

ORIGINAL CONTRIBUTION

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Protective effect of crude sulphated polysaccharides from *Sargassum Swartzii* (Turn.) C.Ag. against acetaminophen induced liver toxicity in rats

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Abstract

Background: Polysaccharides from seaweeds have been reported to possess biological activities with potential medicinal value. Present study was aimed to investigate hepatoprotective effect of crude sulphated polysaccharides extracted from *Sargassum swartzii* against acetaminophen-induced liver injury.

Methods: The polysaccharides from *S. swartzii* was extracted at room temperature and at 70 °C and named as EW₁ and EW₂. These fraction was given orally to rats at 200 mg/kg body weight. Liver injury was induced by single intraperitoneal injection of acetaminophen. Hepatic marker enzymes; alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin and other biochemical parameters; glucose, triglycerides, cholesterol, urea and creatinine were estimated in serum, while hepatic glutathione (GSH) and lipid peroxidation were measured in liver tissue. Histopathology of liver tissues was also carried out.

Results: Treatment with polysaccharides EW₁ & EW₂ fractions significantly ($p < 0.05$) reduced the hepatic marker enzymes and other biochemical parameters along with increased GSH and reduced lipid peroxidation. The EW₁ fraction of crude sulphated polysaccharides produced hepatoprotection more or less equivalent to silymarin (35 mg/kg), a commercial herbal drug, while some parameters showed better results than silymarin. These results were further confirmed with histology of liver.

Conclusion: This study suggests that crude polysaccharides of *S. swartzii* has ability to protect against liver toxicity similar and/or better than silymarin (a standard drug) based on biochemical and histological findings. However toxicological studies would be recommended to evaluate any toxic effect of *Sargassum swartzii*.

Keywords: *Sargassum swartzii*, Sulphated polysaccharides, Hepatoprotective, Acetaminophen, Liver injury, Silymarin

Introduction

Liver is the vital organ and major metabolic hub of the body. An inflammation of liver cells is called hepatitis, if not controlled effectively then may leads to chronic liver diseases [1, 2]. A number of drugs have been used to treat liver diseases but their successful use in treating liver diseases is very small, because the drugs used for treating the liver diseases may have side effects [3]. The

therapies based on natural substances are believed to be safer and better than drugs used in medical practices [4].

The marine environment is believed to be an incomparable reservoir of bioactive natural products, many of which exhibited structural features that have not been found in terrestrial plants [5]. In marine environment algae are of two types, microalgae and macroalgae [6]. Records showed that macroalgae (seaweeds) have been consumed in human diets particularly as a vegetable in Asia, since 600 BC [7]. The Chinese used algae and seaweeds for medicinal purposes as early as 300 BC [8]. The presence of reactive antioxidants (ascorbate and

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glutathione) along with carotenoides (α - and β -carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine) and catechins (e.g., catechin, epigallocatechin), gallate, phlorotannins (e.g., phloroglucinol), eckol and tocopherols was reported in seaweeds [9].

Phytochemicals from marine macroalgae are found to be effective non-toxic substances with wide variety of pharmacological activities [10, 11]. Seaweeds possess biological activities, such as cholesterol lowering, immunomodulatory, anti-hepatotoxic [12]. The polysaccharide from seaweeds specially agaro-oligosaccharides not only prevents the oxidative injury but also boosts host antioxidant enzyme system [13]. Sulphated polysaccharides from *Laminaria japonica* are able to repair damaged or hypofunctional organs and tissues by enhancing the production of immunoregulatory and proinflammatory cytokines [14]. Among different polysaccharides derived from brown and red seaweeds, sulphated polysaccharides (fucoidan) and lambda carrageenan exhibit hepatoprotective, antioxidant and free radical scavenging activity that might be due to their sulfur content [15] and molecular weight [16, 17]. Seaweed, polysaccharide can be used for the development of new drugs due to its many biological activities [18]. The present study, further investigates the hepatoprotective effect of sulphated polysaccharides from *Sargassum swartzii* against acetaminophen (AAP) induced liver damage and associated complication.

Materials and methods

Collection of seaweeds

Seaweeds were collected at low tide from Buleji beach, Karachi coast. These seaweed species were washed thoroughly in order to remove salts, debris and epiphytes. Voucher specimens and herbarium sheets of the seaweeds were prepared and record (KUH-SW) was kept in the Seaweed Herbarium, MAH Qadri Biological Research Center, University of Karachi. Professor Dr. Mustafa Shameel (Department of Botany, University of Karachi) identified the seaweeds. Seaweeds were dried under shade, grinded and fine powder was used for experimental purpose.

