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Research Article

PROTECTIVE EFFECT OF ETHANOL EXTRACT OF *SARGASSUM DENTIFOLIUM* (PHAEOPHYCEAE) IN CARBON TETRACHLORIDE-INDUCED HEPATITIS IN RATS

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ABSTRACT

The present study evaluates the hepatoprotective activity of ethanolic extract of the brown algae *Sargassum dentifolium* in carbon tetrachloride (CCl_4)-induced hepatitis in rats. Protective effect of *S. dentifolium* extract (100 mg kg^{-1} body weight) was investigated in CCl_4 (1 ml kg^{-1} body weight twice weekly for 8 weeks) induced hepatic damage by measuring levels of bilirubin, albumin and diagnostic marker enzymes such as Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT) and alkaline phosphatase (ALP). The histopathological studies were carried out to support the above parameters. Results showed that administration of CCl_4 originated biochemical and histopathological alterations represented as significant elevations ($p< 0.05$) in AST, ALT, ALP and bilirubin, decreasing albumin level, in addition to extensive intralobular fibrosis lesions, which collectively indicate hepatic damage. Administration of ethanol extract of *S. dentifolium* significantly ($p< 0.05$) restored these alterations to normal levels in the manner comparable with standard drug silymarin. Histopathological study revealed that liver treated with *S. dentifolium* extract exhibited nearly normal architecture as compared to CCl_4 treated group. The results of the present study substantiate the potential hepatoprotective effect of ethanol extract of *S. dentifolium*.

Keywords: *Sargassum dentifolium*, Hepatoprotective effect, Carbon tetrachloride, Liver marker enzymes.

INTRODUCTION

Liver is the key organ responsible for drug and xenobiotics metabolism, representing a sensitive target site for substance modulating biotransformation[1]. Drug-metabolizing enzymes detoxify many xenobiotics but bioactivate the toxicity of others causing liver damage[2]. Increase in free radicals and reactive oxygen species enhance the development of various enzymatic and non-enzymatic systems in the cell[3]. This situation creates many complications such as; establishment of oxidative stress, antioxidant alteration of defense mechanisms, reduction of intracellular concentration of Glutathione (GSH), decreasing of catalase activity which cause organ injury and carcinogenesis[4,5]. The chronic liver diseases are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma[6,7]. As life style changes, hepatic disorders have become one of the most prevalent disease worldwide responsible for considerable morbidity and mortality especially in developing countries[8]. Therefore, more efforts have been made recently for exploring new drugs of natural origin, which could limit the drug-induced hepatic injury for the adequate management of liver diseases.

Carbon tetrachloride (CCl_4) is one of the most commonly hepatotoxins that has been reported to show many metabolic and morphologic aberrations in the liver of the experimental animals similar to those observed in human viral hepatitis[9,10,11]. It was found that chronic administration of CCl_4 induces liver cirrhosis by a multiple step mechanism. CCl_4 biotransformed in the liver to trichloromethyl radicals ($-CCl_3$) which reacts with excess O_2 forming reactive free radicals ($-CCl_3OO$). These free radicals initiate peroxidation of membrane polyunsaturated fatty acids and covalently bind microsomal lipids and proteins forming lipid peroxides followed by cellular disorders and pathological changes[12,13]. To prevent and reduce the potential mutation in the cell, reactive oxygen species should be scavenged properly[14].

Recently, there is a lot of interest in drugs from marine origin with potential benefits. Marine algae (seaweeds) have long been recognized in the Orient world for their nutritional value and used as food stuff in the Asian diet for centuries, as it contains carotenoids, proteins, essential fatty acids, vitamins and minerals[15]. Marine algae serve as important resources for bioactive natural products which attract more attention as raw materials for medicative drugs[16,17]. Hence, they have been used for a variety of remedial purposes, such as in eczema, gallstone, renal trouble, scabies, psoriasis, asthma, arteriosclerosis, heart disease, ulcers and cancer[18,19]. Beside their low fat content, they contain bioactive compounds like polysaccharides, which are potential natural antioxidants not common in land plants[20]. Hepatoprotective effect of some marine algae such as *Sargassum polycystum*[21,22], *Ulva lactuca* and *Gracilaria edulis*[23] and *Gracilaria corticata*[24] have been reported. This work was conducted to investigate the hepatoprotective activity and the effect of ethanol extract of the Egyptian isolate seaweed *Sargassum dentifolium* on liver function markers in the serum, as well as on hepatic histopathology of CCl_4 induced hepatotoxicity in Wistar rats.

