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Cardio protective activity of *Sargassum wightii* on isoproterenol induced myocardial stress in rats

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The aim of this investigation is to determine whether the methanolic extracts of *Sargassum wightii* can protect rats against isoproterenol-induced myocardial infarction. Four different groups of rats (6 rats in each group) were taken; where group 1 comprised of normal untreated rats, group 2 was injected with Isoproterenol (synthetic catecholamine), group 3 was considered as standard and hence, was injected with Isoproterenol + Simvastatin and group 4 was treated with Isoproterenol + *Sargassum wightii*'s extract. Cardioprotective effects of *Sargassum wightii* was observed via the changes in the lipid profile, cardio marker enzymes and through histopathological studies. Rats treated with the extract of *S. wightii* showed a significant reduction in total cholesterol, LDL-cholesterol, serum triglycerides and increase in HDL- cholesterol level indicating an undamaged myocardial membrane. Likewise, low enzyme activity in *Sargassum wightii* treated rats clearly indicated the cardioprotective effects of *Sargassum wightii.* Histopathological studies were also done to observe the changes on the rats at the tissue level and no pathological changes were observed in *Sargassum wightii* treated rats. Hence, methanolic extract of *Sargassum wightii* is evidenced to possess cardioprotective activity against myocardial infarction.

[**Keywords**: Acute myocardial infarction, Cardioprotective activity, Isoproterenol, Marine algae, *Sargassum wightii*]

Introduction

Acute Myocardial Infarction (AMI), also known as 'heart attack' happens when the blood supply to the heart and heart muscle is compromised, which is mostly caused by the deposit of unstable cholesterol/fat, white blood cell, etc. in the blood vessels[1]. The occurrence of AMI is the initial indication of heart diseases and is found in approximately 50 to 70 % people and is one of the common causes for hospitalization. However, 64 % of the people with AMI do not experience any chest pain and it is described as 'silent' myocardial infarctions[2]. High levels of LDL (low-density lipoprotein), cholesterol, triglycerides, low levels of HDL high-density lipoprotein (HDL), obesity, alcohol intake, cigarette smoking, and other risk factors are significant contributors to the condition[3]. Hence, there is a continued interest in developing different forms of strategies to combat the risks associated with AMI.

As a result of increased demand for efficient screening and therapeutic treatment of AMI, there is great interest in the investigation of cardioprotective effects of marine algae species where it has been reported to have cardioprotective activities[4,5]. The presence of high protein content along with the essential amino acids and minerals in the marine algae species are said to be a main factor in cardioprotective activity[6,7]. Among the marine algae species, *Sargassum* sp. are known to various metabolites like sterol, glycolipids, phycocolloids etc which responsible for various bioactivities including antimicrobial, antioxidant, anticancer etc[8]. *Sargassum wightii*, linear ovate and macroscopic algae present in large quantities in Tamil Nadu, India[9]. There are few

investigations on its bioactivities and strong evidence of its cardioprotective effects[10-12].

Synthetic catecholamine 'isoproterenol' (isoprenaline) functions as a β-adrenergic agonist and is known to cause myocardial infarction by inducing stress to cardiac myocardial tissues[13]. It also causes lipid peroxidation and increases the level of LDL, VLDL, stress enzymes which is believed to causing through generating free radicals, where these free radicals cause myocardial membrane destruction[14]. Therefore, it is considered for creating myocardial infarction in animal models. Several studies have been conducted on isoproterenol induced myocardial stress on rats for better understanding of the effects of the diseases and for investigation of the therapeutic effects of marine algae species against myocardial infarction[15,16]. The goal of the current investigation was to investigate *Sargassum wightii*'s cardio-protective properties against the myocardial infarction in animal model - Wistar rats.

Materials and Methods

Collection, identification, and extraction of seaweed

Sargassum wightii, a brown-algae, was collected from the Gulf of Mannar's Mandapam coast in the south-east coast of India. Dr. V. Deepak Samuel from the Conservation of Coastal and Marine Resources Division, Ministry of Environment and Forest, Government of India, identified the algae as *Sargassum wightii. Sargassum wightii* was collected, washed under running water, then rinsed with ethanol (70 %), and kept for shade dry. The dried algae were ground to powder, and 20 g of algae was subjected to Soxhlet extraction with methanol as solvent. The distillation was carried out at 40 °C until the methanol was completely drained. The residue was then transferred to another container and dissolved in sufficient quantity of distilled water.