Extraction of polysaccharides

The extraction of polysaccharides from seaweeds was carried out by slightly modified method of Ponce et al. [19]. The coarse powder of dry seaweeds (100 g) was extracted with 500 ml of 80% ethanol first at room temperature and then at 70 °C with constant stirring for 24 h in order to remove proteins, peptides, amino acids, vitamins, polyphenols, mannitols and other monosaccharides. The liquid portion was centrifuged off and residual seaweed was extracted with 250 ml of water at

room temperature and at 70 °C for 7 h with constant stirring and labeled as EW₁ and EW₂ fractions of crude sulphated polysaccharides respectively. The EW₁ and EW₂ fractions of crude sulphated polysaccharides were dried on Eyla FD-1, lyophilizer and stored at -20 °C.

Animals

Healthy male albino rats of *Wistar* strain (140–170 g) were purchased from Dow University of Health Sciences, Karachi. All the animals were housed in prebedded polyethylene cages (3 rats/cage and 6 rats were used per group) with standard laboratory conditions (temperature 25 ± 2 °C and 12 h light/dark cycle), fed with standard pellet diet and water ad libitum. The animals were kept in the laboratory for 1 week before starting the experiment to acclimatize animals with laboratory conditions.

Induction of hepatotoxicity

Acetaminophen (Sigma Aldrich, U.S.A.) was administered intraperitoneally @ 1 g/kg, body weight and the dose was prepared in 40% polyethylene glycol (Sigma Aldrich, U.S.A.) with constant stirring and mild heating.

Experimental design

Group 1 received the distilled water daily for 14 days and served as normal control. Group 2 rats received distilled water daily for 14 days but injected with acetaminophen, 24 h prior to decapitation and served as AAP control. Group 3 orally administered with reference drug, silymarin (Sigma Aldrich, U.S.A.) @ 35 mg/kg b.w. daily for 14 days. Group 4 and 5 received the EW₁ and EW₂ polysaccharide fractions of *S. swartzii* respectively (200 mg/kg b.w.) daily for 14 days. On last day, all the rats from group 2, 3, 4 and 5 received single intraperitoneal injection of acetaminophen. All the animals were fasted overnight and decapitated after 24 h of AAP injection. Normal control rats were injected with vehicle, 1 day before to decapitation.

Assessment of hepatic damage

Serum was separated from blood by centrifugation and used for the determination of alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea, creatinine, bilirubin, triglycerides, cholesterol and glucose using kits from Merck (France) and Ecoline (Germany). The liver tissue was excised immediately after decapitation and washed with ice cold normal saline. Homogenate (10%) was prepared by homogenizing liver tissue with Tris HCl buffer (pH 7.4). Freshly prepared homogenate was used for the estimation of glutathione and lipid peroxidation.

Estimation of hepatic glutathione

Reduced glutathione was estimated according to the method of Samarth et al. [20] with slight modifications. Homogenate (0.1 ml) was mixed with 100 μ l of 25% trichloroacetic acid (TCA) and kept for 5 min at room temperature in order to precipitate protein. The mixture was centrifuged at 3000 rpm for 10 min. 0.2 ml of the supernatant was mixed with 1.8 ml of 0.1 mM DTNB (5,5'-Dithiobis-2-nitrobenzoic acid; in 0.3 M sodium phosphate buffer pH 8). The tubes were incubated for 10 min and absorbance was recorded against reagent blank at 412 nm using UV-visible spectrophotometer (Helios α , Thermo Spectronic, UK). Mixture of all reagents except sample served as reagent blank.

Estimation of lipid peroxidation

The extent of lipid peroxidation was determined by quantifying the concentration of malondialdehyde (MDA) as described by Ohkawa et al. [21]. The tissue homogenate (0.1 ml) was mixed with 0.1 ml of 8.1% SDS and incubated for 10 min at room temperature. 750 μ l of 20% acetic acid (pH 3.5) and 750 μ l of 0.8% thiobarbituric acid (TBA) were added and volume was adjusted to 2 ml with water. The tubes were placed in water bath at 95 °C for 60 min and cooled under tap water. The final volume was adjusted to 2.5 ml with distilled water. The same volume of butanol and pyridine (1:1) mixture was added in each tube, vortex and centrifuged at 4000 rpm for 10 min. The upper organic layer was removed and absorbance was recorded against reagent blank at 532 nm using UV-visible spectrophotometer.

