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Ameliorative effect of marine macro-algae against carbon tetrachloride (CCl_4) induced hepatic fibrosis and associated complications in rats

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ABSTRACT
Objectives: Liver fibrosis is one of the serious health concerns around the globe. Persistent exposure to drugs, toxicants and pathogens may induce liver fibrosis. Marine macro algae have been globally consumed due to nutritive and medicinal value. This study was conducted to evaluate the protective role of two seaweeds *Padina pavonia* and *Caulerpa racemosa* in CCl_4-induced liver fibrosis in rats.
Materials and methods: animal model of hepatic fibrosis was developed by injecting 40% carbon tetrachloride (CCl_4) dissolved in olive oil (2ml/kg, b.w., i.p.) on alternate days for 30 days. Water extracts [(200mg/kg body weight (b.w.) p.o.] of *P. pavonia* and *C. racemosa* were given to rats daily for 30 days. On day 31, rats were sacrificed after 12 h fasting. Serum was used for biochemical estimation. 10% neutral buffered formalin was used to preserve the liver sample for histopathological examination, while the other portion was used for the preparation of tissue homogenate to estimate antioxidant enzymes and malondialdehyde levels.

Results: water extracts of both marine macro-algae significantly abrogates the elevated serum concentration of aminotransferases (alanine aminotransferase and aspartate aminotransferases), alkaline phosphatase and lactate dehydrogenase along with substantial (p<0.05) reduction in serum bilirubin levels. They also showed positive effect against oxidative stress, evident by improvement in reduced glutathione (GSH), catalase (CAT) and glutathione peroxidase (Gpx) activities and down regulating lipid peroxidation (MDA) level, with stabilizing the destructive cellular morphology of liver induced by repeated CCl_4 injection. Both algal extracts also improved kidney function (urea and creatinine) along with lipid metabolism (triglycerides and cholesterol).
Conclusion: Water extract of *C. racemosa* has shown great potential in attenuating liver fibrosis induced by CCl_4.
Keywords: *Padina pavonia; Caulerpa racemosa*; bilirubin; glutathione; lipid peroxidation; kidney

Introduction

The liver is metabolic hub for the degradation, synthesis, detoxification and transformation of drugs and biomolecules. Along with all the vital functions it performs, it is also prone to toxicity [1]. Liver is comprised of a variety of cells, such as hepatocytes, hepatic stellate cells (HSCs), which work as a reservoir of lipid droplet (vitamin A) and kupffer cells usually known as resident macrophages of liver[2]. Viral infections, chemical/drug intoxications or any malfunctioning of liver lead to damage of hepatocytes [3]. Hepatic stellate cells (HSCs), released extracellular matrix components around the injured area to prevent the further damages to the liver [4]. Chronic exposure of the liver with toxicants resulting in continuous activation of HSCs, forming scar tissues in the liver, lead to conditions such as; fibrosis, cirrhosis and hepatocellular carcinoma[5]. Liver fibrosis is one of the serious health concerns around the globe [6] and it is an early stage of cirrhosis [7]. It has been reported that fibrotic liver contains more extracellular matrix (ECM) components, which contains collagens (I, III and IV), fibronectin, elastin, laminin, hyaluronic acid (HA) and proteoglycans [8]. The earlier diagnosis of liver fibrosis is important, since it is treatable and termed as reversible liver fibrosis.[9]

CCl_4 has been widely used for the development of an animal model of hepatic fibrosis, cirrhosis and hepatocellular carcinoma since many decades.[10] The lipid soluble nature of CCl_4 allows it to cross the lipid bilayer membrane, produces hindrance in cellular activities by promoting the lipid peroxidation, activating reactive oxygen species (ROS).[11] Persistent exposure of CCl_4 promotes accumulation of extracellular matrix (ECM) components which either damages the cellular structures and/or disturbs the cellular integrity. [12]

