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Research Article

Protective role of *Ascidia sydneiensis* Stimpson, 1855 on cardiac enzyme biochemistry and histopathology in isoproterenol - induced myocardial ischemia

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Abstract

Myocardial ischemia refers to the condition in which a portion of a heart is starved of oxygen and nutrients as a result of sudden block i the coronary arteries. It may be caused by chemotherapy, complications from anorexia nervosa, adverse effects of heavy metals intake and an incorrectly administered drug. The aim of the study is to investigate the protective role of *Ascidia sydneiensis* in isoproterenol - induced myocardial ischemia in rats. The extract was administered at a dose of 50, 100 and 150 mg/kg bw i.g.c for 28 days. The animals of ISO control and *Ascidia sydneiensis* pretreated groups were given isoproterenol (150 mg/kg bw) at an interval of 24 hours on 29th and 30th day of the experiment. Biochemical parameters like LDH, AST, ALT in serum and lipid peroxidation marker enzyme - MDA in both serum and tissue; enzymatic and non-enzymatic myocardial antioxidant enzymes - SOD, CAT, GPx in heart tissue and GSH in both serum and tissue were analysed following standard procedures. Histopathological architecture of cardiac muscle was also studied. A significant increase in the level of LDH, AST, ALT in the serum and lipid peroxidation marker enzyme - MDA in serum and heart tissue and decrease in the enzymatic and non-enzymatic antioxidant enzymes - SOD, CAT, GPx in heart tissue and GSH in both serum and tissue were noted in ISO treated groups. In the pretreated groups all the above parameters were normal. Restoration of enzyme level observed in the co-treated groups indicate that the extract has protective role in ISO - induced myocardial ischemia. Histopathological studies also confirmed the protective nature of the extract on heart tissue. Based on present findings, it is concluded that *Ascidia sydneiensis* may be a potential and therapeutic agent against the oxidative stress associated ischemic heart disease owing to antioxidant and antiperoxidative activity.

Keywords: *Ascidia sydneiensis*, myocardial ischemia, cardiotoxicity, lipid peroxidation, antioxidant.

1. Introduction

Cardiovascular disease (CVD), a group of disorders of the heart and vasculature, including high blood pressure, coronary heart disease, myocardial ischemia, contractile heart failure, stroke and congenital heart defects are the most prevalent cause of death and disability worldwide [1]. Among these myocardial ischemia (MI) is the most alarming one and it occurs due to imbalance between coronary blood supply and myocardial demand. Free radical mediated myocardial damage is an important etiological mechanism that is associated with increased level of reactive oxygen species and/or inadequate antioxidant defense system [2]. These are responsible for 17.1 million fatalities each year and it will reach upto 20 million in 2020 [3-5]. Despite improvement in clinical care and better awareness, MI still remains the leading cause of mortality [6]. Isoproterenol is a widely used chemical in toxicological studies to provoke cardiac muscle injury, through an exaggerated pharmacological effect [7]. It

causes severe stress in the myocardium resulting in necrosis of heart muscles leading to cardiac dysfunction, increased lipid peroxidation along with an increase in the level of myocardial lipids, altered activities of the cardiac enzymes and antioxidants [8,9]. Many studies specify that ISO - induced MI supplies a well standardized model in considering the beneficial effects of several drugs and heart function. Various reports have shown that a lot of herbal extracts have been screened for their cardio protective effects against ISO - induced toxicity [10-14]. *Ascidia sydneiensis* is a marine sedentary simple ascidian found in plenty in Tuticorin coast. A review of literature shows that studies on taxonomy [15], ecology, distribution, seasonal variation in the occurrence, breeding biology, recruitment and succession in the fouling community, role as bioindicators, food value [16], association with coral reef [17], antibacterial, antimicrobial activity against human pathogens [18,19], chemical investigations [20-34], antipyretic and analgesic [35,36], larvicidal [37], antimicrobial [38,39], antidiabetic [40,41], antiproliferatve [42], toxicity [43-46], antitumour and immunomodulatory [47-52], anaesthetic [53-55], wound healing [56-58], CNS depressant [59], antifertility [60], hepatoprotective [61,62], antiinflammtory [63-65], immunostimulating [66], cardioprotective [67] and anti-hyperlipidemic [68] activity of a large number of ascidians have been studied. But chemical investigations [68-71], toxicity [72], antidiabetic [73], antifertility [74] and hepatoprotective [75] effect of the selected ascidian has only been carried out. However as there is no systematic study on isoproterenol - induced myocardial ischemia to male albino rats, the present investigation focuses on evaluating the protective effect of ethanolic extract of *Ascidia sydneiensis*.

