

PHARMACOLOGICAL ACTIVITY OF *ULVA LACTUCA* POLYPHENOLS FRACTION: CARDIOPROTECTIVE AND ANTIOXIDANT ACTIVITIES AGAINST ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Objective: The objective of this study is to assess the activity of *Ulva lactuca* polyphenols fraction in protecting the myocardial infarction induced by a high dose of isoproterenol.

Methods: This study was carried out using Wistar albino rats divided into six groups. Group 1 was the normal group. Groups 2, 3, 4, 5, and 6 received isoproterenol (85 mg/kg) *i.p.* twice at an interval of 24 h on the 14th and 15th day. In addition to isoproterenol, Group 3 received verapamil (5 µmol/kg) as a single dose intravenously on the 14th day 10 min before isoproterenol and Groups 4, 5, and 6 received *U. lactuca* polyphenols fraction at the doses of 50, 100, and 200 mg/kg, respectively, for 14 days. On the 16th day, serum and heart samples were harvested from the animals and the cardioprotective and antioxidant activities were assessed by studying the levels of cardiac functional heart marker enzymes, lipid profile, reduced glutathione, and antioxidant enzymes.

Results: *U. lactuca* polyphenols fraction, at the tested doses, restored the levels of all serum markers and enzymes (aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase, lactate dehydrogenase, cholesterol, triglycerides, and reduced glutathione) and heart homogenate antioxidant markers (reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase) significantly, in dose-dependent manner.

Conclusion: This study suggests that *U. lactuca* polyphenols fraction has a cardioprotective effect against isoproterenol-induced myocardial infarction and possess antioxidant activities.

Keywords: Cardioprotective activity, Antioxidant activity, Isoproterenol, Marine alga, Edible alga, *Ulva lactuca*, Polyphenols fraction.

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INTRODUCTION

Cardiovascular diseases are presently one of the major causes of disability and mortality in both economically well-developed and developing countries [1]. Cardiovascular diseases are the major cause of 17.1 million fatalities each year and it will reach up to 20 million in 2020 [2]. Isoprenaline, a synthetic catecholamine has a lethal effect on the myocardium. Among the diverse mechanisms proposed to explain isoprenaline-induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important contributing factors [3].

At present, available cardioprotective drugs have been associated with a number of side effects and are very costly. Some safe and cost-effective medicinal plants have been indicated for their cardioprotective effects due to their chemical composition [4]. The medicines currently used to treat myocardial infarction have numerous side effects. Dietary factors play a crucial role in the development of different human diseases including cardiovascular diseases. Common conviction that herbal formulations are safer than modern drugs has led to grow the use of herbal preparations. The World Health Organization (WHO) estimates that 80% of the total world's population presently use medicines of herbal origin for primary health care [5]. Hence, the WHO has recommended the use of herbal medicines as an alternative medicine, especially in developing countries [6].

Since the 1970s, more than 21,855 structurally different, bioactive natural products with an astonishing array of biological activities have been discovered from marine microbes, algae, and invertebrates [7].

Although the ocean covers more than 70% of the earth's surface, we only utilize less than 10% of the total ocean area [8]. In particular, many marine organisms survive in complex habitats exposed to extreme conditions and in adapting to new environmental surroundings, they generate a wide variety of secondary metabolites which cannot be found in other organisms. Moreover, in view of its great taxonomic diversity, investigations related to the search for new bioactive compounds from the marine environment can be viewed as an almost limitless field. In addition, since the biological productivity of terrestrial ecosystems has also perhaps reached what it can achieve; the marine biodiversity of the ocean can be anticipated to yield new therapeutic agents [9].

The green marine alga *Ulva lactuca*, commonly known as sea lettuce, has long been used as food and as a traditional medicinal agent to treat helminthic infections, fever, urinary diseases, and dropsy [10]. *U. lactuca* is rich in flavonoids and has potent antioxidant properties [11]. The hepatoprotective nature of *U. lactuca* against liver injury induced by D-galactosamine/endotoxin in rats has been established [12]. *U. lactuca* has also been shown to have potent hypocholesterolemic and antioxidant effects [13], antibacterial activity [14], nephroprotective activity [15], neuroprotective activity [16], alpha-amylase and alpha-glucosidase inhibitory activity [17], and hepatoprotective and antioxidant activities [18].

