Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J6-78

Hepatoprotective efficiency of methanol extract of red algae against chromium-induced oxidative damage in Wistar rats

Murugesan Subbiah[1*], Bhuvaneswari Sundaresan[1], Kalandar, Ameer[2], Sivamurugan Vajiravelu[3*]

[1]*Division of Algal Biotechnology and Bionano Technology, Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai, 600030. India*

[2]*Faculty of Medical Sciences, Haramaya University, Harar, Ethiopia*

[3]*Post Graduate and Research Department of Chemistry, Pachaiyappa's College, Chennai, 600030, India*

ARTICLE INFO

Article history:
Received 18 Apr 2016
Received in revised form 19 May, 2nd revised form 30 May 2016
Accepted 20 Jun 2016
Available online 13 Jul 2016

Keywords:
Oxidative liver injury
Red algae
Chromium (VI)
Hepatoprotection
Lipid peroxidantion
Antioxidant enzymes

ABSTRACT

Objective: To investigate the hepatoprotective activity of red algae *Portieria hornemannii* (Lyngbye) Silva (*P. hornemannii*) and *Spyridia fusiformis* Boergesen (*S. fusiformis*) by using the chromium treated rat liver as the animal model.
Methods: The extract of red algae at a dosage of 0.200 g/kg of whole body weight was orally administrated to Cr (VI) intoxicated rats for 28 consecutive days. The effect of drug in rats was evaluated by comparing the degree of the production of enzymes responsible for antioxidant activity such lipid peroxidase, superoxide dismutase, catalase and reduced glutathione with Cr (VI) analogs in the absence of any secondary treatment. The overall damage of liver was detected by measuring serum enzymes such as aspartate amino transferase and alanine aminotransferase activities which released into the blood from the damaged cells.
Results: It was observed that these enzyme levels were noticed in the animals treated with methanol extracts of red algae (200 mg/kg) through preventing the leakage of the above enzymes into the blood. The hepatoprotection obtained using LIV 52 (standard reference drug) appeared relatively higher. The antihepatotoxic potential of red algae *P. hornemannii* and *S. fusiformis* might be due to their antioxidative and membrane stabilizing activities.
Conclusions: Our results indicated that the extract of *P. hornemannii* and *S. fusiformis* obtained from methanol could be a promising hepatoprotective agent against chromium (VI)-induced liver damage.

1. Introduction

Marine algae are potentially prolific birthplaces for biologically active natural products that might signify an useful lead for the design of new medicines with high values[1]. In the last few decades, numerous compounds with unique chemical entities have been obtained from marine plants and animals with interesting biological activities[2].

The natural products which provide protection to animals and humans from damage caused by free radicals generated from biological redox processes are classified as antioxidants. The free radicals are known to induce oxidative damage to most of the organs of the body by peroxidation of lipids, damage of proteins and DNA[3]. During the past two decades, several investigations have been carried out to identify potential rich sources of natural antioxidants[4-9].

Oxygen-derived free radicals are common byproducts of the metabolism process. However, these compounds are highly active and can cause severe damages, commonly called oxidative damages, to the cell membranes and other cellular structures[10]. The free radical damages associated with diseases such as atherosclerosis, cataract formation, ageing and carcinogens have been reported[10]. Human body possesses a complex antioxidant defense system that utilizes various vitamins, minerals and other naturally producing substances to counteract the destructiveness of the free radicals[10]. Consequently, it is possible to prevent the radical damage by

*Corresponding author: Dr. Murugesan Subbiah, Division of Algal Biotechnology and Bionano Technology, Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai, 600030. India.
Tel: +919840276446
E-mail: smurugesan5@gmail.com

All procedures followed in this investigation were prescribed by the Institute Animal Ethical Committee (IAEC) (Approval No. IAEC/006/2011).

Foundation Project: Supported by University Grants Commission, New Delhi [Grant No. F1-17.1/2011-12/RGNF-SC-TAM-5342/(SA-III/Website)].

The journal implements double-blind peer review practiced by specially invited international editorial board members.

supplementing the diet with certain food, nutrients and herbs rich in antioxidants.

In the present investigation was focused on the hepatoprotective efficiency of the methanol extract of marine red algae *Portieria hornemannii* (Lyngbye) Silva (*P. hornemannii*) and *Spyridia fusiformis* Boergesen (*S. fusiformis*) against chromium-stimulated liver toxicity in rats.