Hepatic histology

A small portion of liver tissue was fixed in formalin (5%) and kept in laboratory for 48 h. Tissue was embedded in paraffin in order to cut sections of 3 to 4 μ m thick and stained with hematoxylin and eosin (H & E) staining solutions. The extent of damage was assessed by using microscope (Nikon FX-35A, Japan) at 100X and 200X.

Statistical analysis

Data was analyzed and subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's multiple range test (DMRT) according to Armitage & Berry [22]. *P*-value < 0.05 was considered as a level of significance.

Results

Effect of EW₁ & EW₂ fraction of *S. swartzii* on serum markers in acetaminophen intoxicated rats

Serum ALT, AST, ALP, LDH and bilirubin values of normal control rats were 27 \pm 1.7, 88 \pm 8, 45 \pm 6.2, 238.6 \pm 10.2 and 0.56 \pm 0.057 respectively. Acetaminophen administration raised the levels of these enzymes as

compared to control (64 \pm 7.8, 187.6 \pm 9.29, 124 \pm 4.3, 542.6 \pm 17.6 and 0.93 \pm 0.054 respectively). Liver is major metabolic hub of body and controls complete body metabolism. If liver is injured by any toxic substance then glucose, triglyceride, cholesterol, urea and creatinine levels would also be disturbed in blood. As evident from this study; acetaminophen intoxication significantly (*p* < 0.05) elevated the levels of glucose, triglyceride, urea and creatinine as compared to normal control rats. Treatment with crude sulphated polysaccharides (EW₁ and EW₂) fractions of *Sargassum swartzii* significantly (*p* < 0.05) reduced the elevated level of ALT, AST, ALP, LDH and bilirubin along with other metabolites glucose, triglyceride, urea and creatinine. The more potent activity was found in crude sulphated polysaccharides extracted at room temperature (EW₁ fraction) of *Sargassum swartzii* with reduced ALT (33.3 \pm 2.5), AST (121 \pm 12.5), ALP (47.6 \pm 1.5), LDH (318 \pm 10.5), bilirubin (0.76 \pm 0.06), glucose (127 \pm 7.5), triglycerides (108 \pm 17.08), urea (38.6 \pm 2.5) and creatinine (1 \pm 0.1). The effect was more or less equivalent to silymarin (35 mg/kg) while some parameters showed better results than silymarin. The rats pretreated with crude sulphated polysaccharides extracted at 70 °C (EW₂) fraction of *Sargassum swartzii* also showed hepatoprotective activity (Tables 1 and 2) but at lower extent. The silymarin (35 mg/kg) also protected the liver from being injured by acetaminophen, evident by significant (*p* < 0.05) decreased in ALT, AST, ALP, LDH, bilirubin.

Effect of EW₁ & EW₂ fraction on glutathione (GSH) and lipid peroxidation

Oxidative stress was confirmed by observing hepatic reduced glutathione (GSH) status in rats administered with AAP alone (8.16 \pm 0.75 μ mole/gm wet liver tissue) as compared to normal control rats (14.7 \pm 1.1 μ mole/gm w.l.t.). The significant improvement was found in rats pretreated with EW₁ fraction of *S. swartzii* (14.8 \pm 0.15 μ mole/g wet liver tissue) followed by acetaminophen administration and this is almost equal to normal control rats. The EW₂ fraction ameliorated the acetaminophen-induced reduction in reduced glutathione level to 13.7 \pm 0.321 μ mole/gm w.l.t. but was not too much effective (Fig. 1).

Administration of AAP induced significant increase in lipid peroxidation as observed in high concentration of TBARS as compared with normal control rats. However significant decline in TBARS level was found in rats pretreated with EW₁ and EW₂ fraction of *S. swartzii* following the acetaminophen intoxication. But good protective effects were observed in EW₁ fraction of *S. swartzii* followed by EW₂ fraction of *S. swartzii* (Fig. 1). The crude sulphated polysaccharides (EW₁) fraction of *S. swartzii* restored hepatic glutathione level and reduced