MATERIALS & METHODS

Seaweed collection and extraction

The seaweed, *S. dentifolium*, was collected from Suez Canal (Egypt) at Defresswar during spring 2010. The seaweed samples were washed in seawater followed by fresh water to remove the epiphytes and other contaminants. The samples were shade dried, powdered and extracted with ethanol in cold for a period of 5 days with occasional shaking. The extract was filtered then concentrated by drying in vacuum. The resulting concentrated crude extract was used for the animal experimentation.

Animals

The study was performed on adult male Wistar strain albino rats with initial body weights of 100-130 g. Rats were procured from the Animal House Colony of the National Research Center, Dokki, Cairo, Egypt. The animals were housed in cages under proper environmental conditions and received human care in compliance with the guidelines of Scientific Ethical Committee, Faculty of Veterinary Medicine, Suez Canal University. The animals were acclimatized to laboratory conditions at temperature of 25±2°C for a week. They were fed a commercial pellet diet and had free access to water throughout the experimental period.

Experimental protocol

Healthy albino rats were divided into five groups of 5 rats each. Group I served as a vehicle control which received normal diet and water. Group II were given 50% CCl_4 in corn oil (1ml kg^{-1} PO) twice weekly for 8 weeks and kept as CCl_4-induced hepatotoxicity control.

Group III received CCl_4 and ethanol extract of *S. dentifolium* (100 mg kg^{-1} PO) simultaneously for 8 weeks. Groups IV and V received CCl_4 for 8 weeks then group IV were given *S. dentifolium* (100 mg kg^{-1} PO), and group V received standard drug silymarin (100 mg kg^{-1} PO) for a month to assess the hepatoprotective potential.

Biochemical assays

At the end of the experimental period, the animals were deprived of standard diet for 20 h and anesthetized with diethyl ether. Blood samples of each animal were collected by puncturing retro-orbital plexus in separate tubes without anticoagulant. It was kept at room temperature for 1 h then centrifuged at 1000 g for 10 min to separate the serum to assay the diagnostic marker enzymes in plasma. Serum Aspartate aminotransaminase (AST) and Alanine aminotransaminase (ALT) were determined by the method of Reitman & Frankel[25]. Alkaline phosphatase (ALP) was estimated according to Kind & King[26]. Serum bilirubin and albumin levels were estimated according to Malloy & Evelyn[27] and Lowry *et al*. [28] respectively.

Histopathological observation

For histopathological examination, the rats were sacrificed by cervical decapitation and liver from each animal was excised then immersed in neutral buffered formalin for 24 h. Liver tissues were cleaned and embedded in paraffin, cut in 5µm sections, stained with the haematoxylin and eosin and examined microscopically[29]. Special staining by Masson's trichrome stain was performed to assess the degree of fibrosis[30]. The degree of liver fibrosis was graded according to a semi-quantitative scoring system as reported by Ruwart *et al*.[31] (Table 1).

Table 1: The scoring scheme used in the assessment of liver histopathology

Score	Description
0	Normal liver with no collagen or fibrosis
1	Increased collagen, without septa, generally seen as small stellate expansions of the portal fields
2	Definite increase with incomplete septa from the portal tract to the central vein (those septa which do not interconnect with each other)
3	Complete but thin septa (those septa which interconnect with each other so as to divide the parenchyma into separate fragments)
4	Same as grade 3, except for the presence of thick septa

Statistical analysis

All data were expressed as mean±SD and were analyzed by one-way ANOVA to evaluate differences between groups. If significance was observed between groups, Duncan Multiple Range Test was used to compare the means of specific groups with $p<0.05$ considered as significant.

RESULTS

The effect of ethanol extract of *S. dentifolium* on serum transaminases, alkaline phosphatase, bilirubin and albumin levels in CCl_4 intoxicated rats were summarized in Table 2. There was a significant increase ($p< 0.05$) in serum marker enzymes AST, ALT, ALP and bilirubin levels in group II (CCl_4 intoxicated rats), while albumin levels were significantly decreased ($p< 0.05$) when compared to the normal (group I).

Treatment with ethanol extract of *S. dentifolium*, either simultaneously or after 8 weeks of CCl4 administration (groups III and IV), and sylimarin (group V) significantly decreased ($p< 0.05$) the elevated serum marker enzymes and reversed the altered bilirubin and albumin levels to almost normal.

