

## PHARMACOLOGICAL ACTIVITY OF *ULVA LACTUCA* POLYPHENOLS FRACTION: HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES AGAINST PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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### ABSTRACT

**Objective:** The objective of this study was to assess the activity *Ulva lactuca* polyphenols fraction in protecting the liver damage induced by high dose of paracetamol.

**Methods:** This study was performed using Wistar albino rats divided into six groups. Group 1 was the normal group. Groups 2, 3, 4, 5, and 6 received paracetamol (2 g/kg) for 7 days. In addition to paracetamol, Groups 3, 4, 5, and 6 received silymarin (100 mg/kg), *U. lactuca* polyphenols fraction at the doses of 50, 100, and 200 mg/kg, respectively, for 7 days. On the 8<sup>th</sup> day, serum and liver samples were collected from the animals and the hepatoprotective and antioxidant activities were assessed by studying the levels of liver marker enzymes, bilirubin, protein, 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, alkaline phosphatase, lactate dehydrogenase,  $\gamma$ -glutamyl transferase, total bilirubin, total protein, cholesterol, triglycerides, and reduced glutathione) and liver homogenate 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 hepatoprotective effect against paracetamol-induced liver damage and possesses antioxidant activities.

**Keywords:** Hepatoprotective activity, Antioxidant activity, Paracetamol, Marine alga, Edible alga, *Ulva lactuca*, Polyphenols fraction.

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### INTRODUCTION

Liver is an essential organ which is involved in detoxification of the exogenous xenobiotics, drugs and endogenous bile pigments, viral infections, and chronic alcoholism. While performing several detoxifications, liver is subjected to stress, leading to liver diseases resulting in liver damage and serious health problems and death. The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions [1]. Liver damage is a common pathology, which in most cases, involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [2]. As oxidative stress plays a central role in liver pathologies and their development, the use of antioxidants has been proposed as therapeutic agents to counteract liver damage.

In spite of advancements in modern medicine, hepatoprotective drugs are quite limited. The extended usage of remedies available in modern medicine is associated with severe side effects. The development or detection of new molecules effective in treating or preventing hepatic damage remains a challenge in the field of drug development [3]. It is generally recognized that indigenous drugs used traditionally by ethnic tribes or societies across the world can provide relief to patients with hepatic disorders. As a result, a conscious effort is undertaken to screen indigenous drugs used traditionally in different regions of the world, especially India and China. Plant-based drugs exhibiting hepatoprotective and antioxidant activities have been isolated from many species. A well-known potential hepatoprotective drug is, silymarin, isolated from *Silybum marianum*.

In recent times, the pharmaceutical and agri-food industries have been responsible for a great expansion in the demand for marine alga due to their significant applications as ingredients in functional foods and richness in antioxidant ingredients. It has been reported that marine alga are rich source of bioactive compounds such as terpenoids, phlorotannins, fucoidans, sterols and glycolipids, and the extracts or isolated pure components from marine alga possess a wide range of pharmacological properties such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, anticoagulant, antioxidant, hypoglycemic, hypolipidemic, antimelanogenic, hepatoprotective, and neuroprotective activities [4,5]. Apart from these, marine alga are also a rich resource of dietary iodine and fibers which can also play a major role in improving the nutritional quality [6].

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 drowsy [7]. *U. lactuca* is rich in flavonoids and has potent antioxidant properties [8]. The hepatoprotective nature of *U. lactuca* against liver injury induced by D-galactosamine/endotoxin in rats has been established [9]. *U. lactuca* has also been shown to have potent hypocholesterolemic and antioxidant effects [10], antibacterial activity [11], nephroprotective activity [12], neuroprotective activity [13], and alpha-amylase and alpha-glucosidase inhibitory activity [14].

In view of these reports, the present investigation has been undertaken to evaluate the hepatoprotective and antioxidant activities of the polyphenols fraction of *U. lactuca* against paracetamol intoxicated liver damage in rat. 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.

## MATERIALS AND METHODS

### Collection of marine alga 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 then 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 [15]. 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 Swiss albino mice weighing 20–25 g and male Wistar albino rats weighing 150–200 g were used for the study. The animals were maintained in well-ventilated rooms 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 toxicity studies

Acute oral toxicity was performed according to OECD-423 guidelines [16]. Male Swiss albino mice weighing 20–25 g selected by random sampling technique were used in the study. The animals were fasted overnight, provided only water after which polyphenols fraction was administered to the groups (3 mice/group) orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was considered as a toxic dose. However, if mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If no mortality was observed, then higher (50, 300, and 2000 mg/kg) doses of polyphenol fraction were employed for further toxicity studies. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions, and mortality.

### Induction of hepatic damage

Liver damage was induced in rats by paracetamol (acetaminophen) suspended in 0.5% Tween-80 and administered p.o., at a dose of 2 g/kg body weight.