Table 1 Effect of Crude Sulphated Polysaccharides of *Sargassum swartzii* on Liver Enzymes and Bilirubin in Acetaminophen-Intoxicated Rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	Bilirubin (mg/dl)
Normal control	27 ^d ± 1.7	88 ^d ± 8	45 ^c ± 6.2	238.6 ^d ± 10.2	0.56 ^c ± 0.051
Acetaminophen (AAP) control	64 ^a ± 7.8	187.6 ^a ± 9.29	124 ^a ± 4.3	542.6 ^a ± 17.6	0.93 ^a ± 0.054
Silymarin (35 mg/kg) + AAP	37.3 ^{bc} ± 4.5	139 ^{bc} ± 8.88	68.3 ^b ± 7.5	375 ^b ± 17.5	0.76 ^b ± 0.056
<i>S. swartzii</i> EW ₁ + AAP	33.3 ^{cd} ± 2.5	121 ^c ± 12.5	47.6 ^c ± 1.5	318 ^c ± 10.5	0.76 ^b ± 0.06
<i>S. swartzii</i> EW ₂ + AAP	45 ^b ± 4	154 ^b ± 14	60.3 ^b ± 0.57	375 ^b ± 17.5	0.73 ^b ± 0.052

The values are expressed as means ± Standard error (n = 6 animals per group)

The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test

hepatic lipid peroxidation almost near to the standard drug (silymarin).

Effect of crude Sulphated polysaccharides fractions (EW₁ & EW₂) on hepatic histology

Histopathological examination of normal rats showed normal lobular structure. Central vein, portal triads, hepatic sinusoids and cells were normal (Fig. 2a). The rats treated with acetaminophen (AAP) showed prominent hepatic necrosis, abnormal architecture of central vein and dilated sinusoidal structure can be observed. Cells possess vesicular structure in their cytoplasm. Infiltration of macrophages and polymorphonuclear cells were observed (Fig. 2b). Treatment with EW₁ fraction of *S. swartzii* healed the hepatic injuries. The inflammatory cells are very rare with normal sinusoidal pattern (Fig. 2c). Histopathological examination revealed that no fatty changes were observed in rats pretreated with EW₂ fraction of *S. swartzii* prior to acetaminophen intoxication. Very few vacuolization was seen in the cells. However some inflammatory cells and polymorphonuclear cells can be seen, but they are quite less as compared to acetaminophen control rats. Very few damaged cells around centrilobular portion of tissue can be seen (Fig. 2d). The rats pretreated with silymarin (35 mg) prior to acetaminophen administration showed almost complete protection against injury. It showed recovery with no fatty deposition. Hepatic cells and sinusoids appeared to be normal with minimal portal inflammation (Fig. 2e).

