Hepatoprotective and Antioxidant Potential of the Sulphated Polysaccharides fraction of *Turbinaria ornata* against paracetamol-induced liver damage in rats

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ABSTRACT

Aims: To assess the potential of the sulphated polysaccharides fraction from brown marine alga *Turbinaria ornata* in protecting the liver damage induced by high dose of paracetamol.
Study design: In the present work, liver damage was induced in rats with paracetamol. Simultaneously other groups of rats were given standard drug or *Turbinaria ornata* sulphated polysaccharides fraction. From the rats' serum, parameters like alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase, total bilirubin, total protein, cholesterol, triglycerides and reduced glutathione were determined; and from the liver homogenate, reduced glutathione and antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase were determined for the evaluation of the hepatoprotective and antioxidant potential of the sulphated polysaccharides fraction from *Turbinaria ornata*.
Place and Duration of Study: Department of Biochemistry, Adhiparasakthi College of Arts and Science (Autonomous), G.B. Nagar, Kalavai – 632 506, Tamil Nadu, India.
Methodology: This study was performed using Wistar albino rats divided into six groups. Group 1 Control group. Groups 2, 3, 4, 5, and 6 received paracetamol (2 g/kg) for 7 days. In addition to paracetamol, group 3 received silymarin (100 mg/kg), groups 4, 5 and 6 received *Turbinaria ornata* sulphated polysaccharides fraction at the doses of 50, 100 and 200 mg/kg respectively for 7 days. On the 8th 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: *Turbinaria ornata* sulphated polysaccharides fraction, at the tested doses, restored the levels of all serum markers and enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase, total bilirubin, total protein, cholesterol, triglycerides and reduced glutathione) as well as liver homogenate antioxidant markers (reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase) significantly, in dose-dependent manner.
Conclusion: This study suggests that the *Turbinaria ornata* sulphated polysaccharides fraction has a hepatoprotective effect against paracetamol-induced liver damage and possess antioxidant activities.

Keywords: Hepatoprotective activity, Antioxidant activity, Paracetamol, Marine alga, Turbinaria ornata, Sulpahted Polysaccharides fraction

1. INTRODUCTION

The liver is one of the vital organs and largest gland of the human body located in right hypochondriac region below the diaphragm, in the the thoracic region of the abdomen. It is the key organ controlling the regulation of homeostasis in the body, and is involved with

almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The liver is also involved in a broad range of functions including protein synthesis and production of biochemicals required for digestion and metabolism. The liver plays a major role in metabolism and has numerous functions in the body, including glycogen storage, plasma protein synthesis and detoxification. It produces bile, an alkaline compound, which aids in digestion, *via* the emulsification of lipids. It also executes and regulates many biochemical reactions requiring highly specialized tissues, including the synthesis and breakdown of small and complex molecules, many of which are essential for normal vital functions [2].

An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma [3]. Paracetamol (PCM), also known as acetaminophen (APAP), is widely used as an analgesic and antipyretic throughout the world. It is used for different diseases such as headache, muscle pain, tooth pain, arthritis, common cold, fever, and menstrual pain. When taken in amounts higher than the therapeutic doses, it leads to elevation of serum ALT and AST, a clear indication of liver injury [4]. The injury starts with the production of NAPQI, an intermediate molecule, *i.e.,* paracetamol gets metabolized in the liver to an active metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by the cytochrome-P_{450} microsomal enzyme system. NAPQI is detoxified by glutathione (GSH) to form an APAP-GSH conjugate.

After a toxic dose of paracetamol, total hepatic GSH is depleted by as much as 90% and as a result the metabolite covalently binds to cysteine groups on protein, forming paracetamol-protein adducts [5]. So, in cases of overdose, it saturates GSH and starts to bind with mitochondrial proteins and leads to reduced ATP production, mitochondrial permeability transition and increased reactive oxygen species (ROS) production followed by acute liver failure [6]. In paracetamol overdose, APAP-Cys adduct formation was found to be high in both mice and humans [7].

Turbinaria ornata is a tropical brown alga of the order Fucales native to coral reef ecosystems of the South Pacific. Fucoidans were detected in *T. ornata*, collected from the Maldives [8]. Glucosamine, a sulphated fucan-like polysaccharide with amino sugar was isolated from the ethanolic extract of *T. ornata* from coasts of Tahiti [9]. The methanol extract of *T. ornata* collected from Gulf of Mannar (India), was detected to have phenolic content [10].