### 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 paracetamol (2 g/kg) for 7 days.
- Group 3: Treated with paracetamol (2 g/kg) and silymarin (100 mg/kg) dissolved in water for 7 days.
- Group 4: Treated with paracetamol (2 g/kg) and *U. lactuca* polyphenols fraction (50 mg/kg) dissolved in water for 7 days.

- Group 5: Treated with paracetamol (2 g/kg) and *U. lactuca* polyphenols fraction (100 mg/kg) dissolved in water for 7 days.
- Group 6: Treated with paracetamol (2 g/kg) and *U. lactuca* polyphenols fraction (200 mg/kg) dissolved in water for 7 days.

Animals were kept starved overnight on the 7<sup>th</sup> 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. 100 mg of liver issue from each rat was used for antioxidant study.

### Biochemical estimations

The separated serum was subjected to biochemical estimation of different parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) [17], alkaline phosphatase (ALP) [18], lactate dehydrogenase (LDH) [19],  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) [20], total bilirubin [21], total protein [22], cholesterol [23], triglycerides [24], and reduced glutathione [25].

### The antioxidant status

One hundred milligram of liver 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 [25] and assay of antioxidant enzymes such as superoxide dismutase [26], catalase [27], and glutathione peroxidase [28].

### 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

All the doses (5, 50, 300, and 2000 mg/kg) of *U. lactuca* polyphenols fraction tested for acute oral toxicity studies were found to be non-toxic. According to the OECD-423 guidelines for acute oral toxicity, the LD<sub>50</sub> dose of 2000 mg/kg and above is categorized as unclassified. *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.

Liver is the largest organ and is a target for toxicity due to its role in clearing and metabolizing chemicals through the process of detoxification [29]. Drug-induced liver disorders occurring frequently can be life threatening and mimic all forms of liver diseases [30]. Paracetamol is also a drug which is capable of causing liver disorders, if it is given continuously.

The paracetamol-induced liver disorders were treated with *U. lactuca* polyphenols fraction for 7 days. Estimating the activities of serum marker enzymes such as AST, ALT, ALP,  $\gamma$ -GT, and LDH is a tool for the assessment of the liver function. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream. Their estimation is a useful quantitative marker of the extent and type of hepatocellular damage identification [31]. An obvious sign of hepatic injury is the leakage of cellular enzymes such as AST, ALT, and ALP [32].

There was a significant increase ( $p < 0.01$ ) in the levels of AST, ALT, ALP, LDH,  $\gamma$ -GT, total bilirubin, total cholesterol, and triglycerides and a significant decrease ( $p < 0.01$ ) in total protein and plasma reduced glutathione in paracetamol-treated animals from those of the control group. Administration of *U. lactuca* polyphenols fraction (50, 100, and 200 mg/kg) decreased the reduced levels of AST, ALT, ALP, LDH,  $\gamma$ -GT, total bilirubin, total cholesterol, and triglycerides in a dose-dependent manner ( $p < 0.01$ ). The levels of total protein and plasma reduced

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

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
ALT (IU/L)	48.50±2.09	161.00±7.26 <sup>a*</sup>	52.17±1.92 <sup>b*</sup>	65.00±3.07 <sup>b*</sup>	58.00±2.74 <sup>b*</sup>	52.83±2.49 <sup>b*</sup>
AST (IU/L)	82.00±3.71	213.67±10.45 <sup>a*</sup>	84.83±4.13 <sup>b*</sup>	111.83±5.37 <sup>b*</sup>	99.83±4.79 <sup>b*</sup>	90.83±4.25 <sup>b*</sup>
ALP (IU/L)	135.00±6.60	291.33±11.08 <sup>a*</sup>	139.50±6.61 <sup>b*</sup>	181.83±9.52 <sup>b*</sup>	162.33±8.50 <sup>b*</sup>	147.50±7.23 <sup>b*</sup>
LDH (IU/L)	110.33±5.74	182.17±8.69 <sup>a*</sup>	112.67±5.36 <sup>b*</sup>	140.00±7.30 <sup>b*</sup>	125.00±6.52 <sup>b*</sup>	113.67±5.86 <sup>b*</sup>
γ-GT (IU/L)	3.18±0.15	6.58±0.31 <sup>a*</sup>	3.25±0.17 <sup>b*</sup>	4.17±0.31 <sup>b*</sup>	3.72±0.28 <sup>b*</sup>	3.38±0.18 <sup>b*</sup>
Total bilirubin (mg/dL)	0.78±0.04	2.48±0.13 <sup>a*</sup>	0.80±0.04 <sup>b*</sup>	1.02±0.06 <sup>b*</sup>	0.90±0.05 <sup>b*</sup>	0.82±0.05 <sup>b*</sup>
Total protein (g/dL)	7.33±0.35	5.03±0.26 <sup>a*</sup>	7.25±0.30 <sup>b*</sup>	5.80±0.29 <sup>b*</sup>	6.50±0.33 <sup>b*</sup>	7.23±0.39 <sup>b*</sup>
Total cholesterol (g/dL)	96.33±4.33	200.17±8.98 <sup>a*</sup>	101.33±5.22 <sup>b*</sup>	130.50±5.73 <sup>b*</sup>	116.50±5.12 <sup>b*</sup>	105.83±4.37 <sup>b*</sup>
Triglycerides (g/dL)	86.33±4.09	286.50±10.16 <sup>a*</sup>	89.00±4.55 <sup>b*</sup>	115.00±6.19 <sup>b*</sup>	102.67±5.53 <sup>b*</sup>	93.33±4.78 <sup>b*</sup>
Reduced glutathione (mg/dL)	32.00±1.31	17.50±0.98 <sup>a*</sup>	30.83±1.21 <sup>b*</sup>	22.67±0.99 <sup>b*</sup>	25.50±1.10 <sup>b*</sup>	28.33±1.22 <sup>b*</sup>