Table 2: Levels of serum enzymes in plasma of normal and experimental groups of rats

Treatment group	AST ($U\ l^{-1}$)	ALT ($U\ l^{-1}$)	ALP ($U\ 100ml^{-1}$)	Bilirubin ($mg\ d^{-1}\ l^{-1}$)	Albumin ($g\ d^{-1}\ l^{-1}$)
I	53.300 ± 17.321^{cd}	24 ± 6.00^{bc}	252.33 ± 54.647^{c}	0.21 ± 0.110^{b}	3.77 ± 0.153^{a}
II	143.00 ± 27.185^{a}	48 ± 6.00^{ab}	737.67 ± 63.516^{a}	0.83 ± 0.411^{a}	2.73 ± 0.208^{b}
III	72.067 ± 7.852^{bc}	30 ± 12.00^{bc}	375.33 ± 19.035^{bc}	0.28 ± 0.064^{b}	3.77 ± 0.058^{a}
IV	80.867 ± 7.915^{bc}	34 ± 3.46^{b}	468.67 ± 94.342^{b}	0.36 ± 0.168^{b}	3.73 ± 0.058^{a}
V	94.333 ± 7.506^{b}	34 ± 6.93^{b}	435.00 ± 27.221^{b}	0.28 ± 0.127^{b}	3.47 ± 0.252^{a}

All results are mean±SD for 5 animals. Values that have a different superscript letter (a, b, c, d) differ significantly with each other ($p<0.05$; Duncan's Multiple Range Test).

Macroscopically, the appearance of liver in control group (group I) were grossly normal in both color and consistency. In CCl_4 intoxicated rats (group II); liver was small, firm and pale with irregular coarse surface, in addition to the presence of adhesion of the hepatic capsule with the surrounding in some cases. On the other hand, liver specimens of groups III-V were nearly normal in size and slightly firm in consistency with dark-red to mild yellowish discolored hepatic surface.

Histological observation of liver tissue of the normal animal (group I) showed a normal liver architecture of hepatocytes since they were well arranged without any alteration at central vein (Fig. 1). Hepatic cells were polyhedral in shape with defined cell lining in the liver tissue. The cytoplasm was well preserved with prominent nucleus and nucleolus indicating grade 0-fibrosis (Figs. 1a, b). In fibrosis model animals (group II), hepatocytes showed severe and diffuse degenerative changes mainly hydropic and fat degeneration. In addition, focal areas of necrosis and extensive intralobular fibrosis of the two forms porto- portal and porto-central bridging fibrosis were observed (Figs. 1c, d). Moreover, severe infiltration of hepatic areas with mononuclear cells, mainly lymphocytes, along with hyperplasia of bile ducts was also recorded (Fig. 1e). Fibrous tissue of both porto- portal and porto- cental bridging fibrosis was stained blue by Masson's trichrome stain indicating the presence of grades 3 and 4-fibrosis (Fig. 1f).

In group III, fibrous tissue proliferation was observed around portal tracts along with mild mononuclear cell infiltration mainly lymphocytes (Fig. 2a). The mild fibrous tissue proliferation with periportal and minimal intralobular fibrous septa were clearly obvious in Masson's trichrome stained section representing grade 1-fibrosis (Fig. 2b). In group IV, the portal tracts demonstrated moderate infiltration with lymphocytes and few macrophages (Fig. 2c). Fibrous tissue proliferation around the portal areas and incomplete bridging of the hepatic parenchyma were observed which stained blue in Masson's trichrome section indicating grades 1 and 2-fibrosis (Figs. 2d, e). In sylimarin treated rats (group V), the portal tracts displayed moderate infiltration with lymphocytes in addition to the presence of congested portal blood vessels and hepatic sinusoids along with moderate degeneration of hepatocytes (Fig. 2f). Fibrous tissue proliferation around the portal areas and incomplete bridging of the hepatic parenchyma were observed indicating grades 1 and 2-fibrosis (figs. 2g-i).