Therefore an attempt was made to assess the hepatoprotective and antioxidant potential of the sulphated polysaccharides fraction of brown marine alga *Turbinaria ornata* against paracetamol-induced liver damage in rats. In the present work, the Sulphated Polysaccharides fraction from *Turbinaria ornata* (TOSP) was used for this study at the dose of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight. AST, ALT, ALP, γ-GT, LDH, bilirubin, total protein, cholesterol and triglycerides (all in serum), total GSH (in plasma and liver tissue homogenate), SOD, CAT and GPx (in liver tissue homogenate) were successfully used for the evaluation of the hepatoprotective activity of the sulphated polysaccharides fraction from *Turbinaria ornata,* and the results are presented here.

2. MATERIAL AND METHODS

2.1 Chemicals

Paracetamol was purchased from M/s. CIPLA Ltd., Judikalan, Baddi, Himachal Pradesh; Silymarin was obtained from M/s. Micro Labs Ltd., Katha, Baddi, Himachal Pradesh; the rest of the chemicals and biochemicals utilized were obtained from local firms and were of analytical grade. Water was distilled prior to use.

2.2 Collection and Extraction of Sulphated Polysaccharides fractions from *Turbinaria ornata*

Fresh and healthy specimens of *Turbinaria ornata*, a brown marine alga, belonging to Sagassaceae family (Phaeophyceae class) were collected from the intertidal regions of the Mandapam coast of Gulf of Mannar. The collected samples were cleaned well with the seawater until unnecessary impurities, adhering sand particles, extraneous matter like epiphytes, pebbles and shells were removed and they were brought to the laboratory in sterile plastic bags containing sea water in order to prevent evaporation. Then they were washed thoroughly with tap water and distilled water to remove the surface salty materials. They were air dried for 1 week and later ground in an electric mixer. The powdered samples were subsequently stored in the refrigerator for future use.

2.2.1 Extraction of Sulphated Polysaccharides fraction from *Turbinaria ornata*

The powdered samples were depigmented with acetone for 24 h and then the sulphated polysaccharides fraction was extracted by an optimized single-step extraction procedure [11]. The depigmented sample was extracted in 0.03 M HCl with continuous stirring at 200 rpm for 4 h at 90 °C water bath. The suspended sample was filtered, and the extract was precipitated using 60% ethanol, the precipitate collected after centrifugation at 10,000 rpm for 10 min, and the resulting pellet was dried. This dried pellet constituted the sulphated polysaccharides fraction and was stored at 4 °C. When required it was dissolved in distilled water to required concentration.

2.3 Determination of Hepatoprotective and Antioxidant Potential of the Sulphated Polysaccharides fraction of *Turbinaria ornata*

2.3.1 Experimental animals

Male Swiss albino mice weighing 20-25 g were used for the acute toxicity studies; male Wistar albino rats weighing 150-200 g were used for the study on hepatoprotective and antioxidant potential. 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 one week prior to the initiation of the study and their use was conducted under the guidance of the basic standards in the care and use of laboratory animals which has been prepared and published by the National Institute of Health (NIH publication No. 85-23, revised 1985. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted as per the guidelines of CPCSEA (IAEC/APCAS/01/2015/01).

2.3.2 Acute toxicity studies

Acute oral toxicity was performed according to OECD-423 guidelines [12]. 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 sulpahted polypsaccharides 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 2,000 mg/kg) doses of sulpahted polypsaccharides fraction were employed for further toxicity

studies. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality.

2.3.3 Induction of hepatic damage

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

2.3.4 Experimental design

Male Wistar albino rats weighing between 150 and 200 g were randomly divided into 6 groups of 6 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 *Turbinaria ornata sulphated polysaccharides* fraction (50 mg/kg) dissolved in water for 7 days.
Group 5: Treated with paracetamol (2 g/kg) and *Turbinaria ornata sulphated polysaccharides* fraction (100 mg/kg) dissolved in water for 7 days.
Group 6: Treated with paracetamol (2 g/kg) and *Turbinaria ornata sulphated polysaccharides* fraction (200 mg/kg) dissolved in water for 7 days.
Animals were kept starved overnight on the 7^{th} 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 2,500 rpm for 10 min and subjected to various biochemical estimations. Anticoagulant was added to one tube of blood for the collection of plasma. A 100 mg of liver issue from each rat was used for antioxidant study.

