



Original article doi: 10.12980/jclm.4.2016j5-204

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Hepatoprotective potential of three *Sargassum* species from Karachi coast against carbon tetrachloride and acetaminophen intoxication

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ARTICLE INFO

Article history:

Received 19 Oct 2015

Received in revised form 20 Oct 2015

Accepted 28 Nov 2015

Available online 21 Dec 2015

Keywords:

Sargassum spp.

Hepatoprotective potential

Carbon tetrachloride

Acetaminophen

Karachi coast

ABSTRACT

Objective: To assess the hepatoprotective effect of ethanol extracts of *Sargassum variegatum* (*S. variegatum*), *Sargassum tenerrimum* (*S. tenerrimum*) and *Sargassum binderi* occurring at Karachi coast against carbon tetrachloride (CCl₄) and acetaminophen intoxication in rats.

Methods: *Sargassum* species were collected at low tide from Buleji beach at Karachi coast. Effect of ethanol extracts of *Sargassum* spp., on lipid parameter, serum glucose and kidney function was examined. Liver damage in rats was induced by CCl₄ or acetaminophen. Rats were administered with ethanol extracts of *S. tenerrimum*, *S. variegatum* and *Sargassum binderi* at 200 mg/kg body weight daily for 14 days separately. Hepatotoxicity was determined in terms of cardiac and liver enzymes and other biochemical parameters.

Results: *S. variegatum* showed highest activity by reducing the elevated level of hepatic enzymes, bilirubin, serum glucose, triglyceride with restoration of cholesterol. Urea and creatinine concentrations were also significantly ($P < 0.05$) reduced as compared to acetaminophen intoxicated rats. *S. tenerrimum* and *S. variegatum* showed moderate activity against CCl₄ hepatic toxicity.

Conclusions: The protective role of *S. variegatum* against acetaminophen liver damage and its positive impact on disturbed lipid, glucose metabolism, kidney dysfunction and *S. tenerrimum* against CCl₄ liver toxicity suggest that *Sargassum* species offer a non-chemical means for the treatment of toxicity mediated liver damage.

1. Introduction

Liver diseases caused by infectious agents, chemicals or higher doses of the drugs (pain killers, antibiotics, vitamins, medicines are major public health problem worldwide[1]. The most common drugs and chemicals said to be hepatotoxic are acetaminophen (paracetamol), carbon tetrachloride (CCl₄), D-galactosamine, thioacetamide, aflatoxin, cyclosporine A, alcohol, 2-acetyl-aminoflourene, tacrine and rifampicin[2]. Acetaminophen and CCl₄ induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs[3].

When the liver is injured as a result of the introduction of infectious agents or chemicals, the serum levels of glutamic-pyruvate transaminase [alanine aminotransferase (ALT)] and glutamic oxaloacetic transaminase [aspartate aminotransferase (AST)] are raised significantly[4] along with leakage of alkaline phosphate (ALP) and lactate dehydrogenase (LDH)[5]. Similarly liver damage also leads to

elevation of the bilirubin level in serum which is used as a marker for liver function[6]. Liver damage also affects kidney function and lipid metabolism[7]. Medicines used for the treatment of liver diseases have many side effects, with mild to severe toxicities[8]. Herbs play a critical role in the management of many liver disorders and a number of herbs, plant extracts and plant derived natural compounds have been screened for liver management[9]. The therapies based on nature are believed to be safer and better than standard medical practice[10].

Marine algae have remained in use by man in a variety of ways since medieval times and have conventionally been used as marine vegetables[11,12]. Many algae have found to produce a range of complex compounds including some of considerable medicinal value[13-15]. *Sargassum* species are brown algae belong to the order Fucales are commonly found on the coast of many countries including Pakistan[16-18]. Hepatoprotective role of *Sargassum* species have been reported from various part of the world[19-22]. Species of *Sargassum* occurring at Karachi coast are yet not evaluated for hepatoprotective potential, although antibacterial[23], nematicidal[24], hypolipidaemic[25] and antioxidant[26] activities have been reported from the Karachi coast of Pakistan. The present report describes the hepatoprotective effect of ethanol extracts of *Sargassum variegatum* (*S. variegatum*), *Sargassum tenerrimum* (*S. tenerrimum*) and *Sargassum binderi* (*S.*

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binderi) occurring at Karachi coast against CCl₄ and acetaminophen intoxication in rats. Effect of ethanol extracts of *Sargassum* spp., on lipid parameter, serum glucose and kidney function was also examined.