In view of these reports, the present investigation has been undertaken to evaluate the cardioprotective and antioxidant activities of the polyphenols fraction of *U. lactuca* against isoproterenol-induced myocardial infarction in rats. Marine alga *U. lactuca* was selected for the present study as it is an edible marine alga, easily available in the Gulf of Mannar Coast of Mandapam.

METHODS

Collection of marine algae sample

Fresh marine alga *U. lactuca* was collected from the intertidal regions of the Mandapam coast of Gulf of Mannar. The taxonomic identification of species was done using standard literature and taxonomic keys. The alga was identified and authenticated by the Principal Scientist, Central Salt and Marine Chemical Research Institute, Mandapam Camp. The collected samples were cleaned well with the seawater until unnecessary impurities, adhering sand particles, and extraneous matter such as epiphytes, pebbles, and shells were removed and it was brought to the laboratory in sterile plastic bags containing seawater to prevent evaporation. It was subsequently washed thoroughly with tap water and distilled water to remove the surface salty materials. It was air-dried for 1 week and later ground in an electric mixer. The powdered samples were subsequently stored in the refrigerator for future use.

Extraction of polyphenols fraction from *U. lactuca*

The powdered samples were then extracted with 80% ethanol for 24 h under continuous shake at 20°C. The extracts were then concentrated in a rotary evaporator under reduced pressure at 40°C [19]. The solid mass obtained was stored at 4°C. At the time of administration to the rats, the polyphenols fraction was dissolved in distilled water to required concentration.

Experimental animals

Male Wistar albino rats weighing 150–200 g were used in the study. The animals were maintained in well ventilated room with 12:12 light/dark cycle, 24±2°C temperature, and 30–70% relative humidity, in polypropylene cages. Standard rat rodent pellets (M/s. Hindustan Lever Ltd., Mumbai) and water were provided *ad libitum*. Animals were acclimatized to the laboratory conditions 1 week before the initiation of the study. The study was approved by the Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA (IAEC/APCAS/01/2015/01).

Acute oral toxicity performed according to OECD-423 guidelines [20], has already been referred to in our earlier study [20]. *U. lactuca* polyphenols fraction did not produce any mortality even at the highest dose (2000 mg/kg) employed, and hence, *U. lactuca* polyphenols fraction was considered to be safe for further pharmacological screening. Three submaximal doses (50, 100, and 200 mg/kg) were employed for further pharmacological investigations.

Induction of myocardial infarction

Myocardial infarction was induced in rats by dissolving isoproterenol in normal saline and injecting intraperitoneally to rats (85 mg/kg body weight) at an interval of 24 h for 2 days [21].

Experimental design

Male Wistar albino rats (male) weighing between 150 and 200 g were divided into six groups of six animals each. The weight range of the animals was equally distributed throughout the groups.

- Group 1: Control rats received distilled water orally for 7 days.
- Group 2: Treated with isoproterenol (85 mg/kg) i.p. twice at an interval of 24 h on the 14th and 15th day.
- Group 3: Treated with verapamil (5 µmol/kg) as a single dose intravenously on the 14th day, followed after 10 min by isoproterenol (85 mg/kg) i.p. twice at an interval of 24 h on the 14th and 15th day.
- Group 4: Treated with *U. lactuca* polyphenols fraction (50 mg/kg) dissolved in water for 14 days, followed by isoproterenol (85 mg/kg) i.p. twice at an interval of 24 h on the 14th and 15th day.
- Group 5: Treated with *U. lactuca* polyphenols fraction (100 mg/kg) dissolved in water for 14 days, followed by isoproterenol (85 mg/kg) i.p. twice at an interval of 24 h on the 14th and 15th day.
- Group 6: Treated with *U. lactuca* polyphenols fraction (200 mg/kg) dissolved in water for 14 days, followed by isoproterenol (85 mg/kg) i.p. twice at an interval of 24 h on the 14th and 15th day.

Animals were kept starved overnight on the 15th day. The next day, all the animals were sacrificed under light ether anesthesia. Blood was collected by direct cardiac puncture into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and subjected to various biochemical estimations. Hundred milligram of heart tissue from each rat was used for antioxidant study.

