are an indicator of the severity of ISO-induced myocardial membrane necrosis, which is in line with an earlier report [16]. The prior administration of ESS (200 and 400 mg/kg) showed a significant ($p < 0.01$) reduction in ISO 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 was reported that seven kinds of flavonoids and coumarins like esculentin were found in *S.surettense;* particularly esculentin is well proven anti-infalmmatory [17] and antioxidant [18], which possess and take part in the healing of myocardium damaged by isoproterenol. Since, flavonoids are one of the most popular compounds in the plant kingdom and have effectiveness in reducing blood lipid, as an anti-oxidative, in assimilating cholesterol, inhibiting thrombosis, dilating the coronary artery [19].

In the present study the presence of flavonoids, phenolic compounds in ESS were contributing to the reduction of elevated levels of serum cardiac markers to elucidate cardio protective activity.

The cardioprotecctive mechanism of ESS appears to be through modulation of various antioxidant parameters, thereby improving the overall antioxidant defense of the myocardial tissue. In the present study shows a significant decrement in tissue antioxidants likes GSH, SOD, and Catalase and increased levels of LPO in the ISO treated animals. Whereas significant elevation in on GSH, SOD, and Catalase levels and with decreased LPO was observed in pre-treated groups with ESS in doses dependent manner.

Present data on GSH, SOD, and LPO demonstrated that antioxidant capacity of the myocardium on ISO treated group is significantly hindered. Significant fall in GSH levels impaired SOD activity together with increased LPO shows that the tissue is more susceptible to oxidative damage. Increased free radical production in this situation may be responsible for the observed membrane damage as evidenced by the elevated TBARS levels.

GSH levels or the production was increased in the animals pre-treated with ESS in the dose dependent manner. Here the GSH and SOD levels are required for the compensation of free radicals generated by the isoproterenol.

Decrease in activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the myocardium. In the present study the ESS pre- treated groups were found to enhanced SOD activity could effectively scavenge the first free radical superoxide from the myocardium.

Antioxidant properties of ESS could be attributed to its constituents like ω-3 fatty acids, flavonoids, vitamin E, vitamin C, phytosterols and flavonols etc. Pre-treated animals with ESS at the dose of 200 and 400 mg/kg offered a significant at $p < 0.001$ protection against ISO induced MI.

Histopathological studies reveal that there was more myocardial damage and focal myonecrosis and chronic infiltration of inflammatory cells found in the animals treated with isoproterenol 85mg/kg when compared with normal group (Fig: 1a&1b). The animals were pre-treated with propranolol 10mg/kg (Fig:1c) and ESS at low and high doses were found less damage and low fatty infiltration when it was compared with control group (Fig: 1d&1e). This confirming furthers the cardio protective activity of ethanolic extract of *Solanum surettense* in the present study. The present data indicate that ESS may provide potential therapeutic value in the treatment of myocardial infarction.The beneficial effects of *Solanum surettense* can be reproduced in human beings, these findings may represent a novel prophylactic therapy for MI.

Conclusion

This study thus demonstrates the cardio protective effect of ethanolic extract of *Solanum surettense* (200 and 400mg/kg, p.o.). This extract was found to be most effective in the reduction of biomarkers of the heart and restoration of biochemical and histopathological alterations. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Solanum surettense.*

Acknowledgement

The authors are grateful to management of the Sri Padmavathi School of pharmacy. Tirupati, A.P. for their valuable support in this project.

Conflicts of interest

None declared.

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