2. Materials and methods

2.1. Collection and extraction of marine algae

Fresh materials of *P. hornemannii* and *S. fusiformis* were obtained from south east coast of Tamil Nadu as mentioned in our earlier report[7] and the algae were identified by the standard manual[11]. The salt and sand stuck on the surface of the freshly collected samples were removed by using sterilized seawater. The shade-dried seaweeds were finely powdered using a blender and stirred in methanol solvent overnight. The contents were filtered and concentrated to crude extract. The methnol crude algal extract was stored at room temperature for further analysis.

2.2. In vivo antioxidant activity

2.2.1. Selection of animals

Adult Wistar albino rats were obtained from KM College of Pharmacy in Uthangudi, Madurai, India. In our investigation, animals of both sex weighing between 180 and 220 g were used.

2.2.2. Maintenance of animals

The animals housing was made of polypropylene cages and the animals were preserved under standard laboratory conditions with the temperature of (25 ± 2) ℃ and 14 h dark/10 h light cycle. They had free access to standard dry pellet (Amrut, Bangalore) and water *ad libitium*. The rats were acclimatized to the laboratory conditions for 30 days before the commencement of the experiments. All procedures followed in this investigation were prescribed by the Institute Animal Ethical Committee (IAEC) (Approval No. IAEC/006/2011). By following the standard procedure, the rats were divided based on their sex and isolated for 15 days before the experiment. The animals were fed on a healthy food and maintained in a hygienic environment.

2.2.3. Experimental set up

The animals were randomly grouped into five groups with six animals per group and treated for 28 days. The animals in group I received normal saline in a dose of 10 mL/kg and the group is taken as the control; animals in group II were given chromium 30 mg/kg (30% v/v, 1 mL/100 kg) orally and taken as the toxic control; animals in group III were administered with LIV 52 (56 mg/kg) and this group was consider as the standard drug control; animals in group IV were given the methanol extract of *P. hornemannii* (MEP) (200 mg/kg) and taken as the treatment control; and animals in group V were fed with the methanol extract of *S. fusiformis* (MES) (200 mg/kg) and this group was treated as the treatment control as well. Standard drug as well as two extracts were given for 1 h. Groups III–V were treated with the algal extracts after the administration of chromium.

2.2.4. Sample collection and processing

On the 29th day of the experiment, rats were sedated and the blood was taken via intra-cardiac perforation of rats. The rats were starved for 12 h earlier to the withdrawing of the blood sample. The animals were sacrificed by cardiac dislocation, and their liver was removed, cleaned using normal saline and stored in a refrigerator. A tissue homogenate weighing 10% w/v was prepared in 0.9% saline followed by the solution centrifuged at 5 000 r/min for about 10 min at 4 ℃. The supernatant solution was taken for the measurement of various biochemical parameters. A small piece of liver was also preserved in formal saline and used for histopathological studies. All the assays were carried out within 48 h after the sacrifice of the animals.

2.2.5. Biochemical studies

2.2.5.1. Lipid peroxidation assay

The secondary product, malondialdehyde (MDA) formed by lipid peroxidation, reacted with thiobarbituric acid at acidic pH about 3.5. The red colour pigment formed was isolated using n-butanol-pyridine mixture and estimated from measurement of the absorbance fixed at 532 nm.

2.2.5.2. Superoxide dismutase activity (SOD)

SOD was assessed by the method described by Kono[12]. The reaction was commenced by adding hydroxylamine hydrochloride to the nitro blue tetrazolium chloride containing reaction mixture followed by the addition of nuclear fraction of liver homogenate. The reduction of nitro blue tetrazolium chloride was suppressed by the presence of SOD and it was estimated by measuring the absorbance at 560 nm using spectrophotometer. The obtained readings were stated as units per mg of protein with one unit of enzyme defined as the amount of superoxide dismutase needed to suppress the reaction rate by 50%.

2.2.5.3. Activity of catalase enzyme (CAT)

The activity of CAT was examined by the method described by Luck[13], in which the hydrogen peroxide breakdown was estimated at the absorbance of 240 nm. The reaction mixture composed of 3 mL of hydrogen peroxide and phosphate buffer (0.012 5 mol/L of H_2O_2) and 0.05 mL of homogenate supernatant solution of animal liver and the variation in the absorbance at 240 nm was measured. The activity of CAT was determined using the H_2O_2 (0.07) milli molar extension coefficient. The absorbance readings were expressed as micromole of peroxide decomposed per min per milligram of protein.

