Protective role of *Sargassum* species in liver and kidney dysfunctions and associated disorders in rats intoxicated with carbon tetrachloride and acetaminophen

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Abstract: Hepatoprotective and reno-potective effect of *Sargassum* species was investigated in rats against carbon tetrachloride (CCl_4) and acetaminophen (AAP) intoxication. The rats were given ethanol extracts of *Sargassum ilicifolium*, *S. lanceolatum* and *S.swartzii* orally at dose of 200mg/kg b.w. (body weight) daily for 14 days. These seaweed treated rats were then intoxicated with single intra-peritoneal dose of CCl_4 or AAP on14th day. The administration of CCl_4 and AAP caused significant (p<0.05) elevation in liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and other biochemical parameters, bilirubin, glucose, triglyceride and kidney function markers: urea and creatinine. The pre-treatment with ethanol extracts of *S. ilicifolium* and *S. swartzii* protected the liver and kidney significantly (p<0.05) by lowering the elevated level of hepatic enzymes, liver and kidney function markers towards normal range. *Sargassum* species have also showed positive effect on serum glucose, total cholesterol and triglyceride.

Keywords: *Sargassum* spp., hepatoprotective, reno-protective, bilirubin, liver enzymes, creatinine.

INTRODUCTION

Drug-induced hepatic injury is the most frequent reason reported for the withdrawal of an approved drug from the market and it accounts for more than 50% cases of acute liver failure in USA (Lee, 2003). The hepatic biotransformation of drug involves oxidative pathways, primarily via a cytochrome P450 enzyme system (Guengerich, 2001). Acetaminophen, an analgesic and antipyretic drug is sold in market with trade names of Paracetamol, Tylenol and Panadol and considered safe when use at therapeutic doses (Jaeschke *et al*., 2013). But at high doses it causes severe hepatic necrosis leading to acute liver failure (Larson *et al*., 2005).Similarly, carbon tetrachloride (CCl_4) is a prevalent industrial chemical and an organic solvent (Bupesh *et al*., 2012) used in rubber, drug, chemical and paint industries. It is used as fire extinguishers and cleaning agent (Davis, 1934). Carbon tetrachloride-induced liver injury similar to the injury produced by some common liver degenerative substances including viruses, chemicals, alcohol and autoimmune disorders (Rechnagel and Glende, 1973).

Liver damage caused by drugs over dose or chemicals not only affect the liver function but also affect lipid and glucose metabolism besides affecting the kidney function (Dashti *et al.,* 1995; Hira *et al*., 2016).When hepatotoxicity occurs, renal injury is commonly seen and evident from elevation of creatinine from normal level (Mazer and Perrone, 2008).Treatment of this complex disease is an extraordinary challenge for modern medical science.

Seaweeds have been consumed as human diet particularly as a vegetable in Asia, since 600 BC (Tabarsa *et al*., 2012). A large number of epidemiological studies, regarding seaweeds consumption and their health benefits have been reported (Hiqashi *et al*., 1999; Abdel-Wahhab *et al*., 2006; Cassolato *et al*., 2008).Among various biological activity, seaweeds also posses hepatoprotective potential (Kawano *et al.,* 2007; Raghavendran *et al*., 2007; Hwang *et al.,* 2008; Ross *et al*., 2012). The oral administration of ethanol and water extracts and sulfated polysaccharides of *Sargassum polycystum* showed hepatoprotective activity against acetaminophen induced biochemical changes in serum and liver tissues (Raghavendran *et al.,* 2004; 2007; Raghavendran and Srinivasan, 2008). *Sargassum* species are abundantly found at Karachi coast and their antimicrobial (Ara *et al*., 1998; 2002) cytotoxic (Ara *et al*., 1999) and hypolipidaemic (Ruqqia *et al.,* 2015) activities have been reported. Hepatoprotective role of three *Sargassum* species have been reported from Karachi coast by us (Hira *et al.,* 2016). The current report describes the protective effect of some other species of *Sargassum* against carbon tetrachloride (CCl_4) and acetaminophen (AAP) induced hepatic injuries and associated disorders.