2. Materials and methods

2.1. Algal material

Sargassum species were collected at low tide from Buleji beach at Karachi coast. These seaweeds species were washed thoroughly in order to remove salts, debris and epiphytes. Voucher specimens and herbarium sheets of the seaweeds were prepared and record was kept in the seaweed Herbarium, MAH Qadri Biological Research Center, University of Karachi. Professor Dr. Mustafa Shameel (now late), Department of Botany, University of Karachi, Pakistan identified the *Sargassum* spp., as *S. variegatum*, *S. tenerrimum* and *S. binderi*. For experimental use seaweeds were dried under shade, grinded in an electrical grinder and fine powder was stored in polyethylene bags at room temperature.

2.2. Ethanol extract

Dry powder of each seaweed was soaked in distilled ethanol for one week at room temperature, separately. The mixture was filtered through cotton wool twice. The filtrate was concentrated to dryness using rotary vacuum evaporator (Buchi R-200, Japan) at 40 °C. The finally obtained extract was stored at room temperature.

2.3. Animals

Healthy male Wistar rats (140–170 g) were purchased from Dow University of Health Sciences, Karachi. All the animals were housed in prebedded polyethylene cages (3 rats/cage) 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 acclimatized animals with laboratory conditions. The experiment was conducted according to the rules of Institutional Animal Ethics Committee, University of Karachi.

2.4. Induction of hepatotoxicity

Liver damage was induced by CCl₄ or acetaminophen. CCl₄ was purchased from Merck (private) limited (France). Animals were injected with CCl₄, (intraperitoneal) 1:1 in olive oil at 2 mL/kg, body weight whereas acetaminophen (Sigma Aldrich, USA.) was also administered intraperitoneally at 1 g/kg, body weight and the dose was prepared in 40% polyethylene glycol (in normal saline) with constant stirring and mild heating.

2.5. Preventive effect of ethanol extracts of *Sargassum* species in CCl₄ intoxicated rats

To evaluate the effect of *Sargassum* species on CCl₄, intoxication, rats were randomly divided into 5 groups of 6 rats in each group.

Normal control group: Animals were administered with distilled water daily for 14 days.

CCl₄ control group: animals were treated with distilled water daily for 14 days and on Day 14th hepatotoxicity was induced by a single dose of CCl₄.

Seaweeds pretreated group: Animals were administered with ethanol extracts of *S. tenerrimum*, *S. variegatum* and *S. binderi* at 200 mg/kg body weight daily for 14 days separately. On Day 14th rats from each group were injected with single dose of CCl₄ while control rats were injected with vehicle only. The decapitation was carried after 12 h of fasting. The experiment was repeated twice.

2.6. Preventive effect of ethanol extracts of *Sargassum* species in acetaminophen intoxicated rats

In this set, hepatotoxicity was induced by a single intraperitoneal injection of acetaminophen at 1 g/kg prepared in polyethylene glycol 400. Other details are similar to CCl₄ set of experiment.

2.7. Assessment of hepatotoxicity

Hepatotoxicity was determined in terms of cardiac and liver enzymes and other biochemical parameters. For the assessment of these parameters blood was collected in clean centrifuge tubes, and serum was separated after centrifugation at 3000 r/min for 10 min and stored at -20 °C. Serum samples were analyzed for cardiac and liver marker enzymes *viz.*: AST, ALT, ALP, LDH, besides, bilirubin (total and direct), urea, creatinine, glucose, cholesterol and triglycerides on blood chemistry analyzer (Microlab-300, Merck, France) using kits from Merck (France) and Ecoline (Germany).