Group I – Control; Group II – PCM; Group III – PCM+Silymarin; Group IV – PCM+ULPF 50 mg/kg; Group V – PCM+ULPF 100 mg/kg; Group VI – PCM+ULPF 200 mg/kg. The observations are given as Mean±SEM, n=6; \*p<0.01; <sup>a</sup>Group I versus Group II; <sup>b</sup>Group II versus Groups III, IV, V, and VI. PCM: Paracetamol; ULPF: *Ulva lactuca* polyphenols fraction, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, γ-GT: γ-Glutamyl transferase

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

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Liver reduced glutathione (mg/100 g wet tissue)	48.50±2.52	21.00±1.10 <sup>a*</sup>	47.50±2.13 <sup>b*</sup>	35.17±1.94 <sup>b*</sup>	39.33±2.17 <sup>b*</sup>	43.67±2.14 <sup>b*</sup>
1Superoxide dismutase	8.25±0.45	4.30±0.23 <sup>a*</sup>	8.02±0.46 <sup>b*</sup>	6.18±0.39 <sup>b*</sup>	6.93±0.43 <sup>b*</sup>	7.70±0.24 <sup>b*</sup>
2Catalase	57.50±2.20	24.83±1.24 <sup>a*</sup>	55.50±2.92 <sup>b*</sup>	41.17±1.89 <sup>b*</sup>	46.17±2.12 <sup>b*</sup>	51.33±2.45 <sup>b*</sup>
3Glutathione peroxidase	8.73±0.41	4.35±0.24 <sup>a*</sup>	8.42±0.39 <sup>b*</sup>	6.12±0.38 <sup>b*</sup>	6.85±0.43 <sup>b*</sup>	7.62±0.38 <sup>b*</sup>

Group I – Control; Group II – PCM; Group III – PCM+Silymarin; Group IV – PCM+ULPF 50 mg/kg; Group V – PCM+ULPF 100 mg/kg; Group VI – PCM+ULPF 200 mg/kg. 1Superoxide dismutase is expressed as 50% inhibition of epinephrine autoxidation/min/mg protein. 2Catalase is expressed as μmoles of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein. 3Glutathione peroxidase is expressed as μmoles of glutathione oxidized/min/mg protein. The observations are given as Mean±SEM, n=6; \*p<0.01; <sup>a</sup>Group I versus Group II; <sup>b</sup>Group II versus Groups III, IV, V, and VI. PCM: Paracetamol; ULPF: *Ulva lactuca* polyphenols fraction

glutathione were increased significantly by the administration of *U. lactuca* polyphenols fraction in a dose-dependent manner. Silymarin, the reference drug restored the altered levels of enzymes significantly (p<0.01) (Table 1).

Hypoalbuminemia is very common in advanced chronic liver diseases. Hence, decrease in total protein content can be considered as a useful index of the severity of cellular dysfunction in chronic liver diseases. The decreased level of total proteins recorded in the serum of paracetamol-treated rats suggests the severity of hepatotoxicity. The restoration of near normalcy in total protein content of the serum of *U. lactuca* polyphenols fraction treated rats further elucidates the hepatoprotective effect of *U. lactuca* polyphenols fraction.

Decreased activities of the antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase observed in the liver homogenate of paracetamol-treated rats indicate the extensive liver damage induced by the hepatotoxin (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 hepatoprotective 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 [33]. 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 [34]. The body has an effective mechanism to prevent and neutralize the free radical-induced damage. This is achieved by 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 [35].

It is probable 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 can be said that *U. lactuca* polyphenols fraction has exhibited a liver protective effect against paracetamol-induced hepatotoxicity and possessed antioxidant activities. Thus, *U. lactuca*, commonly used as a food and as a traditional medicinal agent, is found to have hepatoprotective effect and antioxidant activities. *U. lactuca* is a seaweed, but it is proven to possess immense potential by this study. Efforts are in progress to isolate and purify the active principle involved in the hepatoprotective efficacy of this marine alga.

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## AUTHORS' CONTRIBUTIONS

Ravindran NT designed study project and performed experiments. He was also involved in manuscript editing and finalization. Mohamed Sadiq A designed study project, contributed in 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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