Among different marine sources, macro-algae have drawn the attention of many researchers as an interesting and unique source of bioactive natural products, besides, a great source of proteins, carbohydrates, minerals and vitamins.[13] They have medicinal importance, including anticancer, antibacterial, anticoagulant, nephroprotective, anti-inflammatory and antioxidant activities.[14,15] Green seaweeds are a rich source of carotenoids, phenolics, terpenoids, essential proteins, vitamins, minerals and sulfated polysaccharides[16]. *Ulva* and *Caulerpa* species have been reported to contain higher content of sulfated polysaccharides and rare sugars (arabinose, rhamnose and iduronic acid).[17] *Caulerpa racemosa,* an edible green seaweed has been reported to have number of biological activities such as antibacterial and hypolipidemic activities against triton induced hyperlipidemic rats.[18,19] However, the

hepatoprotective role of these seaweed against CCl_4 induced hepatic injury has not been evaluated. Brown seaweeds contain phenolic compounds and sulfated polysaccharides (SP) (alginates, fucoidans, and laminarins) and possess tremendous biological activities such as hepatoprotective, anticancer, anticoagulant, wound healing and antiviral.[14] *Padina pavonia*, has been previously reported to have anti-proliferative and pro-apoptotic activities.[20] Its hepatoprotective activity against azoxymethane induced hepatotoxicity has been reported.[21] However, its hepatoprotective activity against CCl_4 has not been explored yet. The current study was designed to elucidate the hepatoprotective potential of *C. racemosa* and *P. pavonia* against CCl_4 induced liver fibrosis. The study also describes the effect of these seaweed on liver fibrosis associated complications, including renal dysfunction and lipid metabolism.

Materials and methods

Algal material

Seaweeds were collected from Karachi coast (Buleji Beach) at low tide during the month of November-April. Collected algal samples were identified by taxonomist (Dr. Aisha Begum, Associate Professor, Department of Botany, University of Karachi). Algal material was washed using running tap water and air dried under a green shade. Dried samples were ground and stored for further use.

Water extract

Water extract was obtained by soaking dry powder (250gm) of seaweed in 1L of deionized water under continuous shaking for 3 hours. The filtrate obtained was lyophilized using a lyophilizer (Eyela FD-1, Japan). The dry powders were stored separately at -20°C until used according to Ismail and Hong.[22]

Experimental animals

Female Wistar rats (120-200gm) acquired from Dow University of Health Sciences, Karachi. Animals were accommodated in polypropylene cages under standard laboratory conditions (23± 2°C and 12 h light/dark cycle). The cages were bedded with wood shaving and rats had free access to normal pellet diet and tap water. Rats were acclimatized for 7 days under the guidelines of the Institutional Bioethical Committee (IBC-KU-132/2020) prior to experimental protocol.

Induction of fibrosis

Method of Iredale[23] was followed to develop hepatic fibrosis in rats with slight modifications. Rats were received 40% carbon tetrachloride (CCl_4) intraperitoneally at 2ml/kg b.w., dissolved in olive oil, on alternative days for 30 days.

Experimental design

Effect of the water extract (WE) of seaweeds in normal and liver fibrotic rat model

To evaluate the efficacy of seaweed extracts in rats, they were randomly divided into 8 groups (n=6).

Group1; Normal control: Rats received distilled water at 1mL/kg b.w., daily for 30 days.

Group2; Water extract of *P. pavonia* treated rats: Water extract of *P. pavonia* was supplemented to rats at 200mg/kg/mL, b.w., in distilled water, daily for 30 days.

Group 3; Water extract of *C. racemosa* treated rats: Water extract of *C. racemosa* was supplemented to rats at 200mg/kg /mL, b.w., in distilled water, daily for 30 days.

Group 4; Silymarin treated rats: Rats were supplemented with silymarin [(Sigma-aldrich (50mg/kg b.w., suspended in normal saline)] suspended in normal saline, daily for 30 days.[24]
Group 5; CCl_4 control: Rats were intraperitoneally (i.p.,) injected with 40% CCl_4 (in olive oil) 2ml/kg b.w., on alternate days for 30 days.
Group 6; Water extract of *P. pavonia* + CCl_4 induced liver fibrosis: Water extract of *P. pavonia* was supplemented to rats at 200mg/kg/mL, b.w., in distilled water, along with administration of CCl_4 (i.p., 2ml/kg, b.w.), on alternate days for 30 days.
Group 7; Water extract of *C. racemosa* + CCl_4 induced liver fibrosis: Water extract of *C. racemosa* was supplemented to rats at 200mg/kg /mL, b.w., in distilled water daily, along with administration of CCl_4 (i.p., 2ml/ kg, b.w.), on alternate days for 30 days.
Group 8; Silymarin treatment +CCl_4 induced liver fibrosis: Rats were supplemented with silymarin [Sigma-aldrich (50mg/kg b.w., suspended in normal saline)] daily, along with administration of CCl_4 (i.p., 2ml/kg, b.w.), on alternate days for 30 days.