2. Materials and Methods

2.1 Animal material

Samples of *Ascidia sydneiensis* were collected from Tuticorin coast and identified using key to identification of Indian ascidians [76]. A voucher specimen AS 2252 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628002.

2.2 Taxonomic status

Ascidia sydneiensis is a simple ascidian belonging to the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascidiidae, Genus: *Ascidia*, Species: *sydneiensis*

2.3 Preparation of extract

For myocardial ischemia studies, 100 gram powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown residue.

2.4 Experimental animals

Normal healthy adult male Wistar albino rats (180-200 g) were obtained from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. They were maintained under standard environmental conditions of temperature - 24±1°C, 12 h dark-light cycle, humidity (60-70%), free access to drinking water and standard pellet diet. Rats were deprived of food except water 16-18 hour prior to the experiments. The rules and regulations of Animal Ethical Committee, Government of India were followed.

2.5 Induction of myocardial ischemia

Myocardial ischemia was induced by subcutaneous injection of isoproterenol hydrochloride (ISO) 150 mg/kg dissolved in saline, once a day for 2 days.

2.6 Experimental protocols

Experimental animals were divided into eight groups of 6 rats each. Forty eight rats were randomly selected. They were acclimatized for an hour, pre and co-treated orally with saline/*Ascidia sydneiensis* along with isoproterenol subcutaneously on the scheduled days as per the groups in table 1. Rats were euthanized twenty four hours after the second dose of isoproterenol (31st day).

2.7 Preparation of serum and heart tissue for biochemical studies

Samples of blood were collected by cardiac puncture. The serum obtained was analysed for lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), reduced glutathione (GSH) and lipid peroxidation marker enzyme-malondialdehyde (MDA). The heart of the animals were dissected out, weighed, washed in ice-chilled physiological saline, frozen and homogenized in a Teflon homogenizer with 10 times (w/v) ice-cold phosphate buffer saline of pH 7.8. The homogenate was centrifuged at 1000 rpm at 4^{o} C and the supernatant was used for the estimation of enzymatic and non-enzymatic myocardial antioxidant enzymes. SOD, CAT, GPx, GSH and MDA were measured spectrophotometrically [77-84].

Table 1: Experimental protocol

Groups	Treatment Days 1-28 (mg/kg bw i.g.c)	Treatment Days 29 & 30
I – Saline	Saline	Saline i.g.c. + saline s.c
II - ISO control	Saline	Saline i.g.c. + ISO150 mg/kg s.c
III - AS 50	*Ascidia sydneiensis* - 50	*Ascidia sydneiensis* 50 mg/kg i.g.c. + saline s.c
IV - AS 100	*Ascidia sydneiensis* - 100	*Ascidia sydneiensis* 100 mg/kg i.g.c. + saline s.c
V - AS 150	*Ascidia sydneiensis* - 150	*Ascidia sydneiensis* 150 mg/kg i.g.c. + saline s.c
VI - AS 50 + ISO 150	*Ascidia sydneiensis* - 50	*Ascidia sydneiensis* 50 mg/kg i.g.c. + ISO 150 mg/kg s.c
VII - AS 100 + ISO 150	*Ascidia sydneiensis* - 100	*Ascidia sydneiensis* 100 mg/kg i.g.c. + ISO 150 mg/kg s.c
VIII - AS 150 + ISO 150	*Ascidia sydneiensis* - 150	*Ascidia sydneiensis* 150 mg/kg i.g.c. + ISO 150 mg/kg s.c

AS - *Ascidia sydneiensis*; **ISO** - Isoproterenol; **i.g.c.** - Intra gastric catheter; **s.c.** - sub cutaneously

2.8 Histopathological studies of heart muscle

Heart tissue was excised immediately, washed with ice-cold saline and preserved in 10% formalin solution for histological study. They were dehydrated with varying percentage of ethanol, cleared in xylene and embedded in molten wax. Thin sections were cut (5 µm), stained with hematoxylin, eosin and viewed under the high power of a microscope. The myocardial architecture was examined under light microscope and photomicrographs were taken [85].

2.9 Statistical analysis

Values are presented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by student's t - test to identify the differences between *control, pretreated groups and co treated groups^{a} and cardiotoxic control and co-treated groups^{b}.