MATERIALS AND METHODS

Algal material

Sargassum species, *S. ilicifolium*, (Turn.) C. Ag., *S. lanceolatum* (Turn.) C. Ag. and *S. swartzii* (Turn.) C. Ag.,

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were collected in March 2011 from Buleji beach at Karachi coast. These seaweeds were washed thoroughly in order to remove salts, debris and record (KUH-SW) was kept in the seaweed Herbarium, MAH Qadri Biological Research Center, University of Karachi. Dr. Mustafa Shameel (Professor, Department of Botany, University of Karachi) identified the seaweeds. The seaweeds were dried under shade, grinded to fine powder and kept in polyethylene bags at ambient temperature.

Preparation of ethanol extract

Dry powder (500g) of *S. ilicifolium, S. lanceolatum* and *S. swartzii* were soaked in 2 L distilled ethanol for 1 week at room temperature, separately. The mixture was percolated through cotton wool (twice), the filtrate was dried under reduced pressure using rotary vacuum evaporator (Buchi R-200). The finally obtained gummy extracts were used for experimental purpose.

Animal

Healthy male albino rats of *Wistar* strain (140-170g) were purchased from Dow University of Health Sciences, Karachi, Pakistan in July 2011. All the animals were kept in prebedded polyethylene cages (3rats/cage) with standard laboratory conditions (temperature 25±2°C and 12h light/dark cycle), fed with standard pellet diet and water ad libitum. The animals were kept in laboratory for 1 week before starting the experiment to acclimatized animals with laboratory condition.

Effect of ethanol extracts of Sargassum species in rats intoxicated with CCl_4

The protective effect of ethanol extract was evaluated in seaweeds pretreated rats by the slightly modified method of (Raghavendran and Srinivasan, 2008). The rats were randomly divided into following group and each group was comprised of 6 rats:

CCl_4 control group

Which were given orally with distilled water daily for 14 days and on day 14th hepatotoxicity was induced by intra-peritoneal injection of a single dose of CCl_4(2ml/kg) prepared in olive oil (1:1).

Seaweed pretreated group intoxicated with CCl_4

The group was further divided into three sub-groups, each group was administered orally with ethanol extract of seaweeds, *S. ilicifolium, S. swartzii* and *S. lanceolatum* dissolved in water at 200mg/kg separately for 14 days followed by a single intra-peritoneal injection of CCl_4 at 2ml/kg on 14th day. Rats of all groups were fasted for 12 hour, decapitated after 24 hour of CCl_4 administration and blood was collected.

Normal control group

They were given distilled water daily for 14 days and received injection of olive oil on last day.

Effect of ethanol extract on Sargassum species in rats intoxicated with acetaminophen

For the determination of protective effect of ethanol extract (EE) of seaweeds in acetaminophen intoxicated rats, the animals were randomly divided into different groups as described in CCl_4model except that liver toxicity was induced by acetaminophen at 1g/kg dissolved in 40% polyethylene glycol (in normal saline). While control group received polyethylene glycol injection.

Assessment of hepatoprotective and Reno-protective effect of Sargassum species

For the assessment of hepatic damage, serum was separated from blood by centrifugation at 3000rpm for 10 minutes and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin (total and direct), urea, creatinine, glucose, total cholesterol and triglycerides by using kits from Merck (France) and Ecoline (Germany).

STATISTICAL ANALYSIS

Data was analyzed and subjected to one way analysis of variance (ANOVA) and means were separated using Duncan's multiple range test (DMRT) according to Armitage and Berry, (1994).