2.8. Statistical analysis

Data was subjected to One-way ANOVA and means were separated using Duncan's multiple range test according to Armitage and Berry[27].

3. Results

Administration of single dose of CCl₄ resulted in hepatic injury as evident from marked elevation in liver enzymes (ALT, AST, ALP and LDH) and bilirubin. The liver toxicity also affected the kidney function and elevated the concentrations of kidney function markers, urea and creatinine. The liver toxicity also increased serum glucose and triglycerides with decreased in the concentration of total cholesterol. *S. tenerrimum* showed significant ($P < 0.05$) positive effect on liver function by reducing the elevated level of ALT, AST, LDH and bilirubin direct (Tables 1 and 2). *S. tenerrimum* also caused a positive effect on lipid parameters by reducing the elevated level of triglycerides with restoration of cholesterol concentration. The kidney function markers, creatinine and urea were also found towards normal in comparison with CCl₄ control group. Similar pattern was observed in *S. binderi* pre-treated group (Table 1, 2). The rats given ethanol extract of *S. variegatum* showed non-significant effect on high level of ALT, AST, LDH, bilirubin (total and direct), glucose and triglycerides. However,

Table 1

Effect of ethanol extract of *S. variegatum*, *S. tenerrimum* and *S. binderi* on liver enzymes and bilirubin in CCl₄ intoxicated rats.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)	Bilirubin total (mg/dL)	Bilirubin direct (mg/dL)
Normal control	27.6 ± 2.5	85.0 ± 7.0	50.5 ± 2.3	322.6 ± 21.6	0.46 ± 0.05	0.166 ± 0.000
CCl ₄ control	63.0 ± 2.6 ^a	327.0 ± 5.5 ^a	84.3 ± 7.2 ^a	475.3 ± 7.4 ^a	1.06 ± 0.06 ^a	0.300 ± 0.000 ^{ab}
<i>S. variegatum</i> + CCl ₄	63.0 ± 3.6 ^a	332.0 ± 7.0 ^a	75.0 ± 6.6 ^{ab}	458.0 ± 2.8 ^b	0.96 ± 0.05 ^b	0.260 ± 0.050 ^{ab}
<i>S. tenerrimum</i> + CCl ₄	36.3 ± 3.2 ^c	158.6 ± 10.2 ^b	64.0 ± 0.7 ^b	353.0 ± 12.2 ^c	1.00 ± 0.00 ^{ab}	0.230 ± 0.050 ^b
<i>S. binderi</i> + CCl ₄	43.6 ± 3.2 ^b	132.6 ± 6.4 ^c	79.0 ± 4.0 ^a	358.0 ± 5.0 ^c	1.00 ± 0.00 ^{ab}	0.330 ± 0.050 ^a

The values were expressed as means ± SE; The values having the same superscript within the column are not significantly ($P < 0.05$) different according to Duncan's multiple range test.

S. variegatum showed significant effect on kidney function markers by decreasing the elevated level of urea and creatinine in CCl₄ intoxicated rats. This group also demonstrated a positive effect on cholesterol level by increasing its concentration towards normal.

Table 2

Effect of ethanol extract of *S. variegatum*, *S. tenerrimum* and *S. binderi* on glucose, creatinine, urea and lipid parameters in CCl₄ intoxicated rats. mg/dL.

Groups	Glucose	Triglycerides	Cholesterol	Creatinine	Urea
Normal control	113.6 ± 4.0	101.3 ± 4.1	91.3 ± 1.5	0.90 ± 0.00	26.3 ± 2.0
CCl ₄ control	142.6 ± 12.5 ^a	135.3 ± 3.0 ^a	60.0 ± 1.7 ^b	1.16 ± 0.05 ^a	51.0 ± 4.0 ^a
<i>S. variegatum</i> + CCl ₄	138.3 ± 6.3 ^a	136.0 ± 4.0 ^a	74.6 ± 5.6 ^a	0.96 ± 0.10 ^b	35.6 ± 3.2 ^b
<i>S. tenerrimum</i> + CCl ₄	130.0 ± 3.0 ^a	117.0 ± 7.0 ^b	75.0 ± 1.0 ^a	0.93 ± 0.05 ^b	36.3 ± 2.0 ^b
<i>S. binderi</i> + CCl ₄	142.6 ± 8.5 ^a	119.0 ± 5.0 ^b	74.3 ± 3.7 ^a	1.03 ± 0.05 ^b	35.6 ± 3.2 ^b