2.2.5.4. Estimation of reduced glutathione (GSH)

The GSH in the liver was determined based on the procedure described by Ellman[14]. The liver homogenate solution of about 0.75 mL was treated with 0.75 mL of 4% sulphosalicyclic acid and the resulting precipitate was subjected to the centrifugation about 1 200 r/min for 10–15 min at 4 °C. The reaction assay consisted of 0.5 mL of supernatant solution and 4.5 mL of 0.01 mol/L DTNB (5-5'-dithio-bis-2-nitro benzoic acid), 0.1 mol/L phosphate buffer about pH 8.0. The intensity of yellow colour formed was immediately measured at the absorbance of 412 nm. The absorbance readings were converted micromole of reduced GSH per mg of proteins.

2.2.5.5. Estimation of aspartate aminotransferase (AST)

AST obtained from the rat liver in the blood sample was determined based on the procedure developed by Reitman and Frankel[15].

Oxaloacetate so formed from AST catalyzed reaction of α-ketoglutarate and L-aspartate was condensed with 2,4-Dimethylbenzaldehyde to give the respective hydrazone derivatives, which gave a brown colour in a basic medium and this was measured photocolorimetrically.

2.2.5.6. Estimation of alanine amino transferase (ALT)

The amount of ALT presenting in the rat liver blood serum was determined according to the procedure prescribed by Reitman and Frankel[15].

The pyruvate so formed from ALT catalyzed reaction of α-ketoglutarate and L-alanine was condensed with 2,4-dinitrophenylhydrazine to give the respective hydrazone derivative, which produced a brown colour in basic medium and this could be estimated using photocolorimetrically.

2.3. Statistical analysis

All the experimental values in this investigation were expressed as mean ± SD. All pairwise multiple comparison procedures were attempted by Student-Newman-Keuls procedure using One-way ANOVA followed by SPSS 17.0. $P < 0.01$ was considered to be significant. Triplicate assays were performed for each set of test conditions.

3. Results

The MEP and MES were investigated for their antioxidant activities in Wister albino rats against chromium-induced liver damage. The *in vivo* antioxidant status including SOD, CAT, reduced GSH, lipid peroxidase, AST and ALT was assayed in the serum sample and liver tissues of all the groups from group I to group V as mentioned above. The results showed that the antioxidant ability have been significantly enhanced in the algal extracts treated animal groups as compared to the disease control group.

3.1. Effect of MEP and MES

The activity of the MEP and MES is shown by change in body weight during the chromium(VI) tempted oxidative stress was summarized in Table 1. Chromium(VI) administration caused significant reduction in the body weight. However, no significant change in the body weight was observed with the animals fed with the algal extracts after chromium intoxification as well as no significant change in the food and water intake.

Table 1
Effect of MEP and MES on body weight of normal and experimental animals.

Group	Initial body weight (gm)	Final body weight (gm)
Group I	215.80 ± 6.70	225.45 ± 4.65
Group II	218.50 ± 6.40	172.40 ± 3.50[a*]
Group III	222.50 ± 7.30	230.70 ± 5.45
Group IV	215.50 ± 5.30	218.12 ± 4.60
Group V	210.12 ± 5.25	220.40 ± 5.90

[a*]: Values were significantly different from the normal control (Goup 1) at $P < 0.01$.

3.2. Activity of MEP and MES on SOD level

The decreased level of SOD [(31.45 ± 2.15) IU/L] in group II indicated the liver damage during chromium intoxication as compared to that of group I which showed (33.70 ± 2.30) IU/L SOD level. Significant increase in the SOD level was observed after the treatment with the algal MEP and MES at a dose of 0.200 g/kg of animal weight. The experimental algae *P. hornemanni* showed an increase in the SOD level [(32.40 ± 2.25) IU/L] as compared to *S. fusiformis* [(31.80 ± 2.45) IU/L) (Table 2). From this observation, it was very interesting to note that the methanol extract of these algae were able to significantly restore the SOD level as that of the normal control group.

3.3. Effect of MEP and MES on GSH and CAT levels

The tissue samples of the disease control group have clearly

Table 2
Effect of *P. hornemannii* and *S. fusiformis* on chromium induced free radicals in rats.