Evaluation of cardio protective effect of algal extract

Healthy male Wistar rats of weight 110 – 150 g and age of 2 to 3 weeks were selected for the study. Food and water were given with commercial pellets and *ad libitum.* The humidity level was kept at 55 to 60 % and the room temperature was controlled at 22 to 26 °C. The cages were used to house the rats were of dimension: 6 ½" × 9 ½" x 7" (L x B x H).

Animals were separated into 4 groups (6 in each group): 1st group - normal untreated rats, 2nd group was control - intraperitoneally treated with Isoproterenol (150 mg/kg.bw) on 20th and 21st day. 3rd group was marked as standard that is treated with Simvastatin intraperitoneally (standard drug 10 mg/kg.bw dissolved in distilled water) for 19 days and treated with Isoproterenol (150 mg/kg.bw) on 20th and 21st day. 4th group was treated with *Sargassum wightii* intraperitoneally (250 mg/kg.bw) for 19 days and treated with Isoproterenol (150 mg/kg.bw dissolved in saline) on 20th and 21st day. The animals were slain while being given chloroform anesthesia, and blood was taken by tail bleeding. Hearts were taken out, prepared, fixed, and used for histopathology studies.

Analysis of cardio protective effect

Blood serum was subjected to various lipid profiles and for cardio marker enzymes including Alanine transaminase (ALT) and aspartate transaminase (AST) activity[17]. Creatine phosphokinase was determined according to method of Broad and Sirota[18] and lactate dehydrogenase was also determined. Histopathological analysis was done for the tissues.

Statistical analysis

Mean ± SD was the unit of expression for all the values. A one-way analysis of variance (ANOVA) using Duncan's multiple range test (DMRT) was conducted, and a significance level of $p < 0.05$ was used.

Results

Analysis of cardio protective effect

Effect of Sargassum wightii extract on lipid profile

In this study, a marked increase in total cholesterol, triglycerides, LDL-cholesterol, and VLDL-cholesterol were observed in isoproterenol exposed rats, whereas there was a decrease in HDL-cholesterol. Cardiovascular damage is thought to be primarily caused by high levels of cholesterol and triglycerides and their accumulation in the heart region. Hence, an increase in total cholesterol and lipid profiles were indicative of myocardial infarction[19,20]. Whereas, the administration of *S. wightii* extract significantly reduced total cholesterol, LDL-cholesterol, serum triglycerides and significantly increased HDL-cholesterol level ($p < 0.05$) (Figs. 1 – 4) and therefore, treatment with *S. wightii* signifies that the myocardial membrane was intact and not damaged. Similar results were observed in a study conducted by Thomes *et al*.[4] where isoproterenol exposed rats showed a dramatic increase in the levels of total cholesterol, triglycerides, LDL and decrease in the levels of HDL whereas the levels of same lipid

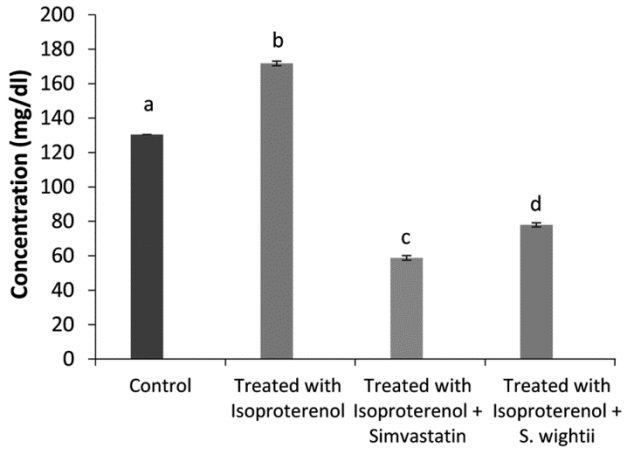

Fig. 1 — Effect of *Sargassum wightii* extract on serum triglyceride of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