Assessment of hepatotoxicity and associated complications

To determine the effect of seaweed on liver fibrosis and other associated complications; rats fasted for 12 hours and decapitated on the 31st day. Blood was centrifugation at 3000 rpm for 15 minutes to obtain serum. Liver enzymes viz; alanine aminotransferase (ALAT) (INO-17531) aspartate aminotransferase (ASAT) (INO-17521), lactate dehydrogenases (LDH)(INO-17653) alkaline phosphatases (ALP)(INO-17541) and other liver markers viz; total-bilirubin(INO-17645) and direct-bilirubin (INO-17646); lipid parameters including cholesterol (INO-17501) and triglycerides (TGs) (INO-17511), renal function markers such as urea (INO-17611) & creatinine (INO-17551) and blood glucose (INO-17602) were estimated on blood chemistry analyzer (Microlab-300, Merck, France) using kits from Merck (*Innoline*), France as per manufacturer's instructions.
For histopathological studies, the liver was excised, washed with normal saline. Right lobe was preserved in 10% neutral buffered formalin. Remaining portion of liver was used for preparation of liver tissue homogenate [Tris-HCl buffer (pH: 7.4) using Polytron (Kinematica) PT-MR 2100 homogenizer]. Tissue homogenate was used for measuring the antioxidant parameters reduced glutathione (GSH), catalase (CAT), malondialdehyde (MDA) glutathione peroxidase (Gpx).

Assessment of hepatic Reduce glutathione (GSH)

Reduced glutathione (GSH) was estimated by using the method of Moron [25]. Briefly, 0.1mL of homogenate was mixed with 0.1mL of TCA (25%) and allowed to stand at room temperature for 5 minutes. Mixture sample was centrifuged at 3000 rpm for 10 minutes, supernatant was collected and mixed with 1.8ml of 0.1mM DTNB. Samples were allowed to incubate in dark at room temperature for 10 minutes and absorbance was recorded at 412nm against the reagent blank.

Assessment of hepatic glutathione peroxidase (GPx)

Activity of GPx was estimated by method Flohé and Günzler[26]. Briefly 300 µl of liver homogenate was mixed with 300µl of phosphate buffer (pH= 7.4), 200µl of GSH (2mM) , 100µl of sodium azide (1mM) and 100µl of hydrogen peroxide (1mM). The mixture was allowed to stand for 15 minutes at 37°C in a water bath. 500µl TCA (15%) was added in the mixture and centrifuged at

1500 rpm for 5 minutes, supernatant was mixed with 200µl of phosphate buffer and 700µl of DTNB (0.1 mM). The absorbance was recorded at 412nm against the reagent blank.

Assessment of hepatic catalase (CAT)

Catalase activity was evaluated by the method of Sinha [27]. Briefly, 100µl of the homogenate was mixed with 1ml of phosphate buffer (pH=7.4) and 500µl of hydrogen peroxide (0.2M). The mixture was allowed to incubate at 37ºC for 15 minute. 2ml of dichromate solution (5%) was added to the mixture and absorbance was recorded at 570nm against the reagent blank.

Assessment of lipid peroxidation (MDA)

Lipid peroxidation was estimated by the method of Ohkawa [28]. Briefly, 100µl of tissue homogenate was mixed with 100µl of SDS (8.1%) and incubated at room temperature for 10 minutes. 750µl of 20% acetic acid and 750µl TBA (0.8%) were added into the mixture and volume was adjusted upto 2ml. Mixture was allowed to stand at 95ºC for 1hour. A 2.5 ml of butanol and pyridine (1:1) solution was added in a mixture and volume was adjusted upto 5ml with distilled water, and centrifuged (4000 rpm) for 10 minutes. Upper organic layer collected and absorbance was recorded at 532nm against the reagent blank.