Group ($n = 6$)	SOD (IU/L)	CAT (μg/min/mg protein)	Reduced GSH (mg/dL)	Lipid peroxidation (nmol/mL)	AST (IU/L)	ALT (IU/L)
Group I	33.70 ± 2.30	282.80 ± 4.55	116.40 ± 4.40	170.50 ± 3.60	192.45 ± 3.20	87.80 ± 2.85
Group II	31.45 ± 2.15	190.25 ± 3.45[a*]	65.25 ± 1.45[a*]	264.50 ± 4.80[a*]	332.10 ± 7.80[a*]	230.35 ± 5.20[a*]
Group III	30.65 ± 2.20	240.45 ± 4.40[b*]	98.5 ± 3.30[b*]	228.80 ± 3.50[b*]	232.30 ± 4.20[b*]	130.45 ± 3.40[b*]
Group IV	32.40 ± 2.25	210.20 ± 2.80[b*]	85.50 ± 2.50[b*]	210.80 ± 2.90[b*]	265.75 ± 5.30[b*]	165.52 ± 2.45[b*]
Group V	31.80 ± 2.45	214.30 ± 2.90[b*]	88.40 ± 3.45[b*]	212.65 ± 3.24[b*]	255.65 ± 4.80[b*]	155.80 ± 2.85[b*]

Values are expressed as mean ± SEM and found out by using One-way ANOVA followed by Newman Keul's multiple range test.
[a*]: Values were significantly different from the normal control (Group 1) at $P < 0.01$; [b*]: Values were significantly different from toxic group (Group 2) at $P < 0.01$.

showed that chromium intoxication significantly reduced the CAT and GSH levels. On the other hand, both the GSH and CAT levels were significantly increased after the oral administration of MEP and MES at 0.200 g/kg of the animal weight. The highest CAT activity [(214.30 ± 2.90) µm/min/mg protein] was observed in the tissues of *S. fusiformis* treated animal group as compared to *P. hornemannii* [(210.20 ± 2.80) µm/min/mg protein] analogous. Similarly, *S. fusiformis* treated group showed the reduced GSH level [(88.40 ± 3.45) mg/dL] as compared to *P. hornemannii* [(85.50 ± 2.50) mg/dL]. The results were summarized in Table 2. The observed results were comparatively similar to that of the LIV 52 administrator group which showed (240.45 ± 4.40) µg/min/mg proteins of CAT and (98.5 ± 3.30) mg/dL of GSH levels.

3.4. Effect of MEP and MES on lipid peroxidation

Increased MDA level in the liver tissues indicated the hepatic tissue damage through the over lipid peroxidation caused by the chromium intoxication. The MDA levels were enhanced in group II and this was significantly reduced by the experimental algae *S. fusiformis* by (212.65 ± 3.24) nmol/mL and *P. hornemannii* by (210.80 ± 2.90) nmoles/mL when compared to the normal control (170.50 ± 3.60) nmol/mL) (Table 2). The drug control group showed more or less similar results.

3.5. Effect of MEP and MES on ALT and AST levels

The ALT and AST levels of group II was found to comparatively develop higher levels than group I. The oral administration of the algal extracts of the experimental algae MEP and MES at 0.200 g/kg of the animal body weight was able to restore the levels of AST and ALT as compare to that of the normal control. In both the cases, *P. hornemannii* showed the highest AST and ALT activities [(265.75 ± 5.30) IU/L and (165.52 ± 2.45) IU/L] and comparatively low activities were observed in *S. fusiformis* [(255.65 ± 4.80) IU/L and (155.80 ± 2.85) IU/L] (Table 2). The LIV 52 treated group showed similar results which proved its efficiency.

3.6. Histopathology

Histopathological investigation revealed that the chromium intoxication resulted in severe liver damage showing sinusoidal hepatic congestion and the portal vessel and severe malformation leading to the formation of malignant hepatocytes (Figure I). Chromium intoxicated animals treated with LIV 52 (a commercial drug for liver cancer) had shown a reduction in malignancy to some extent. However, the chromium intoxicated animals treated with the MEP and MES exhibited higher extent of malignancy with less hepatocyte diffuse necrosis and mononuclear infiltrates as compared to LIV 52 drug treatment. The histopathological observation made on the animals treated with the experimental algal extracts showed similar features to that of the control.