3. Results and Discussion

Table 2 represents the effect of extract on serum and lipid peroxidation marker enzyme. ISO administration at a dose of 150 mg/kg induced myocardial ischemia which is proved by a significant elevation in the levels of serum LDH, AST, ALT and lipid peroxidation marker enzyme MDA in serum and heart tissue. Oxidative stress has been linked with diverse pathophysiological events including cancer, renal disease and neuro-degeneration [86]. It has been established that excessive oxidative stress caused by either increased ROS production or inadequate antioxidant defenses can lead to cardiac lesions [87]. Administration with large dose of ISO kindles morphological and functional changes in the heart leading to myocardial necrosis [88]. As a result of myocardium destruction, cytosolic enzymes (LDH, AST & ALT) are released in to blood which function are diagnostic biomarkers of cardiotoxicity. Cell necrosis, contractile failure, ventricular arrhythmias and subcellular changes of pathophysiology in rats are analogous to those taking place in human myocardial ischemia [89-91]. The present observations on treatment with ISO are in unison with the earlier report using plant extract [92]. An increase was observed in the enzyme levels of the pretreated groups restored to normal in the group which received the highest dose. In the current study, pretreatment of *Ascidia sydneiensis* to myocardial ischemia promoted rats reduce the cardiac damage and restrict the leakage of enzymes as is evident from a significant reduction in the activities of cardiac marker enzymes in plasma. Lower levels of enzyme indicated during co-treatment could be attributed to the reduction in the myocardial damage by the extract.

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation [93]. It is a marker of membrane lipid peroxidation resulting from the interaction of ROS and the cellular membrane. The final membrane damage can lead to loss of cellular homeostasis by altering the membrane characteristics [94]. Data in this study showed that MDA content significantly increased in the heart tissues of rats treated with ISO. This raise in MDA content in the heart tissues may be due to an elevation in free radicals resulting from the induction of oxidative stress in rats treated with ISO. In the pretreated groups, no significant change in the level of MDA in the serum and heart tissue was noted indicating the non toxic nature of the extract. The decreased level of MDA in serum and heart might be due to growth of endogenous antioxidants by the bioactive components in *Ascidia sydneiensis*. The results support anti-lipidperoxidative property of the extract similar to previous reports using plant extracts [95].

The activities of enzymatic and non - enzymatic antioxidants - SOD, CAT, GPx and GSH are illustrated in Table 3. The antioxidant enzymes decreased significantly in ISO treated rats when compared to those of control group. SOD catalyzes the conversion of superoxide anion free radical to H_2O_2 through dismutation reaction. Catalase catalyzes the exchange of H_2O_2 to water and oxygen. The activity of SOD and CAT is found to be increased in the

groups treated with the extract. Endogenous enzymes such as SOD and CAT are the first line cellular defense free radical scavenging enzymes against oxidative injury. Increased activity of SOD and CAT indicates increased removal of superoxide radicals thereby reducing myocardial damage originated by free radicals [96]. The antioxidant properties exhibited may be by the presence of compounds such as Tetradecanoic acid, n-Hexadecanoic acid, 9-Hexyl-heptadecane, 3-ethyl-5-(2-ethylbutyl)-octadecane, Cholest-5-en-3-ol(3∝)-carbonochloridate, Cholesterol [69]. The rise in the activities of SOD and CAT in co-treated ISO challenged group spotlight the safety against oxidative stress. It is suggested that dose as low as 150 mg/kg of extract of *Ascidia sydneiensis* enhanced the antioxidant activity of SOD and contribute to the inhibition of free radical mediated cell injury.

GSH is a very important non-enzymatic antioxidant which can react directly with free radicals or act as an electron donor in the reduction of peroxides catalyzed by GPx [97]. Vitamin C readily reacts with GSH radicals arising from the reaction of GSH and free radicals. Thereby vitamin C spares GSH first by competing with GSH for free radicals and second by converting thiol radicals back to GSH [98]. A significant decrease in the level of GSH observed in the serum and heart tissue of ISO induced rats may be either due to decreased synthesis or increased degradation of glutathione. ROS are reduced by GSH in the presence of GSH peroxidase. GSH plays important role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by GPx. Lipid peroxidation can generate large amounts of electrophilic and oxidizing reactive species which can lead to a variety of DNA and tissue damage [99]. Therefore significant reduction causes accumulation of oxidised glutathione which in turn blocks protein synthesis by inactivating many enzymes containing the SH group. The obtained information of bringing back the plasma and heart (GPx and GSH) levels towards the normal values demonstrates that *Ascidia sydneiensis* can be considered as a potent antioxidant agent when given simultaneously with ISO which is indicative of prophylatic nature of the extract during pretreatment. A gradual increase was observed in the activities of GPx and GSH in co-treated groups which underline the antioxidant components present in the extract. Similar observation of cardioprotective effect is reported on treatment with the ethanolic extract of simple ascidian *Microcosmus exasperatus* [67]. Present study reveals the protective role of ethanolic extract of *Ascidia sydneiensis* in isoproterenol - induced myocardial ischemia as confirmed by improved antioxidant defense as well as inhibition of lipid peroxidation and prevention of leakage of myocytes injury marker enzymes from heart.