RESULTS

Hepatoprotective effect of ethanol extracts of Sargassum species in carbon tetrachloride (CCl_4) intoxicated rats

Liver enzymes ALT, AST, ALP and LDH levels were significantly (p<0.05) increased (2-3 folds) in CCl_4-intoxicated rats when compared with normal control group. Other biochemical markers bilirubin, glucose and triglycerides were also elevated whereas cholesterol level was depleted after intra-peritoneal injection of CCl_4. Pretreatment with ethanol extracts of *S.ilicifolium, S. swartzii* exhibited significant (p<0.05) inhibition of CCl_4induced increase in the level of AST, ALP, LDH and bilirubin, however, *S. lanceolatum* did not show lowering effect on ALT (table 1). Pretreatment with *S. ilicifolium* and *S. swartzii* reduced the blood glucose level upto 94.6 and 122mg/dl in comparison with CCl_4 control group (142 mg/dl) and brought the level to normal range. Similar pattern was observed for triglycerides, creatinine and urea.CCl_4 treatment reduced the cholesterol level to 61±4.5 compared with normal control (82±2.6). *Sargassum ilicifolium* and *S. swartzii* reversed the CCl_4 mediated effect on blood cholesterol (table 2).

Preventive effect of ethanol extracts of Sargassum species in acetaminophen intoxicated rats

Intoxication with acetaminophen (AAP) significantly (p<0.05) elevated the level of ALT, AST, ALP, LDH and total bilirubin up to 65±1, 133.6±6, 224±7.2, 354.6±12

Table 1: Effect of ethanol extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* on liver enzymes and bilirubin in carbon tetrachloride (CCl_4) intoxicated rats.

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	LDH(U/L)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)
Normal control	33^{d}±2	84^{d}±2.6	139.3^{d}±6.8	207^{d}±2.8	0.46^{d}±0.057	0.1^{b}±0
CCl_4 control	130^{a}±14	258^{a}±1	271^{a}±14	507^{a}±28.5	1.06^{a}±0.06	0.3^{a}±0
Sargassumilicifolium+CCl_4	41^{c}±2.6	118^{c}±7.2	174^{bc}±3.4	258.6^{c}±10	0.73^{c}±0.057	0.23^{a}±0
S. swartzii +CCl_4	96^{b}±3.5	152^{b}±2	163^{c}±11.5	314.6^{b}±9.5	0.8^{bc}±0.051	0.23^{a}±0.054
S.lanceolatum +CCl_4	125^{a}±5	140^{b}±13.2	193.6^{b}±4.5	267^{c}±7.7	0.86^{b}±0.055	0.23^{a}±0.05

Table 2: Effect of ethanol extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* on glucose, triglycerides, cholesterol, urea and creatinine in carbon tetrachloride (CCl_4) intoxicated rats.

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Urea(mg/dl)	Creatinine (mg/dl)
Normal control	109^{bc}±5.1	107^{d}±7.6	82^{a}±2.6	26.3^{d} ±2.08	0.93^{b}±0.056
CCl_4 control	142^{a} ±5.6	162^{a}±8.5	61^{c}±4.5	48^{a}±1.5	1.3^{a}±0.05
Sargassumilicifolium+CCl_4	94.6^{c}±2.5	142.6^{b}±4	72^{b}±4.5	39^{b}±3.2	0.83^{c}±0.057
S.swartzii+CCl_4	122^{b} ±1	133.6^{bc}±5	89.6^{a}±7.5	38^{b}±1.7	0.83^{c}±0.053
S. lanceolatum +CCl_4	145^{a} ±1.1	117^{d} ±7.5	61.6^{c}±4	33.6^{c}±3	0.96^{b}±0.052

Table 3: Effect of ethanol extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* on liver enzymes and bilirubin in acetaminophen intoxicated rats.