Biochemical estimations

The separated sera were subjected to biochemical estimation of different parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) [22], creatine phosphokinase (CPK) [23], lactate dehydrogenase (LDH) [24], cholesterol [25], triglycerides [26], and reduced glutathione [27].

The antioxidant status

One hundred milligram of heart tissue was weighed and homogenate was prepared in 10 ml Tris hydrochloric acid buffer (0.5 M; pH 7.4) at 4°C. The homogenate was centrifuged and the supernatant was used for the estimation of reduced glutathione [27] and assay of antioxidant enzymes such as superoxide dismutase [28], catalase [29], and glutathione peroxidase [30].

Statistical analysis

One-way analysis of variance followed by Dunnett's t-test was applied for determining the statistical significance of difference between experimental groups. $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction and once metabolically damaged, it releases its contents into the extracellular fluid [31]. In our study, isoproterenol is used to induce myocardial damage. It is a synthetic catecholamine and beta-adrenergic agonist, and has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle cells and is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane in experimental myocardial infarction [32].

The rats were pre-treated with *U. lactuca* polyphenols fraction for 14 days before isoproterenol administration. Estimating the activities of serum marker enzymes such as AST, ALT, CPK, and LDH are a tool for the assessment of the cardiac function. Isoproterenol is well known cardiotoxic agent due to its ability to destruct myocardial cells. As a result of this, cytosolic enzymes such as LDH, transaminases (ALT and AST), and CPK were released into the bloodstream and serve as diagnostic markers of myocardial tissue damage [33]. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability.

There was a significant increase ($p < 0.01$) in the levels of AST, ALT, CPK, LDH, total cholesterol, and triglycerides as well as a significant decrease ($p < 0.01$) in plasma reduced glutathione in isoproterenol-treated animals from those of the control group. Administration of *U. lactuca* polyphenols fraction (50, 100, and 200 mg/kg) brought down the levels of AST, ALT, CPK, LDH, total cholesterol, and triglycerides to near-normal levels, in a dose-dependent manner ($p < 0.01$). The levels of plasma reduced glutathione were increased significantly by the administration of *U. lactuca* polyphenols fraction in a dose-dependent manner. Verapamil, the reference drug restored the altered levels of enzymes significantly ($p < 0.01$) (Table 1).

Increased activities of CPK, transaminases, and LDH in serum, "the diagnostic markers," were due to the leakage of these enzymes as a result of necrosis induced by isoproterenol (β -adrenergic stimulant) in rats [34]. CPK is a muscle-specific enzyme mainly for heart and brain; therefore, its increase in serum is the result of myocarditis, cardiac insufficiency, arrhythmias, and myocardial infarction [35].

Table 1: Effect of *Ulva lactuca* polyphenols fraction on serum marker enzymes and reduced glutathione

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AST (IU/L)	83.50±3.48	221.33±9.39 ^{a*}	88.50±4.07 ^{b*}	105.67±4.87 ^{b*}	98.33±4.68 ^{b*}	89.33±4.24 ^{b*}
ALT (IU/L)	47.50±2.32	110.50±5.21 ^{a*}	51.50±2.55 ^{b*}	67.50±3.17 ^{b*}	62.83±2.50 ^{b*}	57.17±2.18 ^{b*}
CPK (IU/L)	189.33±6.60	381.83±12.28 ^{a*}	190.17±6.70 ^{b*}	245.33±10.25 ^{b*}	228.17±9.76 ^{b*}	207.50±8.98 ^{b*}
LDH (IU/L)	112.00±5.94	196.67±9.48 ^{a*}	117.17±5.38 ^{b*}	143.17±6.70 ^{b*}	133.17±6.27 ^{b*}	121.17±5.25 ^{b*}
Total cholesterol (g/dL)	97.00±4.70	162.00±7.34 ^{a*}	101.83±4.82 ^{b*}	126.00±5.83 ^{b*}	117.17±5.25 ^{b*}	106.50±4.98 ^{b*}
Triglycerides (g/dL)	91.00±3.55	196.50±8.87 ^{a*}	96.50±4.96 ^{b*}	116.33±5.61 ^{b*}	108.17±5.33 ^{b*}	98.33±4.58 ^{b*}
Reduced glutathione (mg/dL)	30.12±1.43	17.43±0.78 ^{a*}	29.33±1.41 ^{b*}	22.87±1.09 ^{b*}	24.58±1.31 ^{b*}	27.32±1.32 ^{b*}