2.3.5 Biochemical estimations

The separated serum was subjected to biochemical estimation of different parameters like ALT (alanine aminotransferase), AST (aspartate aminotransferase) [13], ALP (alkaline phosphatase) [14], LDH (lactate dehydrogenase) [15], γ-GT (γ-glutamyl transferase) [16], total bilirubin [17], total protein [18], cholesterol [19] and triglycerides [20]; plasma was subjected to the estimation of reduced glutathione [21].

2.3.6 Assay of antioxidants

Liver tissue (100 mg) 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 [21] and assay of antioxidant enzymes such as superoxide dismutase [22], catalase [23] and glutathione peroxidase [24].

2.3.7 Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnet's t-test was applied for determining the statistical significance of difference between experimental groups. P values <0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

3.1 Determination of Hepatoprotective and Antioxidant Potential of the Sulphated Polysaccharides fraction of *Turbinaria ornata*

3.1.1 Acute toxicity studies

All the doses (5, 50, 300 and 2000 mg/kg) of *Turbinaria ornata sulphated polysaccharides* 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_{50} dose of 2,000 mg/kg and above is categorized as unclassified. *Turbinaria ornata sulphated polysaccharides* fraction did not produce any mortality even at the highest dose (2000 mg/kg) employed, and hence the *Turbinaria ornata sulphated polysaccharides* 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.

3.1.2 Biochemical estimations

Liver is the largest organ and is a target for toxicity because of its role in clearing and metabolizing chemicals through the process of detoxification [25]. Drug-induced liver disorders occurring frequently can be life-threatening and mimic all forms of liver diseases [26]. Paracetamol is also a drug which is capable of causing liver disorders, if it is given continuously.

Hepatic biomarkers are protein and non-protein components of cell structures released into the blood stream when hepatic injury occurs and they can be measured in the systemic circulation. Elevation of hepatic markers in plasma is one of the criteria being used for the diagnosis of acute liver injury. The optimal and ideal hepatic marker should be present in high concentration in liver and should be absent from non-hepatic tissues. It should be rapidly released into the blood stream at the time of hepatic injury and there should be a direct relation between the plasma level of the marker and the extent of hepatic injury. The marker should persist in plasma for a sufficient length of time to provide a convenient diagnosis time, and the measurement of the marker should be easy, inexpensive and rapid. In this regard, the serum diagnostic marker enzymes are of particular interest because of their catalytic activity and tissue specificity.

The laboratory findings of paracetamol (PCM)-induced hepatotoxicity are similar as other acute hepatic inflammation and enhancement of liver ailment, with major increase of AST, ALP, ALT, LDH, cholesterol, bilirubin and decrease of total protein [27]. The paracetamol-induced liver disorders were treated with *Turbinaria ornata sulphated polysaccharides* fraction for 7 days. Estimating the activities of serum marker enzymes, like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase and lactae dehydrogenase can be used for the assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol of hepatocytes are released into the blood stream. Their estimation is a useful quantitative marker of the extent and type of hepatocellular damage identification [28].

An obvious sign of hepatic injury is the leakage of cellular enzymes such as AST, ALT and ALP [29]. Aminotransferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-keto acids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds [30]. Liver and bone diseases are the most common causes of pathological elevation of

alkaline phosphatase levels. Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface [31]. Elevated levels of serum LDH_5 isoenzyme activity was observed in patients with various liver diseases [32]. The whole spectrum of liver diseases, regardless of cause, may be responsible for altered GGT serum levels {33]. Elevations in GGT and alkaline phosphatase usually suggest bile duct disease. Measurement of GGT is an extremely sensitive test; it can be elevated when there is any liver disease.