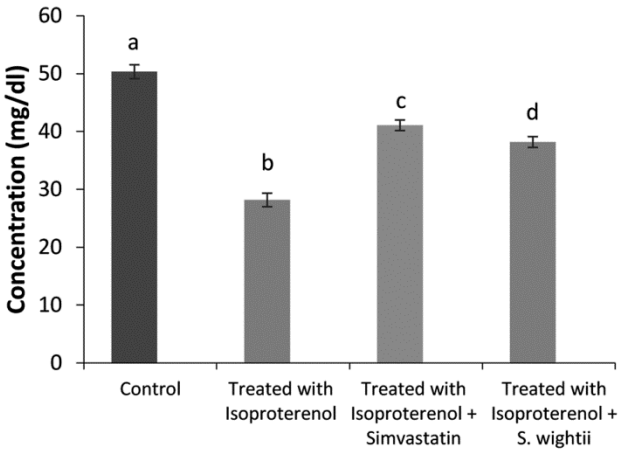

Fig. 3 — Effect of S*argassum wightii* extract on serum HDL-cholesterol of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

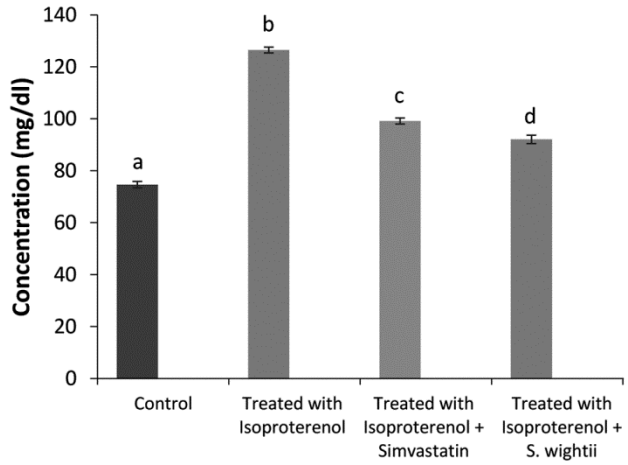

Fig. 2 — Effect of *Sargassum wightii* extract on serum total cholesterol of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

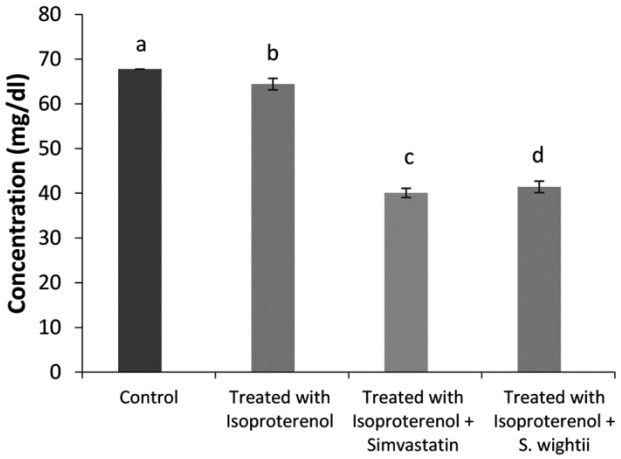

Fig. 4 — Effect of *Sargassum wightii* extract on serum LDL-cholesterol of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

profiles were reversed in Fucoidan (sulfated polysaccharides of brown algae) treated rats.

Effect of Sargassum wightii extract on cardiac marker enzymes

ALP, ALT, AST, and CK are a few of the cardiac marker enzymes whose activity has been investigated. Hepatic necrosis has been measured using serum ALT, which is known to rise in liver diseases[21]. The activity of these enzymes will be at higher levels in cases where tissue damage has occurred. In contrast to the isoproterenol exposed rats, the reversal of these enzyme activities in *S. wightii* treated rats is a clear indication of cardioprotective activity against myocardial stress (Figs. 5 – 9). Similar results were observed on isoproterenol exposed rats treated with Syringic acid where a reduction of enzyme activity was reported[22]. Likewise, in another study, a significant drop in cardiac markers[23] such as Creatinine kinase (CK), cardiac troponin-T (CTT), and lactate dehydrogenase (LD) was observed in Rhapontigenin (RPG) treated rats which was already exposed to Isoproterenol to induce myocardial stress[24]. The reduction in the enzyme activity in *S. wightii* treated rats was maybe due to the stabilizing effects of *S. wightii* on myocardial membrane and thus, protecting the membrane from enzyme leakage.