Histological study

The liver tissue was preserved in 10% neutral buffered formalin and embedded in paraffin wax. Then 3-4µm thin sections were cut using microtome. The tissues were either stained with Hematoxylin and Eosin (H&E) or Masson's trichrome for the evaluation of architectural changes and evaluate abundance of collagen in liver tissue [29]. The slides were studied under the light microscope (Nikon FX-35A, Japan) and pictures were taken from an attached Nikon camera (DS FI1). Batts and Ludwig[30] scoring system was used to grade hepatic fibrosis.

Statistical analysis

The data were represented as a means ±standard deviation. The statistical analysis was executed using SPSS software (version 16). The differences between the means were subjected to one-way ANOVA followed by Tukey's post-hoc test. *P-value* ≤0.05 was considered as a level of significance.

Results

Effect of water extracts (WE) of *Padina pavonia* and *Caulerpa racemosa* on liver profile

CCl_4 remarkably increased serum levels of serum aminotransferases ALAT (623.8%) and ASAT (246.3%) and ALP (392.4%), serum LDH (228.2%), total bilirubin (370%) and direct bilirubin (552.1%) levels as compared to normal control rats. Intoxicated rats treated with water extract of *P. pavonia* decreased ALAT (62.0%), ASAT (49.0%), ALP (29.3%), LDH (65.7%), total bilirubin (70%) and direct bilirubin (79.3%) as compared to CCl_4 treated control rats. Same pattern was observed in intoxicated rats treated with water extracts of *C. racemosa* which significantly ($P \leq 0.05$) reduced the elevation of ALAT (82.2%), ASAT (46.7%), ALP (41.3%)), LDH (25.8%), total bilirubin (69.6%) and direct bilirubin (69.3%) levels. Fibrotic rats concomitantly treated with silymarin showed significant reduction in serum ALAT (71.0%), ASAT (41.3%), ALP (40.7%), LDH (45.6%), total bilirubin (31.2%) and direct bilirubin (43.3%). In general water extract of *C. racemosa* showed more promising effect as compared to water extract of *P. pavonia* on liver function markers in CCl_4 induced liver fibrotic rats. Further fibrotic rats showed significant elevation in blood glucose (25%)

levels as compared with normal rats. Further, treatment with water extract *P. pavonia* reversed the elevated blood glucose level (51.3%) (Table 1 and 2). Overall results demonstrated that water extracts of both seaweeds may reciprocate the elevated level of hepatic enzymes, metabolites and also ameliorates increased blood glucose levels in response to persistent liver assault.

Effect of water extracts (WE) of *Padina pavonia* and *Caulerpa racemosa* on renal function and lipid profile

Table 3.demonstrated that CCl4 has significantly ($P \leq 0.05$) impaired the kidney function and glucose metabolism by increasing serum urea (151.8%) and creatinine (816%) levels. CCl_4 was also responsible to reduce the serum cholesterol (75%) and TGs (62.5%) when compared with normal rats. Rats treated with water extracts of *P. pavonia* significantly ($P \leq 0.05$) reciprocated the elevation of serum urea (59.2%) and creatinine (88.8%) with remarkable elevation in serum lipid levels i.e. cholesterol (140.6%) and TGs (143.5%). Water extracts of *C. racemosa* showed significant ($P \leq 0.05$) reduction in serum urea (56.8%) and creatinine (86.1%). It also produced elevation in serum lipid levels i.e. cholesterol (74.4%) and TGs (50.5%) against CCl_4 induced liver fibrosis in rats. Silymarin also showed reduction in serum urea and creatinine levels i.e. (43.8%, 58.3%, respectively and increased the serum cholesterol and TGs levels i.e., (164.2%, 30.3%). Conclusively, both seaweed extracts have potential to maintain the renal and lipid metabolites which are enhanced in response to CCl_4 administration.