Histopathological studies of the heart muscle of wistar albino rats treated with *Ascidia sydneiensis* extract is shown in Plate 1. In the control rats' heart muscle myocardial fibers were arranged regularly with clear striations, without any damage or necrosis in the tissue. Confluent necrosis of myofibrils, edema and infiltration of inflammatory cells were noted in ISO control. In the extract pretreated groups normal histoarchitecture of myocardium with insignificant edematous intramuscular space was evident. Group co-treated with 50 mg/kg bw, indicated a moderate to marked myonecrosis and infiltration of lymphocytes whereas in the groups which received 100 mg/kg bw the histomorphology of myocardium showed minimal myonecrotic patches with minimal inflammatory cell infiltration. The group which received 150 mg/kg bw exhibited normal histoarchitecture of myocardium and absence of inflammatory cells.

Table 2: Effect on serum LDH, AST, ALT and lipid peroxidation marker enzyme - MDA

Groups & Treatment	LDH IU mg^{-1}	AST IU mg^{-1}	ALT IU mg^{-1}	MDA	
				Serum n mol. ml^{-1} protein	Heart Tissue n mol. mg^{-1} protein
I – Control	143.34±8.18	13.36±2.48	9.31±1.65	6.36±3.16	9.65±2.94
II- ISO 150 mg	279.34±7.24^{aa}	23.68±4.15^{aa}	19.31±4.18^{aa}	18.31±3.84^{aa}	17.54±1.15^{aa}
III- AS 50 mg	126.15±8.15	11.16±3.18	12.16±1.62	5.16±1.15	7.54±1.16
IV- AS 100 mg	131.80±4.16	12.26±4.84	12.65±1.54	6.14±1.06	7.26±2.15
V- AS 150 mg	144.65±3.18	13.12±6.18	13.31±1.18	6.86±1.27	8.05±1.92
VI - AS 50 mg + ISO	206.31±8.84^{ab}	19.31±6.05^{ab}	18.16±4.16^{aa}	16.31±3.18^{aa}	14.54±2.16^{aa}
VII- AS 100 mg + ISO	165.16±6.40^{bb}	16.16±5.18^{bb}	14.80±4.65^{ab}	12.34±2.15^{ab}	13.16±1.65^{ab}
VIII- AS 150 mg + ISO	158.24±3.16^{bbb}	13.31±4.06^{bbb}	12.88±2.16^{bb}	11.16±1.15^{bb}	12.84±1.36^{bb}

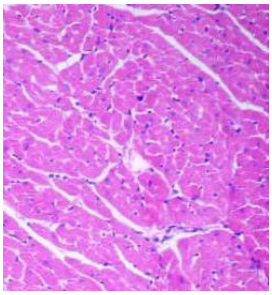
Data represented as mean± S.E.M (n=6). Significance between control, pretreated groups and co-treated groups a < 0.05; aa < 0.01. Significance between cardiotoxic control and co-treated groups b < 0.05; bb < 0.01; bbb < 0.001.