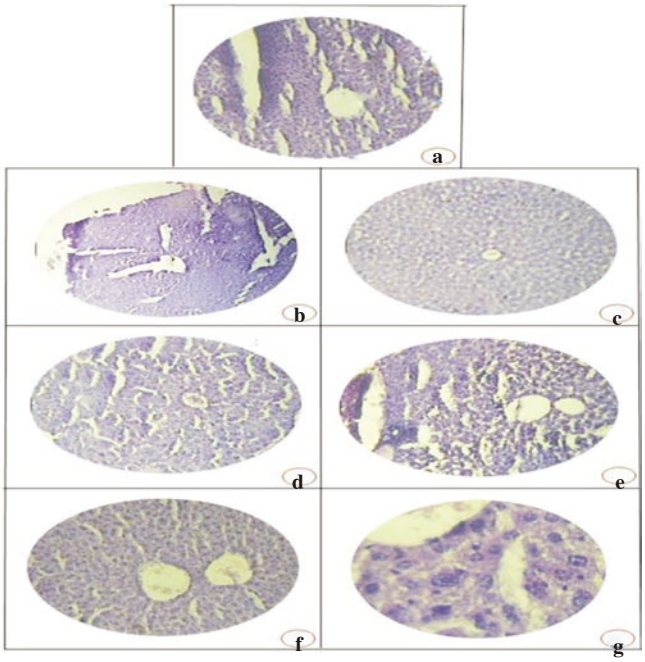

Figure 1. Photomicrograph of a cross section in liver stained with haematoxylin and eosin (magnification × 400x).
a: Animals in the normal control show structure of liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appears normal; b: Chromium-intoxicated animals show structure of liver presented hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate; c: Animals treated with LIV52 show structure of liver presented mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, no cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate; d and e: Animals treated with MEP show structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate; f and g: Animals treated with MES shows structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

The present investigation has demonstrated that experimental algae treated groups showed the significantly improved antioxidant activity and antioxidant enzymes levels in the chromium intoxicated liver tissue samples as good as the standard LIV 52.

4. Discussion

The seaweed extracts have received increased attention, due to their pharmacological effects, particularly *in vivo* hypolipidemic, antioxidant, immunological and antitumour activities[16-19]. The secondary metabolites containing phenolic groups are efficient antioxidant molecules, and a fraction of these compounds may be used as an antioxidant supplement in healthy diets.

No attempts have been made to use MEP and MES with chromium intoxication. Hence, the present investigation was focused to examine the antioxidant efficiency against the chromium(VI) stimulated oxidative damage in male Swiss albino rats. This investigation demonstrates that the methanol extract of the selected marine red algae at a dosage of 0.200 g/kg of animal weight protected the tested animals significantly against the oxidative damage.

Oral administration of chromium(VI) has significantly reduce the body weight. The well-known oxidizing agents derived from chromium(VI) species are capable of causing tissue damage through carcinogenic, mutagenic and teratogenic activities[20]. Chromium(VI) compounds are easily reduced to chromium(III) species during the biological oxidation process happening in cells, which generates free radicals causing the adverse biological effects[21].

The peroxidation of polyunsaturated fatty acid produces a major oxidized product, MDA, and acts as indicator for lipid peroxidation[22]. Intoxication of a heavy metal such as chromium significantly escalates the amount of lipid peroxidation which indicated by MDA levels in all organs which got significantly increased with respect to aging. MEP and MES effectively inhibit the MDA formation.

GSH is considered to be tripeptide made of amino acids such as glycine, glutamic acid and cysteine. It is a naturally available antioxidant. Thus, GSH levels have crucial role in tissue injury caused by toxic substances and offer protection from them[12].

CAT is antioxidant which destroyes H_2O_2 and prevents the tissues from reactive hydroxyl radicals[23]. As observed in the present investigation, SOD and GSH-Px activities marginally lowered with age, but no statistical significance changes observed in other organs[24-26]. This is expected that the lowering activity of SOD and GSH-Px have no influence in damage caused by lipid peroxidation. There was a substantial decrease in GSH and CAT levels observed in the liver tissues to overcome the chromium(VI) induced oxidative stress. However, the changes in the SOD levels not observed in the chromium(VI) intoxicated animals and the present study are similar results as observed by earlier reports[27]. On the other hand, chromium(VI) intoxication showed increased levels in ALT and AST raveling hepatic damage caused by the chromium. Many researchers have also demonstrated the hepato-toxic effect of chromium(VI) [28,29], which is mainly caused by lipid peroxidation. It is very interesting to note that pretreating the animals with MEP and MES protected them from the adverse liver damage caused by the chromium heavy metal.