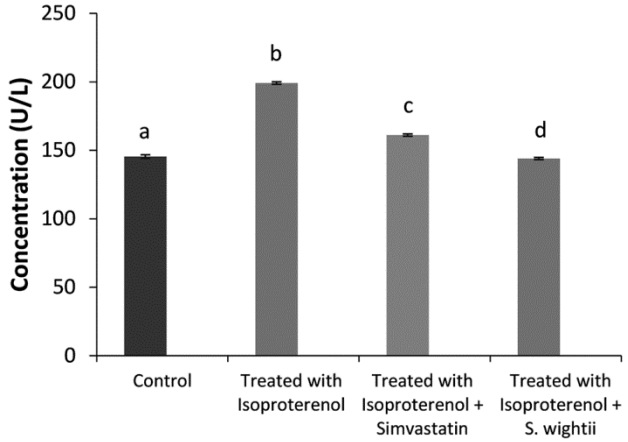

Fig. 5 — Effect of *Sargassum wightii* extract on serum ALP of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

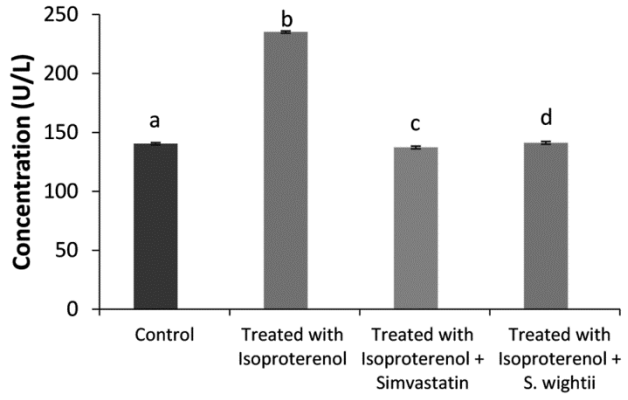

Fig. 6 — Effect of *Sargassum wightii* extract on serum CPK of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

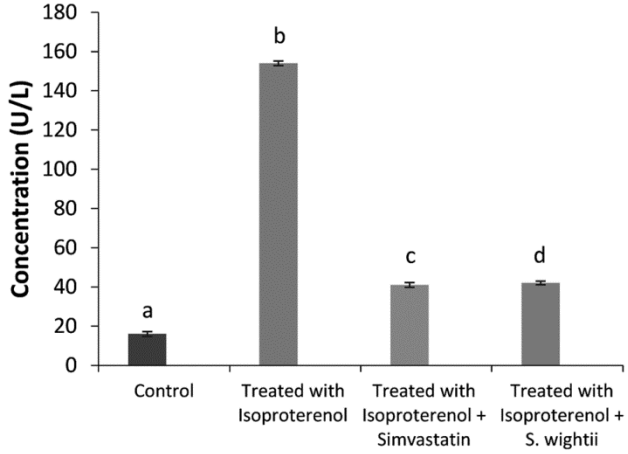

Fig. 7 — Effect of *Sargassum wightii* extract on serum AST of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

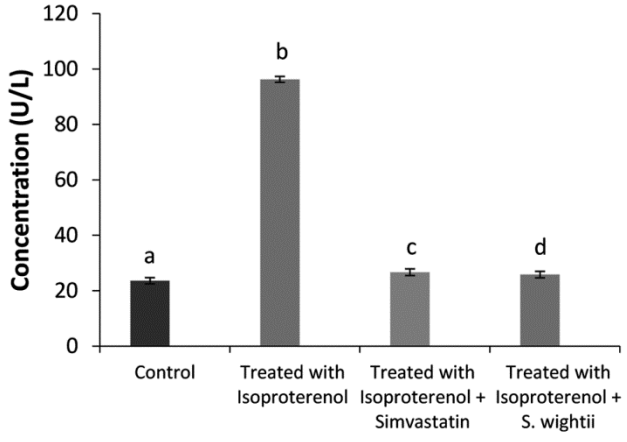

Fig. 8 — Effect of *Sargassum wightii* extract on serum ALT of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

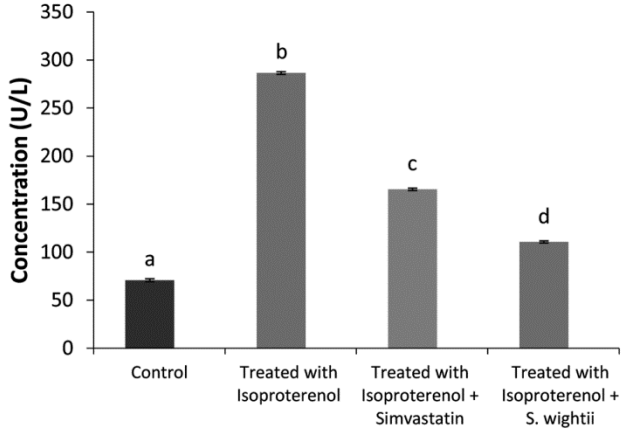

Fig. 9 — Effect of *Sargassum wightii* extract on serum LDH of Isoproterenol induced rats. Different font denotes the significant difference ($p < 0.05$)