Effect of water extracts (WE) of *Padina pavonia* and *Caulerpa racemosa* on liver antioxidant profile

CCl_4 administration significantly damaged the liver tissues, which ultimately depleted the GSH (-76%) and elevated the MDA (551%) concentrations. The reduction in Gpx (49%) and CAT (31%) activity were also observed after administration of CCl_4, when compared with normal rats. The CCl_4 intoxicated rats concomitantly treated with water extract of *P. pavonia* showed increased hepatic antioxidant enzymes activities viz; Gpx (32.2), CAT (18.5%) along with improvement in hepatic GSH concentration (141.5%), and decreased MDA (57.4%) respectively. Same trend was observed for water extract of *C. racemosa* which significantly ($P \leq 0.05$) combated the adverse effect of CCl_4 induced oxidative stress indices by enhancing the activities of CAT (52.6%), Gpx (23%) and improved GSH (89.2%) concentration. Whereas reduced MDA (55.8%) level. Silymarin treated group showed significant ($P \leq 0.05$) reduction in oxidative stress indices i.e. GSH (115.3%), Gpx (87.7%), CAT (23.8%), and MDA (41.7%) (Figure 1 and 2). These results validated the antioxidant capability of water extracts of seaweeds, as they have potential to attenuate the oxidative stress indices in response to CCl4 administration.

Histological changes in liver tissues of normal and fibrotic rats treated water extract of *Padina pavonia* and *Caulerpa racemosa*

Normal control rats, showed stabilized hepatic lobules, consisted of normal central vein and peripheral 4 to 5 portal triads along with no evidence of portal expansion or necrosis (Figure 3A and 3B). CCl_4 intoxicated rats showed portal fibrosis with evidence of expansion in portal tract (grade -I) with massive acidophilic bodies (Figure 3C and 3D). In addition, they also showed macrophages infiltration, evidence of hepatic lobular and portal inflammation (grade-II & I) along with lipid deposition evident by clear vacuoles, abundance of loosely aggregated collagen fibers also seen around portal tract. Intoxicated rats treated with water extract of *C. racemosa* didn't show any presence of collagen fibers, with no evidence of lobular inflammation or piecemeal necrosis (Figure 3E and

3F). Whereas *P. pavonia* demonstrated mild portal expansion with no evidence of lobular inflammation or piecemeal necrosis, they also showed the deposition of collagen fibers around portal tract in lesser intensity (Figure 3G and 3H). The water extracts of both seaweeds have potential to stabilize the normal cellular morphology. However, further investigations are required to unveil the potent component(s) of seaweed, which may have a role to reciprocate the toxic effect induced by CCl_4 administration.

Discussion

Liver fibrosis is one of the serious health problems around the globe and it causes apoptosis or necrosis, inflammation, tissue remodeling and repair processes.[31,6] Excessive deposition of extracellular matrix (ECM), particularly deposition of collagen type (I & III) are major cause of liver fibrosis.[32]

In the present study, animal models of liver fibrosis have been developed via repeated administration of CCl_4. Chronic exposure of CCl_4 promotes degeneration of hepatocytes, which results in excessive secretion of aminotransferases (ALAT & ASAT).[33] In the harmony of previous findings, current data demonstrated significant ($P \leq 0.05$) elevation in serum levels of transaminases (ALAT & ASAT) in CCl_4 intoxicated model. Alkaline phosphatase is usually distributed in microvilli of liver sinusoids and bile duct capillary. CCl_4 administration induces hepatocytes degradation which is responsible to exerts pressure on bile duct capillaries and promotes excessive release of ALP in serum.[34] Persistent liver insults by CCl_4 administration significantly ($P \leq 0.05$) elevated the serum ALP level. Moreover, the CCl_4 intoxicated model showed significant ($P \leq 0.05$) increase in serum lactate dehydrogenases (LDH) levels. It usually releases under hypoxic conditions, responsible to shift metabolic cellular dependency on anaerobic glycolytic pathway. Persistent exposure of toxicants (CCl_4) induces hepatocytes stress, and promotes necrosis, which results in leakage of LDH into the serum. CCl_4 responsible for activation of HSCs (stored retinoid) may contribute to excessive bile secretion in blood[43] along with disturbance in redox mechanism, impaired mitochondrial integrity and promotes apoptosis [35]. In present work, CCl_4 intoxicated model also showed a remarkable elevation in serum total bilirubin and direct bilirubin besides increasing liver enzymes. A previous study also revealed that CCl_4 induced liver fibrosis increased serum liver markers; Aspartate transaminase, alanine transaminase, and total bilirubin. Current study showed that, supplementation of water extract of both seaweeds *C. racemosa* and *P. pavonia* caused significant ($P \leq 0.05$) alleviation in serum levels of ALAT, ASAT, ALP and LDH along with bilirubin in CCl_4 induced liver fibrotic rats. Further, both seaweeds have potential to reduce degradation of hepatocytes, and might have a role in deactivation of activated hepatic stellate cells evident by remarkable reduction in serum aminotransferases, dehydrogenases and phosphatase.