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	LDH(U/L)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)
Normal control	33.6^{d}±3.2	46^{c}±1.2	86.6^{e}±3	278^{b}±105	0.46^{b}±0.057	0.13^{b}±0.057
Acetaminophen (AAP) control	65^{b}±1	133.6^{a} ±6	224^{b} ±7.2	354.6^{a} ±12	0.8^{a} ±0	0.3^{a} ±0.055
Sargassumilicifolium +AAP	40^{c}±3	43^{c} ±2	179^{d} ±10	202.6^{c} ±8.7	0.53^{b} ±0.05	0.1^{b} ±0.06
S. swartzii +AAP	40.6^{c}±1.5	76^{b} ±5.5	158^{c} ±13.1	179^{d} ±132	0.46^{b} ±0	0.1^{b} ±0
S. lanceolatum +AAP	84^{a}±5.1	130^{a} ±3.7	322.6^{a}±19.7	267^{b} ±20	0.83^{a} ±0.054	0.27^{a} ±0

Table 4: Effect of ethanol extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* 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	106.6^{d}±2.5	76.6^{e}±1.5	75.3^{b}±3.7	27^{c}±1.7	1^{b}±0.57
Acetaminophen (AAP) control	129^{a} ±5.7	201^{a} ±12	54.6^{c} ±4	43.6^{a} ±2	1.4^{a} ±0.057
Sargassumilicifolium +AAP	113^{c} ±3	165^{c} ±10.9	73.6^{b} ±5.5	24.3^{d}±0.6	1.26^{b} ±0.052
S.swartzii +AAP	119.6^{b} ±3.5	138.6^{d} ±5	85.6^{a} ±5	31.3^{b} ±0.57	1.06^{b} ±0.054
S.lanceolatum +AAP	128^{a} ±3.6	180^{bc} ±12.4	78.3^{ab} ±4.9	41.3^{a} ±1.5	1.23^{bc} ±0.05

Table 5: Effect of ethanol extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* on liver enzymes andbilirubin in normal rats.

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	LDH(U/L)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)
Normal control	33^{a} ±2	84^{a} ±2.6	99.3^{c}±6.8	207^{b} ±2.8	0.46^{a} ±0.057	0.1^{a} ±0
Sargassumilicifolium	21.6^{c} ±0.57	73.3^{b} ±2.5	101.6^{c}±3.5	206.3^{b} ±2.5	0.5^{a} ±0.0561	0.1^{a} ±0
S. swartzii	30.3^{ab} ±2.08	81.3^{a} ±2.8	214^{a} ±2.5	298^{a} ±9	0.53^{a} ±0.054	0.16^{a} ±0.05
S. lanceolatum	28^{b} ±3	54.3^{c} ±6.6	155^{b} ±8.5	196^{b} ±5.2	0.46^{a} ±0.059	0.16^{a} ±0.057

Table 6: Effect of Ethanol Extract of *Sargassumilicifolium, S. swartzii* and *S. lanceolatum* on glucose, urea, creatinine, triglycerides and cholesterol in normal rats.

Groups	Glucose(mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Urea(mg/dl)	Creatinine (mg/dl)
Normal control	109^{a} ±5.1	107^{a} ±7.6	82^{b} ±2.6	26.3^{a} ±2.08	0.93^{a} ±0.056
Sargassumilicifolium	114^{a} ±6.2	97^{b} ±4.7	92^{a} ±5.5	27^{a} ±2	0.76^{b} ±0.054
S. swartzii	114^{a} ±2	98^{b} ±4	82.6^{b} ±3.5	20^{b} ±1	0.86^{ab} ±0.056
S. lanceolatum	113^{a} ±7	73^{c} ±4.3	82^{b} ±5.1	28^{a} ±1	0.93^{a} ±0.05

The values were 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.

and 0.8±0 respectively. The increase was many folds as compared to normal control rats which indicated liver damage. The treatment of animals with ethanol extracts of *S. ilicifolium* and *S. swartzii* exhibited a significant reduction in the AAP-induced elevated level of above enzymes and total bilirubin (table 3). Intoxication with AAP in AAP control group rats significantly (p<0.05) increased the glucose, triglycerides and kidney function markers: urea and creatinine level. Animals that received ethanol extracts of *S. ilicifolium* and *S. swartzii* @ 200 mg/kg prior to AAP showed a marked decrease in triglyceride, urea and creatinine concentration which was significant (p<0.05) when compared with AAP control animals. Rats pretreated with *S. ilicifolium* and *S. swartzii* dropped the serum glucose upto 113±3 and 119.6±3.5 respectively which was increased after acetaminophen administration to 129 ±5.7 (table 4).