Discussion

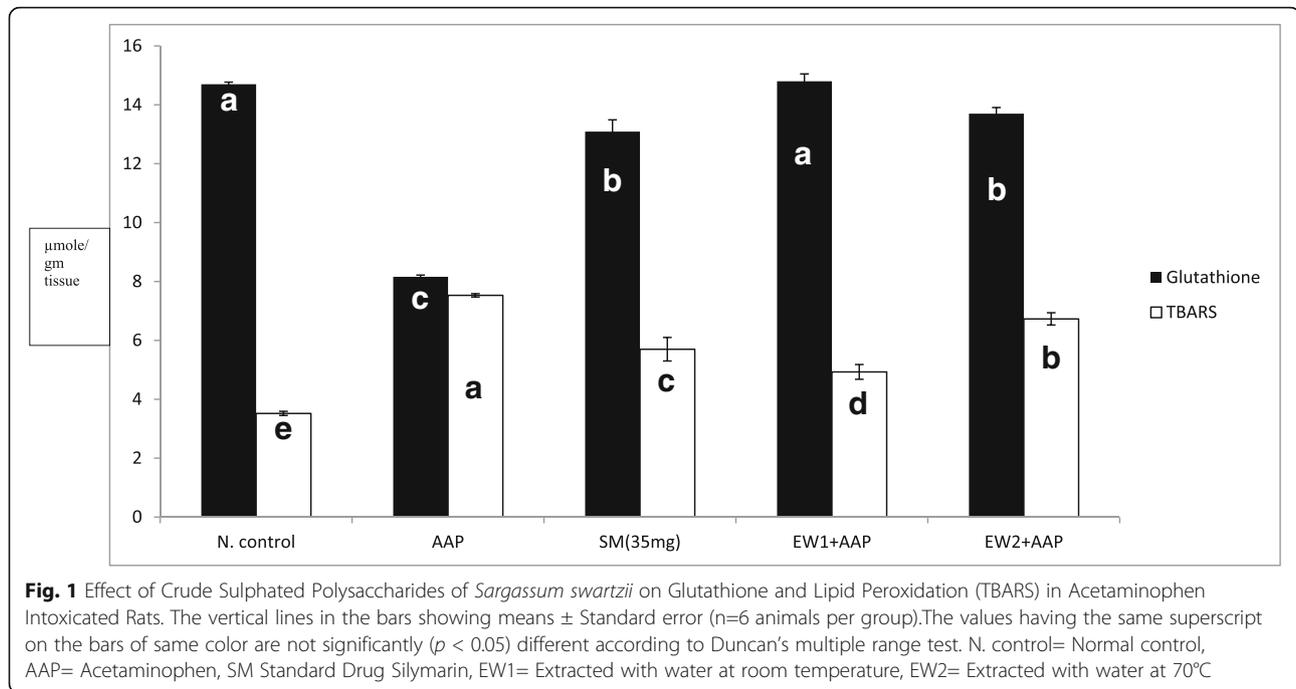
Presence of polysaccharides in seaweeds has been reported for various biological activities including cholesterol lowering, immunomodulatory, In vitro and In vivo anti-hepatotoxic activity [23, 24] and antiproliferative activity [25]. Seaweeds contain commercially important polysaccharides, named as agar, fucoidan, alginate, carrageenan, laminaran and sulphated galactan [12]. In the present study intoxication of acetaminophen (AAP) in experimental rats caused liver damage as evident from the increase in the level of hepatic enzymes; AST, ALT, ALP and LDH along with increase in serum bilirubin, glucose, triglycerides, urea and creatinine. It is well established that when the liver is injured as a result of introduction of infectious agents or chemicals, the serum levels of GPT (ALT) and GOT (AST) are raised significantly [26], along with leakage of ALP and LDH [27] reflecting the severity of liver injury [28]. In present study the treatment with both fractions of crude sulphated polysaccharides, EW₁ and EW₂ fraction of *Sargassum swartzii* significantly ($p < 0.05$) reduced the elevated level of ALT, AST, ALP, and LDH and all other biochemical parameters. The highest activity was found in EW₁ fraction of *S. swartzii*. All parameters including liver marker enzymes, bilirubin, triglycerides, cholesterol and urea were statistically equal in both silymarin and EW₁ fraction of *S. swartzii* in acetaminophen-induced liver injury. Silymarin regenerates liver tissues, help in protein synthesis, reverses glutathione

Table 2 Effect of Crude Sulphated Polysaccharides of *Sargassum swartzii* on Glucose, Urea, Creatinine, Triglycerides and Cholesterol in Acetaminophen-Intoxicated Rats

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	108.6 ^c ± 3.5	84 ^b ± 5.2	84.6 ^a ± 2.3	30 ^d ± 1	0.9 ^c ± 0.1
Acetaminophen (AAP) control	162 ^a ± 6.5	167.3 ^a ± 10.06	64.6 ^{bc} ± 5.8	64 ^a ± 6.2	1.23 ^a ± 0.11
Silymarin (35 mg/kg) + AAP	118.3 ^{bc} ± 8.6	92 ^b ± 23.06	68.6 ^b ± 4.04	35 ^{cd} ± 2.6	1.03 ^{bc} ± 0.057
<i>S. swartzii</i> EW ₁ + AAP	127 ^b ± 7.5	108 ^b ± 17.08	65 ^{ac} ± 3.6	38.6 ^{bc} ± 2.5	1 ^{bc} ± 0.1
<i>S. swartzii</i> EW ₂ + AAP	169 ^a ± 8	106 ^b ± 13.11	58.3 ^c ± 3.7	43.6 ^b ± 2.08	1.1 ^{ab} ± 0

The values are expressed as means ± Standard error (n = 6 animals per group)