Seaweeds, *Caulerpa* species have been used around the globe due to their high nutritive value. Whereas, brown seaweeds have been recognized by researchers due to their medicinal importance [15,18.] Marine macro algae reported to have antioxidant activity and tendency to reciprocate the effect induced through various toxicants [1,15.] *Padina pavonia* also demonstrated a significant effect against thioacetamide induced hepatic fibrosis.[36] The hepatoprotective effect of *C. racemosa* has not been reported yet. Repeated episodes of liver insult by CCl_4 administration caused generation of ROS, usually they are short span molecules generated by partial reduction of oxygen.[37] Chronic exposure of toxicants disrupts balance between ROS and cellular antioxidant defense results in induction in oxidative stress. Further elevated levels of ROS caused depletion in antioxidants molecules and parameters i.e. GSH, Gpx and CAT and increased lipid peroxidation in terms of MDA. Nevertheless, ROS activates HSCs which promotes production of collagen and

contributes in progression of liver fibrosis. Present study showed a remarkable increase in malondialdehyde levels in CCl_4 controls rats as compared to normal rats. The following findings support our results that liver fibrosis promotes generation of ROS which ultimately caused reduction in GSH, Gpx and CAT activities[38]. CCl_4 treated rats administered with water extracts of both algal species significantly attenuate lipid peroxidation by decreasing the formation of MDA and improved hepatic GSH levels and elevated the Gpx and CAT activities as compared to CCl_4 control rats. *Padina pavonia* has been reported to improve the activities of superoxide dismutase and glutathione peroxidase induced by azoxymethane intoxication.[21] However, these results showed that seaweeds might play a role in down-regulation of ROS induced by repeated administration of CCl_4 as they showed decreased levels of MDA and improved GSH concentration and enhanced Gpx and CAT activity.

Previous findings suggested that fibrotic liver tissue showed significant morphological destruction characterized by expansion of central vein, portal vein and necrosis in CCl_4 treated rats.[45] Current findings validate the previous findings, as repeated episodes of CCl_4 treated rats showed expansion of central vein, portal vein and necrosis whereas there were no changes found in normal liver tissue. In-addition intoxicated rats showed abundance of collagen fibers which can clearly be visualized by Masson's staining. The current study was designed to elucidate the efficacy of water extract of *C. racemosa* and *P. pavonia* against liver fibrosis. Furthermore, water extract of *C. racemosa* and *P. pavonia* showed reciprocal effect against CCl_4 intoxication and improved the abnormal cellular architecture of liver tissue. Both extracts showed notable reduction in collagen fibers. Overall, results showed that these seaweeds have a tendency to attenuate the liver fibrosis. Moreover, there were several reports that showed that *P. pavonia* has potential to down regulate anti-apoptotic and pro-apoptotic pathways which may relate with morphological repairs of cellular architecture.[20] Hepatoprotective activity of seaweeds were found comparable to the silymarin, in improving the cellular architecture distorted by CCl_4 intoxication.

Previous studies showed that liver associated problems are not localized and it also affects the activity of nearby organs such as impairment in renal, lipid and glucose metabolism[1]. Liver damage also affects renal function by increasing the concentrations of urea and creatinine. In this study, CCl_4 administration showed remarkable increases in kidney markers i.e., urea and creatinine as compared to normal control rats. Sohail [15] reported that drug induced hepatotoxicity and nephrotoxicity has been suppressed by supplementation of marine macro algae. Another report also showed that *Padina pavonia* reversed renal dysfunction induced by CCl_4 administration[40]. Current findings showed that both algal species (*C. racemosa* and *P. pavonia*) extracts have potential to attenuate the elevated levels of serum urea and creatinine. Water extracts of both seaweeds have potential to reciprocate the renal toxicity induced as consequences of repeated administration of CCl_4.