The values were expressed as means ± SE; The values having the same superscript within the column are not significantly ($P < 0.05$) different according to Duncan's multiple range test.

In another set of experiment, where the rats were intoxicated with acetaminophen, ethanol extract of *S. variegatum* showed significant ($P < 0.05$) hepatoprotective effect as evident by significant reduction in elevated level of liver enzymes and other biochemical parameters towards normal range in comparison with acetaminophen control. However, ethanol extracts of *S. binderi* and *S. tenerrimum* were not found effective against acetaminophen mediated liver damage (Tables 3 and 4).

Table 3

Effect of ethanol extract of *S. variegatum*, *S. tenerrimum* and *S. binderi* on liver enzymes and bilirubin in acetaminophen intoxicated rats.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)	Bilirubin total (mg/dL)	Bilirubin direct (mg/dL)
Normal control	33.6 ± 3.2	46.0 ± 1.2	86.6 ± 3.0	278.0 ± 10.5	0.46 ± 0.05	0.13 ± 0.05
Acetaminophen control	85.0 ± 2.6 ^b	193.0 ± 12.5 ^b	118.6 ± 9.2 ^b	440.0 ± 11.0 ^b	0.80 ± 0.00 ^b	0.30 ± 0.00 ^b
<i>S. variegatum</i> + Acetaminophen	43.6 ± 4.6 ^c	90.3 ± 6.5 ^c	70.0 ± 2.0 ^c	239.6 ± 7.5 ^c	0.60 ± 0.00 ^c	0.20 ± 0.00 ^c
<i>S. tenerrimum</i> + Acetaminophen	94.0 ± 4.0 ^a	225.6 ± 4.5 ^a	143.0 ± 6.6 ^a	477.0 ± 11.6 ^a	1.06 ± 0.05 ^a	0.36 ± 0.05 ^a
<i>S. binderi</i> + Acetaminophen	84.6 ± 2.3 ^b	181.0 ± 9.8 ^b	79.0 ± 7.0 ^c	481.0 ± 11.0 ^a	0.70 ± 0.00 ^{bc}	0.20 ± 0.00 ^c

The values were expressed as means ± SE; The values having the same superscript within the column are not significantly ($P < 0.05$) different according to Duncan's multiple range test.

Table 4

Effect of ethanol extract of *S. variegatum*, *S. tenerrimum* and *S. binderi* on glucose, creatinine, urea and lipid parameters in acetaminophen intoxicated rats. mg/dL.

Groups	Glucose	Triglycerides	Cholesterol	Urea	Creatinine
Normal control	106.6 ± 2.5	76.6 ± 1.5	75.3 ± 3.7	27.0 ± 1.7	1.00 ± 0.57
Acetaminophen	129.3 ± 5.7 ^a	201.0 ± 12.0 ^a	54.6 ± 4.0 ^b	43.6 ± 2.0 ^a	1.40 ± 0.05 ^a
<i>S. variegatum</i> + AAP	114.4 ± 4.9 ^b	97.6 ± 5.0 ^c	60.0 ± 0.5 ^a	25.3 ± 2.0 ^b	0.96 ± 0.05 ^b
<i>S. tenerrimum</i> + AAP	119.6 ± 4.5 ^{ab}	155.0 ± 5.0 ^b	64.3 ± 2.0 ^a	42.3 ± 2.5 ^a	1.00 ± 0.00 ^b
<i>S. binderi</i> + AAP	110.0 ± 5.5 ^b	161.0 ± 11.2 ^b	46.0 ± 2.6 ^c	28.0 ± 1.0 ^b	1.30 ± 0.05 ^a

The values were expressed as means ± SE; The values having the same superscript within the column are not significantly ($P < 0.05$) different according to Duncan's multiple range test.