Effect of Sargassum species in normal rats

Administration of ethanol extracts of *S.ilicifolium* and *S. lanceolatum* in normal rats resulted in decreased level of alanine aminotransferase (ALT) when compared with normal control group. *Sargassum swartzii* increased the ALP and LDH enzymes while *S. ilicifolium* decreased ALP and showed no effect on LDH levels in serum. Bilirubin level was found near normal range in all treated groups and showed no significant difference between normal and treated groups (table 5). All the seaweeds had no effect on glucose level in seaweed treated group, whereas triglyceride level was slightly decreased by all the three seaweed treatment. *Sargassum ilicifolium* and *S. swartzii* decreased creatinine level significantly in comparison with normal control while urea was also found decreased in *S.swartzii* treatment (table 6).

DISCUSSION

Beside the autoimmune diseases and hepatitis, wide range of drugs (pain killers, antibiotics, vitamins, medicines used against T.B., epilepsy and diabetes) can induceliver injury. The screening of hepato-protective drugs is done by producing liver injury in animals most commonly by using acetaminophen and CCl_4 (Gilani and Janbaz, 1995). In our study administration of both hepato-toxins significantly (p<0.05) raised the liver enzymes in serum. The levels of bilirubin, urea, creatinine, glucose and triglycerides were also increased while cholesterol levels were drastically decreased in acetaminophen and CCl_4 intoxicated rats. There are reports that acetaminophen and CCl_4at high doses or overdoses produce major hepatic lesions (Hinson *et al*., 1995) with massive increase in transaminases followed by liver and kidney damage (Rusu *et al*., 1999). The liver injury as a result of infectious agents or chemicals, produces higher levels of ALT and AST (Zhang *et al*., 2013), along with ALP and LDH. The extent and type of hepatocellular damage can be estimated by assessing hepatic enzymes in the blood (Ansari *et al*., 1999).In our study pretreatment with ethanol extract of *S. ilicifolium*, *S. swartzii* prevented the leakage of these enzymes after hepatotoxins administration.

Another biochemical parameter, bilirubin is elevated after hepatic damage. The increased concentration of bilirubin in serum may be a result of its increased production, decreased uptake by liver, decreased conjugation, decreased secretion from the liver or blockage of bile duct (Bun *et al*., 2006). In this study ethanol extract of *S. ilicifolium* and *S. swartzii* showed significant decreased in the elevated levels of total bilirubin in both hepato-toxins (CCl_4 and AAP) treated groups. Khotimchenko and Khotimchenko (2004) 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% as compared to CCl_4 control group.

It has been reported that acute liver damage also affect kidney function and concentrations of urea and creatinine elevated in serum (Karakus *et al*., 2011). In our study, rats given ethanol extract of *S. ilicifolium* and *S. swartzii*. reduced the elevated levels of both kidney function markers in both model of intoxicated rats. This reduction may be due to decreased oxidative stress or increased elimination of hepato-toxicants from body. Pushpavalli *et al*., (2010) reported that urea and creatinine level were elevated in serum after D-galactoseamine induced liver injury that was decreased in rats treated with flavonoid and it was found at various concentrations in seaweeds (Farasat *et al.,* 2014).