The values having the same superscript within the column are not significantly ($p < 0.05$) different according to Duncan's multiple range test



fall in tissue, enhanced glucuronidation and reduced inflammation, hence it acts as antioxidant, antifibrotic, anti-inflammatory, membrane stabilization and immunomodulatory agent [29]. In current study the crude sulphated polysaccharides (EW₁) extracted at room temperature showed its maximum protective effect might be due to its antioxidant properties and presence of higher concentration of sulphated polysaccharide as reported by Ponce et al. [19] in a brown seaweed *Adenocystis utricularis*. They have reported 30% sulphate in EW₁ as compared to 8% in EW₂. Vijayabaskar et al. [30] reported antioxidant activity of *Sargassum swartzii* due to presence of 10.43% crude sulfated polysaccharide in seaweed. The hepatoprotective effect of crude sulphated polysaccharides have also been reported by other researchers [31–33]. The sulphated polysaccharides from *Sargassum polycystum* showed protective effect against paracetamol induced toxic hepatitis in rats [31]. Ross et al. [33] also reported that sulphated polysaccharides from *Sargassum siliquosum* produced hepatoprotective activity against acetaminophen induced liver damage. Sulphated polysaccharides have ability to inhibit viral replication against HIV, hepatitis and herpes viruses [11], to stimulate immune system [14] and to protect against hepatocellular carcinomas [34], may be due to the prevention of increased pro-inflammatory cytokines, IL-6 and IL-12 [35].

Glutathione is most abundant tripeptide endogenous antioxidant present in liver. It protects liver by binding with reactive metabolites formed by AAP, thus preventing their covalent binding with liver proteins. Glutathione

helps in scavenging free radical species and maintenance of membrane protein thiols [36]. Taye & Abdel-Raheem, [37] reported significant reduction in GSH level of kidney and lungs tissues after CCl₄ intoxication. Other researcher also proved the significant decrease in the GSH content in different organs of rats when injected with CCl₄ [38]. The rats pretreated with crude polysaccharides (EW₁ and EW₂ fraction) of *S. swartzii* significantly ameliorated the acetaminophen-induced reduction in glutathione level. The glutathione status of body was significantly improved and lipid peroxidation was reduced in rats pretreated with crude polysaccharides extracted at room temperature (EW₁ fraction) when compared to silymarin (35 mg/kg) pretreated rats intoxicated with acetaminophen. The polysaccharides present in seaweeds prevent the elevation of pro-inflammatory cytokines IL-6 and IL-12 [35] and showed hepatoprotective effect [17, 31]. The sulphated polysaccharides from *S. wightii* restored the disturbed level of hepatic enzymes, antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), non-enzymatic antioxidants (glutathione, vitamin C and E), lipid peroxidation, protein carbonyls and ATPases [32]. Kawano et al. [39, 40] reported that dietary fiber from *Laminaria sp.*, *Sargassum fulvellum* and *Eisenia bicyclis* have ability to suppress D-galactosamine (D-GalN)-induced liver malfunction, might be due to the presence of fucoidan. The crude sulphated polysaccharides from *Laminaria japonica* and *Ulva lactuca* have ability to improve liver function by scavenging free radicals [41–43].