Present study showed that CCl_4 administration disturbed the lipid metabolism by decreasing the cholesterol and triglyceride levels. Ishikawa[38] reported that CCl4-induced liver fibrosis caused alteration in lipid metabolism evident by decreased level of cholesterol and TGs. Distortion of hepatic parenchymal cells responsible to impaired lipid metabolism. However, algal extracts of both seaweeds significantly elevated the cholesterol and TGs levels toward the normal range. CCl_4 toxicity also affects the glucose metabolism resulting in hyperglycemia in CCl_4 control rats, which were reduced in seaweed treated groups. The ability of seaweed extracts to

abrogate the elevated concentration of glucose in serum may be due to protection of hepatocytes from toxic substances and their hypoglycemic potential [1].

CONCLUSION

Present study showed that both seaweeds (*C. racemosa* and *P. pavonia*) can reciprocate the cellular damages and associated complications produced by repeated administration of CCl_4. Both algal species showed significant positive effects on fibrotic rats. Efficacy of seaweed was compared with commonly known herbal medicines silymarin showed more or less similar effect on serum enzymes and hepatic antioxidant enzymes. In general, water extract of *C. racemosa* has great potential to attenuate the liver fibrosis induced by CCl_4. Hepatoprotective activity of *C.racemosa* may be due to presence of polysaccharides, which has been reported from other seaweed. *Caulerpa racemosa* is an edible seaweed and its use as diet supplement may be supportive for liver health.

Availability of data and materials

The data will be provided on request.

Compliance with ethical standards

Competing interests: The authors declare that they have no conflict interests.

Research involving human participants and/or animals: The experiment was conducted according to the rules of the Institutional Animal Ethics Committee (IAEC)/Advanced Studies and Research Board, University of Karachi.

Informed consent: Not applicable

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Authors' contributions

MA and KH collected the seaweed, prepared extracts and wrote the manuscript. Experiments on animals were conducted by MA and JA. Histopathology was carried out by MA and NK. SAQ and SE conceived and designed the experiments, helped in seaweed collection, supervised research work and improved the quality of the final version of the manuscript. All authors read and approved the final manuscript.

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Table 1. Effect of water extract of *Padina pavonia* and *Caulerpa racemosa* on liver enzymes in normal and carbon tetrachloride (CCl_4) intoxicated rats.

TREATMENTS	NORMAL RATS MODEL				FIBROTIC RATS MODEL			
	Normal Control	*P.padina (W.E)*	*C.racemosa* (W.E)	Silymarin	CCl_4 Control	*P.padina (W.E)*+CCl_4	*C.racemosa* (W.E)+CCl_4	Silymarin + CCl_4
ALAT (u/l)	42±7.3	50.5±3.25[a] (20.2%)	44.5±6.59[a] (5.9%)	38.02±0.28[a] (-9.4%)	304.2±34.06[a]* (623.8%)	115.5±14.45[b]* (-62.0%)	54.6±7.2[b]* (-82.2%)	88.2±5.03[b]* (-71.0%)
ASAT (u/l)	123±16.11	100.5±15.5[a] (-18.6%)	64±8.6[a] (-47%)	64.6±5.2[a]* (-47.4%)	426±54.39[a]* (246%)	217±23.7[b]* (-49.0%)	227±28.34[b]* (-46.7%)	140±7.2[b]* (-65.7%)

ALP (u/l)	79±8.85	114±14.5[a] (44.3%)	72±14.3.6[a] (-8.8%)	67.6±5.2[a] (-15.1%)	389±49.1[a]* (392.4%)	275±14.6 [b]* (-29.3%)	250± 1.5[b*] (-41.3%)	230.3±47.5[b*] (-40.7%)
LDH (u/l)	124.6±8.1	146.3±19.6[a] (17.4%)	184.0±25.4[a] (48.1%)	170.3±0.57[a] (36.9%)	409.1±15.07[a]* (228.25%)	162.3±15.6[b]* (-58.3%)	303.33±24.9[b]* (-25.8%)	222.3±14.04[b*] (-45.6%)

The data were analyzed by one-way ANOVA followed by Tukey's Post Hoc test
*=p(< 0.05),
[a] indicates the comparison with control rats, [b] indicates the comparison with CCl_4 control rats.
Data were expressed in means ± Standard deviation (n=6). The values in parenthesis represent percentage (%) increased or decreased as compared to their respective control

Table 2. Effect of water extract of *Padina pavonia* and *Caulerpa racemosa* on liver metabolite and blood glucose in normal and carbon tetrachloride (CCl_4) intoxicated rats.