The present study showed that acetaminophen and CCl_4 administration disturbed the lipid and glucose metabolism beside disturbing the hepatic enzymes. Disturbed lipid metabolism after D-galactosamine induced liver damage has been reported (Sathivel *et al*., 2008).The elevated triglyceride levels in hepato-toxin treated rats may be due to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis (Khan *et al*., 2012). In the study reduction in the elevated level of serum triglycerides was also found in rats pretreated with ethanol extracts of *S. ilicifolium*, *S. swartzii* and *S. lanceolatum*. The total cholesterol level was decreased in serum due to acetaminophen and CCl_4induced hepatic damage. The recovery in cholesterol level was found in rats treated with ethanol extracts of *S. ilicifolium* in AAP hepatotoxicity in rats. Acetyl CoA is responsible for the *de novo* synthesis of triglycerides and cholesterol in liver. The disturbance in cholesterol metabolism may be due to hepatic parenchymal cell death which ultimately leads to disturbance of lipid metabolism in liver (Havel, 1986). It is also reported that seaweeds *Ecklonia cava*, *Colpomenia sinuosa* and *Sargassum hemiphyllum* raised serum cholesterol level might be due to the enhanced

endogenous liver cholesterol synthesis (Sathivel *et al*., 2008). CCl_4 induced hepatic damage not only cause hypertriglyceridemia but also resulted in hyperglycemia (Al-waili, 2004). The hyperglycemia may be due to increased lipid peroxidation or damage of cellular membranes which ultimately results in less functioning of membrane bound enzyme, glucose-6-phosphatase (DeGroot *et al*., 1985). The ability of brown seaweeds *S. swartzii* and *S. ilicifolium* to decrease the elevated concentration of glucose in serum may be due to protection of hepatocytes from toxic substances. Out of three *Sargassum* species tested, ethanol extract of *S. ilicifolium* and *S. swartzii* produced protective effect on hepatic enzymes and other biochemical markers in acetaminophen and CCl_4 intoxicated rats. The maximum protection against CCl_4 induced hepatic toxicity was achieved with the *S. ilicifolium*. The results clearly indicated that both seaweeds i.e. *S. ilicifolium* and *S. swartzii* have potency to protect against chemical induced liver damage.

Seaweeds contain fucoidan, alginates, polyphenols and triterpenes which are found to have broad spectrum biological properties (Chotigeat *et al*., 2004). The hepato-protective effect of seaweeds observed in our study may be due to polysaccharides or polyphenols, as both of these compounds have strong antioxidant activity (Ananthi *et al*., 2010). Phloroglucinol and phloroglucinol derivatives eckstolonol, eckol, phlorofucofuroeckol A and dieckol were isolated from the brown alga *Ecklonia stolonifera* and found to possess hepato-protective activity (Kim *et al*., 2005). It may be concluded that these brown seaweeds may have such type of compounds responsible for the hepato-protection. Studies have demonstrated that possible hepato-protective effect of calcium alginate might be due to inhibition of lipid peroxidation and/or triggering antioxidant activity (Khotimchenko and Khotimchenko, 2004). Presence of alginates may be one of the factor for the protective effect against hepato-toxins. Fucoidan (a seaweed polysaccharide) has been reported to interact with transforming growth factor-β and to scavenge reactive oxygen species (Xue *et al*., 2001). Fucoidan reduce the growth of hepatic stellate cells, which is thought to be essential for the resolution phase of fibrosis, and prevent hepatocytes from injury (Hayashi *et al*., 2008).

CONCLUSIONS

It was found that ethanol extracts of *Sargassum ilicifolium* and *S. swartzii* have hepato-protective as well as reno-protective effects as was demonstrated by decreased in liver and cardiac enzymes and kidney function markers (urea and creatinine). *Sargassum* spp., also showed protective effect on associated disorders as evident from reduction of glucose and triglycerides levels with normalization of cholesterol in animals intoxicated with hepato-toxins (carbon tetrachloride & acetaminophen).In view of available studies and present study seaweeds are expected to play a major role in providing protection against kidney and liver dysfunction besides associated disorders.

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