Group A – Control, Group B – ISO, Group C – ISO+Verapamil, Group D – ISO+ULPF 50 mg/kg, Group E – ISO+ULPF 100 mg/kg, Group F – ISO+ULPF 200 mg/kg. ISO: Isoproterenol, ULPF: *Ulva lactuca* polyphenols fraction. The observations are given as Mean±SEM, n=6; *p<0.01, a: Group I versus Group II, b: Group II versus Groups III, IV, V, and VI, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CPK: Creatine phosphokinase, LDH: Lactate dehydrogenase

Table 2: Effect of *Ulva lactuca* polyphenols fraction on heart homogenate reduced glutathione and antioxidant enzymes

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Heart reduced glutathione (mg/100 g wet tissue)	47.18±2.34	22.87±1.10 ^{a*}	46.05±2.27 ^{b*}	36.30±1.65 ^{b*}	39.02±1.87 ^{b*}	43.35±1.99 ^{b*}
¹ Superoxide dismutase	8.42±0.49	4.25±0.33 ^{a*}	8.20±0.43 ^{b*}	6.70±0.33 ^{b*}	7.20±0.35 ^{b*}	8.00±0.41 ^{b*}
² Catalase	61.85±2.66	32.52±1.76 ^{a*}	59.37±2.59 ^{b*}	47.27±1.98 ^{b*}	50.82±2.35 ^{b*}	56.45±2.58 ^{b*}
³ Glutathione peroxidase	8.45±0.39	3.92±0.28 ^{a*}	8.04±0.35 ^{b*}	6.18±0.28 ^{b*}	6.65±0.32 ^{b*}	7.38±0.36 ^{b*}

Group A – Control, Group B – ISO, Group C – ISO+Verapamil, Group D – ISO+ULPF 50 mg/kg, Group E – ISO+ULPF 100 mg/kg, Group F – ISO+ULPF 200 mg/kg, ISO: Isoproterenol, ULPF: *Ulva lactuca* polyphenols fraction, ¹Superoxide dismutase is expressed as 50% inhibition of epinephrine auto-oxidation/min/mg protein. ²Catalase is expressed as μ moles of H₂O₂ decomposed/min/mg protein. ³Glutathione peroxidase is expressed as μ moles of glutathione oxidized/min/mg protein. The observations are given as Mean±SEM, n=6; *p<0.01, a: Group I versus Group II, b: Group II versus Groups III, IV, V, and VI

Reduced activities of the antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase, observed in the heart homogenate of isoproterenol-treated rats, indicate the extensive myocardial damage induced by the cardiotoxin (p<0.01). The tendency of these enzymes to return to near normalcy in *U. lactuca* polyphenols fraction administered groups (50, 100, and 200 mg/kg) in a dose-dependent manner is a clear indication of cardioprotective effect of *U. lactuca* polyphenols fraction extract through antioxidant mechanism (p<0.01) (Table 2).

Experimental and epidemiological studies indicate the involvement of oxidative stress in the pathogenesis and progression of many chronic diseases [36]. It is known that oxygen, essential for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS may activate a host of disorders in the body resulting in tissue damage and necrosis in many instances [37]. The body has an effective mechanism to avoid and neutralize the free radical-induced damage. This is done through a set of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. When the equilibrium between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events, deregulate the cellular functions, leading to various pathological conditions [38].

It is possible that natural antioxidants strengthen the endogenous antioxidant defense from ROS damage and restore the optimal equilibrium by neutralizing the reactive species. They are gaining enormous importance by virtue of their critical role in disease prevention.

CONCLUSION

It may be said that the polyphenols fraction of *U. lactuca*, commonly used as a food and as a traditional medicinal agent, has exhibited a cardioprotective effect against isoproterenol-induced cardiotoxicity and possessed antioxidant activities. *U. lactuca* is seaweed, but it is proven to possess immense potential by this study. Efforts are underway to isolate and purify the active principle involved in the cardioprotective efficacy of this marine alga.

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AUTHORS' CONTRIBUTIONS

Ravindran N T designed study project and performed experiments. He was also engaged in manuscript editing and finalization. Mohamed Sadiq A designed study project, contributed to experiment finalization and implementation, manuscript editing, and finalization.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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