There was a significant increase (P = 0.01) in the levels of AST, ALT, ALP, LDH, γ-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 *Turbinaria ornata sulphated polysaccharides* fraction (50, 100 and 200 mg/kg) decreased the reduced levels of AST, ALT, ALP, LDH, γ-GT, total bilirubin, total cholesterol and triglycerides in a dose-dependent manner (P = 0.01). The levels of total protein and plasma reduced glutathione were increased significantly by the administration of *Turbinaria ornata sulphated polysaccharides* 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. Serum bilirubin is considered to be one of the true tests of liver functions since it reflects the ability of the liver to take-up and process bilirubin into bile. Many different liver diseases can cause elevated bilirubin levels. Elevated levels may indicate severe illness [34]. Paracetamol seems to cause impairment in lipoprotein metabolism [35] and also alterations in cholesterol and triglycerides metabolism. The restoration of near normalcy in total protein, bilirubin, cholesterol and triglycerides content of serum of *Turbinaria ornata* sulphated polysaccharides fraction-treated rats further demonstrates the hepatoprotective activity.

Thus the treatment with *Turbinaria ornata* sulphated polysaccharides brought normalcy in the paracetamol-induced rats to near normal condition and was also comparable to those rats that received silymarin, the standard hepatoprotective drug. This establishes the hepatoprotective activity of the *Turbinaria ornata sulphated polysaccharides* fraction.

Table 1. Effect of *Turbinaria ornata* Sulphated Polysaccharides fraction on serum marker enzymes, protein, bilirubin and reduced glutathione

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ALT (IU/L)	48.50 ± 2.09	161.00 ± 7.26 [a*]	52.17 ± 1.92 [b*]	68.50 ± 3.10 [b*]	61.67 ± 2.79 [b*]	56.17 ± 2.56 [b*]
AST (IU/L)	82.00 ± 3.71	213.67 ± 10.45 [a*]	84.83 ± 4.13 [b*]	108.67 ± 5.30 [b*]	97.83 ± 4.78 [b*]	89.00 ± 4.34 [b*]
ALP (IU/L)	135.00 ± 6.60	291.33 ± 11.08 [a*]	139.50 ± 6.61 [b*]	176.50 ± 7.64 [b*]	159.00 ± 6.88 [b*]	144.50 ± 7.71 [b*]
LDH (IU/L)	110.33 ± 5.74	182.17 ± 8.69 [a*]	112.67 ± 5.36 [b*]	147.33 ± 7.28 [b*]	133.00 ± 6.56 [b*]	120.83 ± 6.50 [b*]
γ-GT (IU/L)	3.18 ± 0.15	6.58 ± 0.31 [a*]	3.25 ± 0.17 [b*]	4.05 ± 0.21 [b*]	3.65 ± 0.19 [b*]	3.32 ± 0.17 [b*]

Total Bilirubin (mg/dL)	0.78 ± 0.04	2.48 ± 0.13 a*	0.80 ± 0.04 b*	1.05 ± 0.06 b*	0.95 ± 0.05 b*	0.87 ± 0.04 b*
Total Protein (g/dL)	7.33 ± 0.35	5.03 ± 0.26 a*	7.25 ± 0.30 b*	5.73 ± 0.21 b*	6.37 ± 0.23 b*	7.08 ± 0.44 b*
Total Cholesterol (g/dL)	96.33 ± 4.33	200.17 ± 8.98 a*	101.33 ± 5.22 b*	126.50 ± 5.56 b*	114.00 ± 5.01 b*	103.67 ± 4.46 b*
Triglycerides (g/dL)	86.33 ± 4.09	286.50 ± 10.16 a*	89.00 ± 4.55 b*	111.67 ± 6.02 b*	100.67 ± 5.42 b*	91.50 ± 4.77 b*
Reduced Glutathione (mg/dL)	32.00 ± 1.31	17.50 ± 0.98 a*	30.83 ± 1.21 b*	24.33 ± 1.54 b*	27.00 ± 1.71 b*	30.00 ± 1.03 b*

Group 1 – Control; Group 2 – PCM; Group 3 – PCM + Silymarin; Group 4 – PCM + TOSP 50 mg/kg; Group 5 – PCM + TOSP 100 mg/kg; Group 6 – PCM + TOSP 200 mg/kg.

[PCM = paracetamol; TOSP – *Turbinaria ornata* sulphated polysaccharides fraction]

The observations are given as Mean ± SEM, n=6; *P = 0.01; a - Group I vs. Group II; b - Group II vs. Groups III, IV V and VI.