Histopathology study

Isoproterenol-exposed animal's tissue samples demonstrated localized myocyte degeneration along with intense lymphocyte and macrophage infiltration. The rats that had standard exposure displayed a minor edema and a few scathed lymphocytes, but no myocyte degeneration was seen (Fig. 10). *Sargsassum wightii* exposure showed no pathology in the myocytes. Histopathological studies demonstrated that *S. wightii* (250 mg/kg) reversed the deterioration of normal cardiac structure caused by isoproterenol[21]. In another study conducted on isoproterenol exposed rats treated with agnucastoside C (glycoside isolated from the leaves of *Moringa oliefera*), intact myofibrilis was observed with no sign of enema and inflammation. This indicates the protective effects of

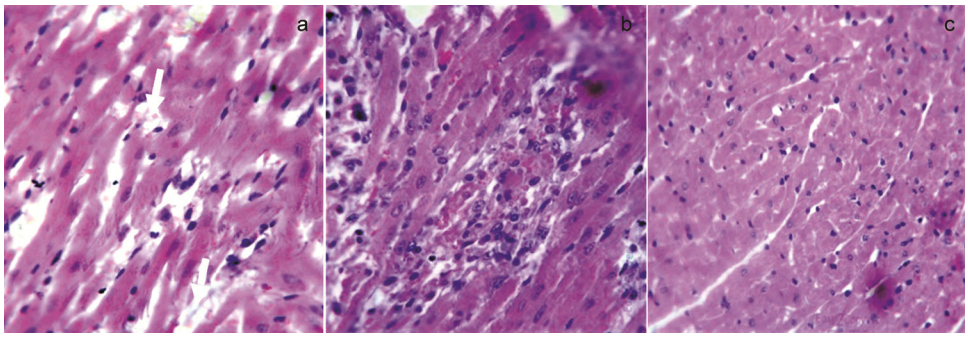

Fig. 10 — Hematoxylin & eosin staining of rat hearts (Magnification 40 X): a) Group II-Rats induced with Isoproterenol that serves as control, b) with Simvastatin and then induced with Isoproterenol, and c) *Sargassum wightii* for 21 days and then induced with Isoproterenol. White arrow denotes tissue degeneration

agnucastoside C against myocardial necrosis which can be due to its antioxidant effects[25]. As mentioned before, *S. wightii* is reported to possess antioxidant activity which could be a major factor in the prevention of pathology in myocytes[26-28]. As a result, these findings show that *S. wightii* may protect against isoproterenol-induced myocardial infarction.

Conclusion

In the present study, methanolic extracts of *S. wightii* was prepared and was given orally at a concentration on 250 mg/kg body weight to the animals in group IV in order to study their cardioprotective role against Isoproterenol induction. Isoproterenol was used to induce myocardial infarction at the concentration of 150 mg/kg body weight. The study on cardio-protective role of *S. wightii* was carried out for 21 days. Animals were orally given with Cardiotonic at concentration of 250 mg/kg on 20th and 21th day, Isoproterenol was given intraperitoneally. On 21th day, animals were sacrificed by cervical dislocation and the serum and cardiac tissue were collected. Alterations in cholesterol, triglycerides, HDL, and LDL levels were determined. The variations in activity of cardiac marker enzymes like ALT, AST, CK, LDH were analyzed. The histopathology studies were carried out to determine the pattern of inflammation in the heart tissue.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

All studies were carried out in strict accordance with the animal ethical committee approval (IAEC/KU/BT/14/08).

Author Contributions

RER: Conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, and manuscript preparation; SR: Conceived and designed the analysis, collected the data, contributed data analysis tools, and performed the analysis; PJJC, AVS, SD & SA: Conceived and designed the analysis, collected the data, and contributed in data or analysis tools; and PP: Conceived and designed the analysis, and contributed data or analysis tools.

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