Table 3: Effect on enzymatic and non - enzymatic myocardial antioxidant enzymes

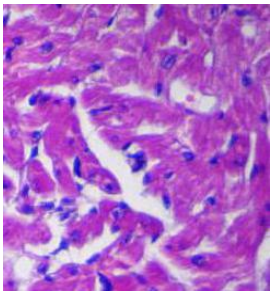
Groups & Treatment	SOD U mg^{-1} protein	Catalase U mg^{-1} protein	Gpx U mg^{-1} protein	GSH	
				Serum n mol. ml^{-1}	Heart Tissue n mol. mg^{-1} protein
I - Control	8.26±0.06	3.81±0.16	6.31±0.15	4.31±0.26	2.18±0.11
II- ISO 150 mg	$4.81±0.65^{aa}$	$2.16±0.24^{aa}$	$5.02±1.68^{aa}$	$2.68±0.16^{aa}$	$1.18±0.15^{aa}$
III- AS 50 mg	5.92±0.55	3.12±0.11	5.12±1.98	4.36±0.26	2.54±0.11
IV- AS 100 mg	6.39±0.48	3.65±0.15	5.24±0.11	4.66±0.11	2.92±0.27
V- AS 150 mg	7.94±0.26	4.06±0.05	6.16±0.31	4.98±0.18	2.97±0.11
VI - AS 50 mg + ISO	$6.63±1.54^{ab}$	$2.63±0.18^{ab}$	$4.26±1.08^{ab}$	$2.93±0.018^{ab}$	$2.16±0.026^{bbb}$
VII- AS 100 mg + ISO	$7.22±1.86^{bb}$	$3.14±0.13^{bb}$	$5.65±1.98^{bb}$	$3.48±0.015^{bb}$	$2.29±0.015^{bbb}$
VIII- AS 150 mg + ISO	$8.55±1.95^{bbb}$	$3.96±0.25a^{bbb}$	$6.11±1.31^{bbb}$	$4.51±0.018^{bbb}$	$2.84±0.036^{bbb}$

Data represented as mean± S.E.M (n=6). Significance between control, pretreated groups and co-treated groups a < 0.05; aa < 0.01. Significance between cardiotoxic control and co-treated groups b < 0.05; bb < 0.01; bbb < 0.001.

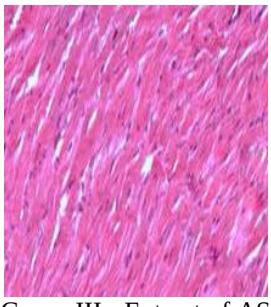
Plate 1: Photomicrograph showing histoarchitecture of cardiac muscle



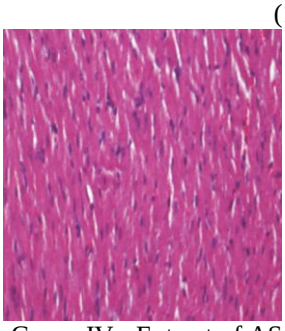
Group I - Normal control



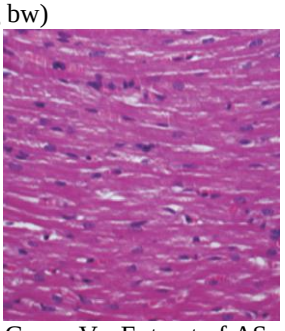
Group II - ISO control (150 mg/kg bw)



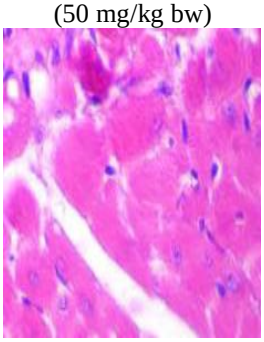
Group III - Extract of AS (50 mg/kg bw)



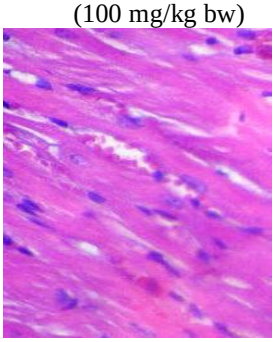
Group IV - Extract of AS (100 mg/kg bw)



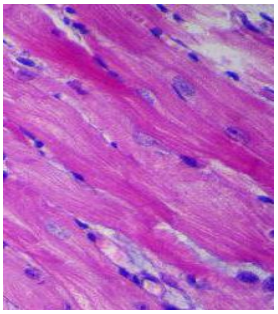
Group V - Extract of AS (150 mg/kg bw)



Group VI - Extract of AS+ISO (50 mg/kg bw)



Group VII - Extract of AS+ISO (100 mg/kg bw)



Group VIII - Extract of AS+ISO (150/kg bw)

4. Conclusion

The present study concludes that the protective role of ethanolic extract of *Ascidia sydneiensis* in ISO - induced myocardial ischemia in rats are related to its effects on counteraction of free radicals. These properties could be due to membrane protective role of the extract by scavenging the free radicals and its antioxidant action. Further works are needed to identify the active principles present in marine ascidians and elucidate its possible mode of action. The observations emphasized that *Ascidia sydneiensis* may be one of the promising drug for improving defense mechanisms in the physiological

systems against oxidative stress caused during myocardial ischemia.

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