Liver damage was identified by measuring levels of enzymes such as AST, ALT, alkaline phosphatase (ALP) and gamma glutamyl transferase and those have been released into the blood stream from the damaged cells. The levels of those enzymes could be used as markers for hepatic cell damage. The elevated serum levels of ALT, ALP and AST may be due to hepatocellular necrosis, which caused an increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream. The increase in ALP activities represents general hepatic toxicity[30]. Induction of ALP synthesis is the usual response of the liver to any form of biliary obstruction[31]. Similar finding was observed before[32-34]. There was a significant decrease in ALT, ALP and AST levels when co-treated with chromium(VI), *P. hornemannii* and *S. fusiformis*. The normalization of the above enzyme levels in rat treated with MEP and MES (200 mg/kg body weight) clearly establishes the hepatoprotective effect of these experimental algal extracts.

Treatment of chromium-caused hepatitic damage in animals fed with algal extracts lowers the serum thiobarbituric acid-reactive substances levels and increase the hepatic glutathione concentrations when compared to the chromium-intoxicated animal group. The most of the oxidative stress investigations in rats have used tissue damage indicator thiobarbituric acid reactive substances[27,28]. In addition, a marked growth in CAT activity was observed after treatment with the algal extracts. The levels of other marker enzymes have coming back to the near normal levels when rats are treated with *P. hornemannii* and *S. fusiformis,* which is a clear exhibition of anti-hepatotoxic activity due to the presence of various kinds of phytochemicals in the seaweed extract. The results obtained in the present investigation suggest that *P. hornemannii* and *S. fusiformis* could be a potential natural antioxidants and have a potential to design new drug compositions.

Chromium(VI) could damage liver and kidney, the two important vital organs. The free radicals generated in the chromium-induced liver damage could initiate the lipid peroxidation in liver[35]. Thus, Chromium(VI) intoxication may cause fatty liver as well as cell necrosis and play a vital role in the depletion of reduced GSH, increased lipid peroxidation, membrane damage and depression of protein synthesis and loss of enzyme activity.

Meena *et al.*[36] stated that the extract of *Sargassum polycystem* at 125 mg/kg body weight significantly altered the D-galactosamine-induced liver damage in rats. The oral treatment using *Sargassum polycystem* alcoholic extracts at a dosage of 200 mg/kg showed liver protection against acetaminophen-induced damages in liver tissue and reduced the inflammatory infiltration[37]. Karthikeyan *et al.*[38] reported that the diethyl ether extract of *Padina boergesenii* significantly reduced the liver damage like cytoplasmic swelling, nuclear damage and edematous hepatocytes. In conclusion, concentration-dependent antioxidant properties as well as substantial protection against liver damage was observed in the methanol extract of both the experimental algae. The protections against the liver damage by the seaweed *P. hornemannii* and *S. fusiformis* are comparable to LIV-52. MEP and MES might follow the mechanism for the protection against liver damage involving its action by scavenging the free radicals and interrupting those radicals involved in Chromium(VI) metabolism through microsomal enzymes[39]. By suppressing the reactive oxygen free radical species, the *P. hornemannii* and *S. fusiformis* extracts might delay their reaction with the polyunsaturated fatty acids and eliminate the lipid peroxidative processes enhancement[40]. The secondary metabolites with antioxidant properties isolated from the natural marine resources are highly valuable and offer wide scope by correcting the imbalance through a proper diet.

The current investigation demonstrated that MEP and MES could be a promising hepatoprotective agent against chromium(VI) induced liver damage. The *in vivo* hepatoprotective activity of *P. hornemannii* and *S. fusiformis* might be owing to the presence of antioxidant phytochemicals.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We are thankful to Dr. N. Chidambaranathan, Vice Principal and Head of the Department of Pharmacology, KM College of Pharmacy, Madurai, Tamilnadu, India, for his support extended by providing the experimental animal facilities. The financial support received from University Grants Commission, New Delhi [Grant No. F1-17.1/2011-12/RGNF-SC-TAM-5342/(SA-III/Website)] is gratefully

acknowledged.

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