Experimental and epidemiological studies indicate the involvement of oxidative stress in the pathogenesis and progression of many chronic diseases [36]. It is initiated by the reaction of free radicals with biological macromolecules such as proteins, lipids and DNA [37]. It is known that oxygen, essential for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as ROS. The high reactivity of ROS may activate a host of disorders in the body resulting in tissue damage and necrosis in many instances [38].

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, deregulates the cellular functions leading to various pathological conditions [39].

Generally antioxidants, preferably from natural sources, have been considered as effective therapeutic agents [40]. 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.

Tissue glutathione plays a central role in antioxidant defence [41] Reduced glutathione detoxifies reactive oxygen species such as hydrogen peroxide and lipid peroxides directly or in a glutathione peroxidase (GPx)-catalyzed mechanism. The major hepatic antioxidant defence system against free radicals includes SOD, catalase, GPx and GSH redox cycle. Superoxide dismutase, catalase and glutathione peroxidase, constitute a mutually supportive team of defence against ROS, which remove free radicals *in vivo* [42]. Superoxide dismutase acts as scavenger of free radicals and reduces the toxicity of oxygen. Tissues are protected from superoxides by the specific enzyme superoxide dismutase [43]. Catalase catalyzes the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and OH [44]. Glutathione peroxidase catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulphide (GSSG) and the

reduction product of the hydroperoxide. Thus, the enhancement of the hepatic antioxidant system capacity may be an effective therapeutic strategy for the alleviation and treatment of liver damage [45].

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 *Turbinaria ornata sulphated polysaccharides* fraction-administered groups (50, 100 and 200 mg/kg) in a dose-dependent manner is a clear indication of hepatoprotective effect of *Turbinaria ornata sulphated polysaccharides* fraction extract through antioxidant mechanism (P = 0.01) (Table 2).

Table 2. Effect of *Turbinaria ornata* Sulphated Polysaccharides 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 [a*]	47.50 ± 2.13 [b*]	34.67 ± 1.92 [b*]	38.50 ± 2.13 [b*]	42.83 ± 2.36 [b*]
[1] Superoxide Dismutase	8.25 ± 0.45	4.30 ± 0.23 [a*]	8.02 ± 0.46 [b*]	6.13 ± 0.39 [b*]	6.80 ± 0.43 [b*]	7.55 ± 0.41 [b*]
[2] Catalase	57.50 ± 2.20	24.83 ± 1.24 [a*]	55.50 ± 2.92 [b*]	40.83 ± 2.35 [b*]	45.33 ± 2.61 [b*]	50.33 ± 2.04 [b*]
[3] Glutathione Peroxidase	8.73 ± 0.41	4.35 ± 0.24 [a*]	8.42 ± 0.39 [b*]	6.58 ± 0.30 [b*]	7.30 ± 0.33 [b*]	8.10 ± 0.44 [b*]

Group 1 – Control; Group 2 – PCM; Group 3 – PCM + Silymarin; Group 4 – PCM + TOSP 50 mg/kg; Group 5 – PCM + TOSP 100 mg/kg; Group 6 – PCM + TOSP 200 mg/kg.

[PCM = paracetamol; TOSP – *Turbinaria ornata* sulphated polysaccharides fraction]

[1] Superoixde dismutase is expressed as 50 % inhibition of epinephrine autooxidation/min/mg protein.

[2] Catalase is expressed as µmoles of H_2O_2 decomposed/min/mg protein.

[3] Glutathione peroxidase is expressed as µmoles of glutathione oxidised/min/mg protein.

The observations are given as Mean ± SEM, n=6; *P = 0.01; a - Group I vs. Group II; b - Group II vs. Groups III, IV V and VI.

Among the therapeutics for liver diseases, protective drugs such as antioxidants have attracted more and more attention and proton radical-scavenging action is an important mechanism of antioxidation.

4. CONCLUSION

On the basis of results in this study, it can be concluded that the *Turbinaria ornata sulphated polysaccharides* fraction has exhibited a liver protective effect against paracetamol-induced hepatotoxicity and possessed antioxidant activities. Thus, *Turbinaria ornata,* a marine alga, is found to have hepatoprotective effect and antioxidant activities, and thus 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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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Both authors contributed in the design, analysis, interpretation and preparation of the manuscript. Both authors read and approved the final manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by Institutional Animal Ethical Committee (IAEC) constituted as per the guidelines of CPCSEA (IAEC/APCAS/01/2015/01).

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