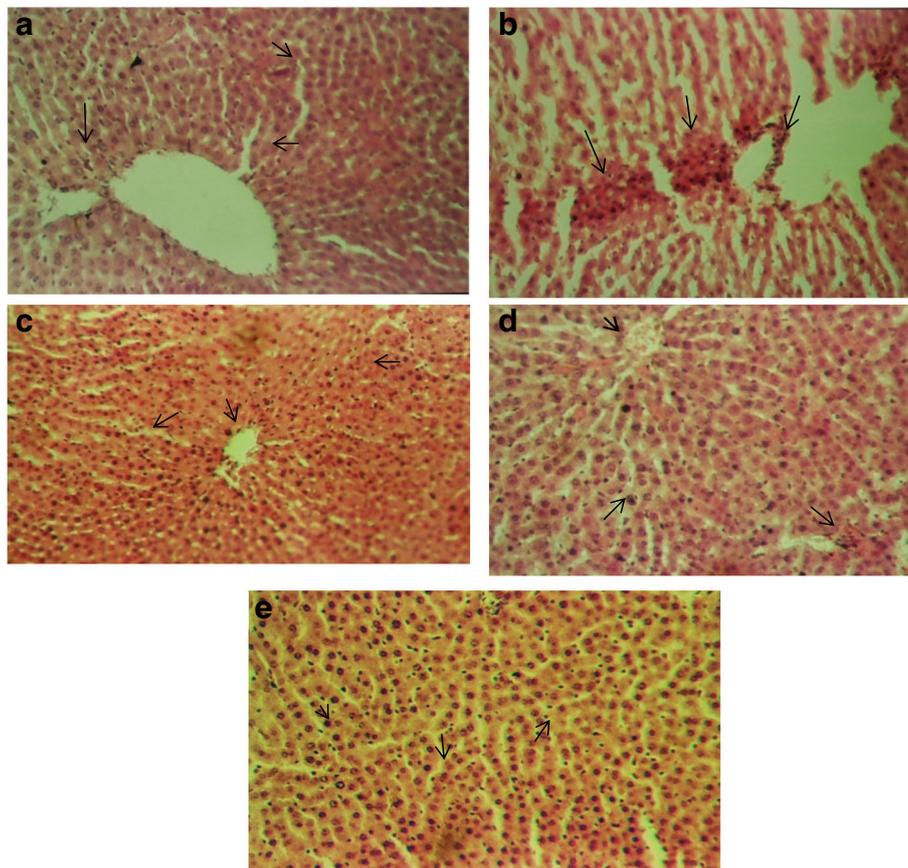


Fig. 2 Histopathological studies showing architecture of rats liver tissues in different pretreated groups. Magnification; 100X and 200X. **a.** Light micrograph of normal liver cells. **b.** Acetaminophen (AAP) treated rats. **c.** EW₁ fraction of *S. swartzii* + AAP. **d.** EW₂ fraction of *S. swartzii* + AAP. **e.** Liver cells of silymarin + AAP

The intraperitoneal administration of acetaminophen caused marked hepatic necrosis with fatty changes, infiltration of polymorphonuclear cells, abnormal central vein and sinusoidal morphological pattern. Crude sulphated polysaccharides from *S. swartzii* (EW₁ fraction) showed considerable reduction in pathological changes that produced after acetaminophen administration. The sulphated polysaccharides from *S. swartzii* extracted at room temperature (EW₁ fraction) prevented hepatic necrosis similar to the rats pretreated with silymarin (35 mg/kg) in acetaminophen-intoxicated rats. Raghavendran & Srinivasan [17], reported that a number of necrotic cells were depleted in rats co-treated with sulphated polysaccharides and acetaminophen. This might be due to the presence of high sulfate content in seaweed polysaccharides, which inhibit oxidative stress [44]. Ross et al. [33] reported that administration of sulphated polysaccharides from *Sargassum siliquosum* produced mild hepatocellular degeneration suggesting their protective role. The sulphated polysaccharides from *Sargassum wightii* showed considerable reduction in the pathological

changes produced after cyclosporine A-induced liver injury animals [32]. This protective action may possibly be due to its effect of preserving the cellular membrane of the hepatocytes from breakage by the reactive metabolites [45]. The sulphated polysaccharides extracted at room temperature and at 70 °C had completely different chemical compositions [19].

Conclusion

Nature based treatment of liver diseases has gained tremendous attention worldwide in past few decades. Seaweeds have characteristic of having unique polysaccharides in their cell walls that are not found in land plants. Current study has focused on the extraction of polysaccharides and their hepatoprotective role as polysaccharides are assumed to be good antioxidants. Results of present study showed that polysaccharides extracted at room temperature (EW₁ fraction) from *Sargassum swartzii* have reduced the acetaminophen attenuated disturbed liver marker enzymes and other biochemical parameters, supported by histopathological findings. The polysaccharides from *Sargassum swartzii* also increased

the antioxidant status of body. This study suggests that crude polysaccharide of *S. swartzii* has ability to protect against liver toxicity similar and/or better than silymarin (a standard drug). However toxicological studies would be recommended to evaluate any toxic effect of *Sargassum swartzii*.

Abbreviations

AAP: Acetaminophen; ALP: Alkaline phosphatase; ALT: Alanine aminotransferases; AST: Aspartate aminotransferases; DTNB: 5,5'-Dithiobis-2-nitrobenzoic acid; EW₁: Polysaccharides extracted at room temperature; EW₂: Polysaccharides extracted at 70 °C; GSH: Glutathione; GSH: Hepatic glutathione; H & E: Hematoxylin and eosin; LDH: Lactate dehydrogenase; MDA: Malondialdehyde; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid; w.l.t.: Wet liver tissue

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Availability of data and materials

Research data (Lab notebook) and materials can be provided on request.

Authors' contributions

KH & VS conceived and designed the study. KH performed experimental work. KH & SE analyzed and interpreted the results. NK supervised histo-pathological work. KH wrote the manuscript. KH, SE & JA improved the manuscript. All authors read and approved the final manuscript.

Ethics approval

The experiment was conducted according to the rules of Institutional Animal Ethics Committee (IAEC), University of Karachi.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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