4. Discussion

Marine algal community signifies a huge source of compound endowed with ingenious structure and potential biological activities[14,18,25,28]. In the present study ethanol extracts of *S. tenerrimum* and *S. binderi* in CCl₄ intoxicated rats showed significant hepatoprotective potential via lowering the elevated level of hepatic marker enzymes ALT, AST, ALP and LDH along with bilirubin. *S. variegatum* showed significant hepatoprotective effect against acetaminophen induced liver injury. Raghavendran *et al.*[29] and Madkour *et al.*[30] also reported hepatoprotective effect of alcoholic extracts of seaweeds. In our study, elevated concentration of bilirubin was recorded in both models of intoxicated rats evident of liver damage. The increased concentration of bilirubin in serum have been reported due to increased production, decreased uptake by liver, decreased conjugation, decreased secretion from the liver or blockage of bile duct[31,32]. Khotimchenko and Khotimchenko[33] reported that administration of calcium alginate (a major component of seaweeds)

decreased blood total bilirubin concentration by 64.3% and blood conjugated bilirubin concentration by 46%.

Kidney and intestine are responsible for elimination of unmodified drugs and metabolites that have processed in liver[34]. It has been reported that acute liver damage also affect kidney function and concentrations of urea and creatinine elevated in serum[35]. In our study, rats given ethanol extract of *S. variegatum* reduced the elevated levels of both kidney function markers in both model of intoxicated rats. This reduction may be due to decrease oxidative stress or increased elimination of hepatotoxicants from body. Pushpavalli *et al.*,[32] reported that urea and creatinine level were elevated in serum after D-galactoseamine induced liver injury that was decreased in rats treated with chrysin (a flavonoid).

In this study *S. variegatum* in acetaminophen intoxicated rats and *S. tenerrimum* in CCl₄ model caused a positive impact on lipid parameters by reducing the elevated level of triglyceride and restoring the cholesterol level. Disturbed lipid metabolism after D-galactosamine induced liver damage has been reported earlier[36]. The elevated triglyceride levels may be due to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis[37]. Similarly disturbance in cholesterol metabolism may be due to hepatic parenchymal cell death which ultimately leads to disturbance of lipid metabolism in liver[38]. In this study rats pretreated with ethanol extract of *S. variegatum* and *S. tenerrimum* restored the cholesterol levels

after both hepatotoxins treatment, these findings are in agreement with findings of Bigoniya and Rana[39]. This restoration of serum cholesterol may be due to increase in endogenous synthesis of cholesterol in the liver as reported about other seaweeds *Ecklonia cava*, *Colpomenia sinuosa* and *S. hemiphyllum*[40].

In this study rats fed with ethanol extract of *S. binderi* and *S. variegatum* caused a reduction in elevated level of serum glucose in acetaminophen intoxicated rats. Hepatic intoxication besides affecting liver, kidney functions and lipid metabolism also disturb glucose metabolism resulted in hyperglycemia[41]. The hyperglycemia may be due to increased lipid peroxidation and damage of cellular membranes which leads to decrease in the activity of membrane bound enzymes such as glucose-6-phosphatase[42]. The acute live damage cause abnormal lipid and glucose metabolism and ultimately kidney dysfunction. Treatment of this complex disease is an extraordinary challenge for modern medical science. The protective role of *S. variegatum* against acetaminophen liver damage and its positive impact on disturbed lipid, glucose metabolism, kidney dysfunction and *S. tenerrimum* against CCl₄ liver toxicity suggest that *Sargassum* species offers a non-chemical means for the treatment of this complex problem.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Financial support provided by the Dean, Faculty of Science, University of Karachi is sincerely acknowledged. We are also thankful to Dr. Mustafa Shameel (now late), Professor Department of Botany, University of Karachi for identifying the *Sargassum* species.

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