TREATMENTS	NORMAL RATS MODEL				FIBROTIC RATS MODEL			
	Normal Control	*P.padina* (W.E)	*C.racemosa* (W.E)	Silymarin	CCl_4 Control	*P.padina* (W.E)+CCl_4	*C.racemosa* (W.E)+CCl_4	Silymarin + CCl_4
Glucose (mg/dl)	116.18±8.07	89.16±5.7 [a] (-23.3%)	115±14.30 [a] (0.94%)	98±3.0 [a] (-15.59%)	146.3±14.3 [a]* (25.8%)	71.16±7.55[b]* (-51.3%)	147±22.4[b] (-0.47%)	100±1.5 [b]* (-31.64%)

Total Bilirubin (mg/dl)	0.34±0.04	0.21±0.04 [a] (-38.2%)	0.23±0.05 [a] (-32.3%)	0.6±0.05[a] (76.4%)	1.6±0.12[a]* (370%)	0.48±0.08 [b]* (-70%)	0.5±0.06 [b]* (-69.6%)	1.1±0.05 [b*] (-31.2%)
Direct. Bilirubin (mg/dl)	0.23±0.05	0.21±0.04[a] (-8.6%)	0.51±0.07[a] (121.7%)	0.3±0.05[a] (30.4%)	1.5±0.16[a]* (552%)	0.31±0.04[b]* (-79.3%)	0.46±0.08[b]* (-69.3%)	0.85±0.04 [b*] (-43.3%)

The data were analyzed by one-way ANOVA followed by Tukey's Post Hoc test
*=p(< 0.05),
[a] indicates the comparison with control rats, [b] indicates the comparison with CCl_4 control rats.
Data were expressed in means ± Standard deviation (n=6). The values in parenthesis represent percentage (%) increased or decreased as compared to their respective control

Table 3. Effect of water extract of *Padina pavonia* and *Caulerpa racemosa* on renal and lipid profile in normal and carbon tetrachloride (CCl_4) intoxicated rats.

TREATMENTS	NORMAL RATS MODEL				FIBROTIC RATS MODEL			
	Normal Control	*P.padina(W.E)*	*C.racemosa* (W.E)	Silymarin	CCl_4 Control	*P.padina* (W.E)+CCl_4	*C.racemosa* (W.E)+CCl_4	Silymarin + CCl_4
Urea (mg/dl)	27.6±6.8	23.3±4.0[a] (-15.5%)	25.6±5.4[a] (-7.2%)	22.00±1.00 [a]	69.5±10[a]* (151.8%)	28.3±5.4[b]* (-59.2%)	30±3.07 [b]* (-56.8%)	39±0.57 [b]* (-43.8%)

				(-20.2%)				
creatinine (mg/dl)	0.2±0.04	0.2±0.05[a] (0%)	0.43±0.05[a] (115%)	0.7±0.05 [a] (250%)	1.8±0.14[a]* (816%)	0.2± 0.02 [b]* (-88.88%)	0.25±0.039[b]* (-86.1%)	0.75±0.05[b]* (-58.3%)
cholesterol (mg/dl)	107±9.6	79±7.6[a] (-26.1%)	137.5±10.6[a] (28.03%)	80.3±0.5 [a] (-24.9%)	26.6±8.6[a]* (-75.1%)	64± 5.5 [b]* (140.6%)	46.4±4.3[b]* (74.4%)	70.3±11.5[b]* (164.2%)
TGs (mg/dl)	103.1±16.7	84.16±5.8[a] (-18.4%)	126±3.8[a] (-22.3%)	90±6.00[a] (-12.6%)	38.6±3.2[a]* (-62.5%)	94±6.3[b]* (143.5%)	58.1±6.6[b]* (50.5%)	50.3±3.5[b]* (30.3%)

The data were analyzed by one-way ANOVA followed by Tukey's Post Hoc test
*=p(< 0.05),
[a] indicates the comparison with control rats, [b] indicates the comparison with CCl_4 control rats.
Data were expressed in means ± Standard deviation (n=6). The values in parenthesis represent percentage (%) increased or decreased as compared to their respective control