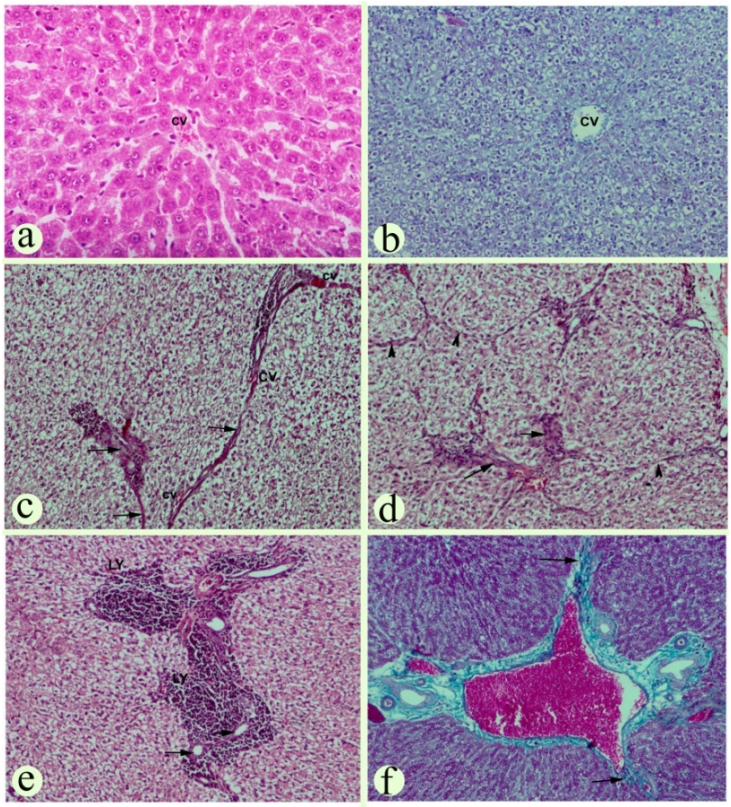

Fig. 1: Structural features of liver examined by light microscopy at X 100 for group I (a, b) showing grade 0-fibrosis, and group II (c-f) showing grades 3 and 4-fibrosis. (Note: sections in figures b and f were stained with Masson's trichrome while the rest with H&E; CV, central vein; LY, lymphocytes)

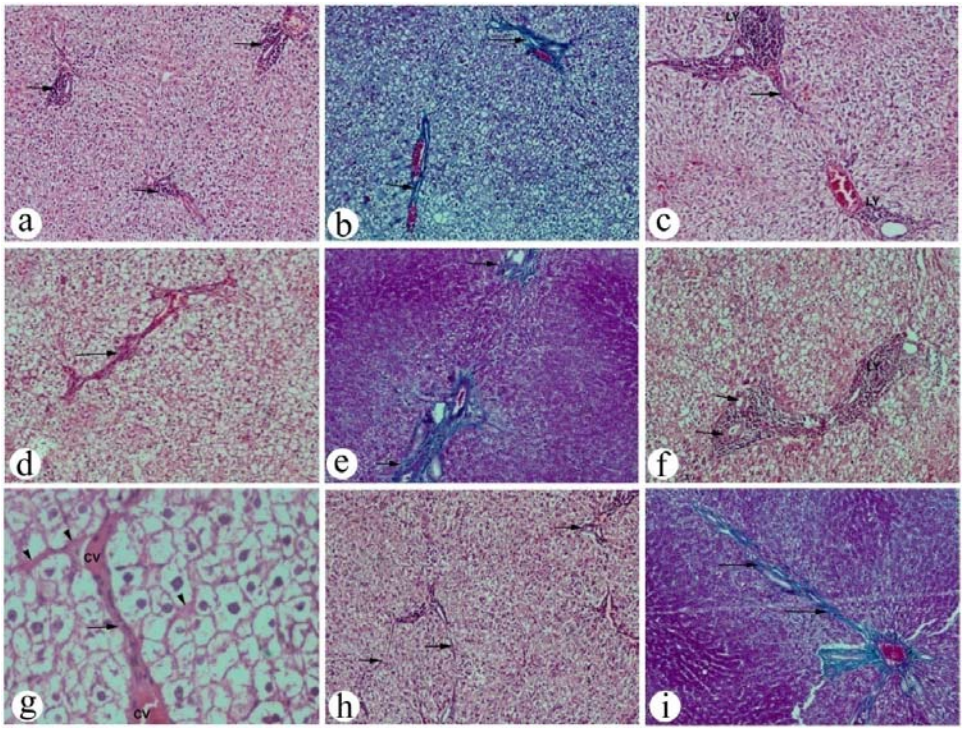

Fig. 2: Structural features of liver examined by light microscopy at X 100 for group III (a, b) showing grade 1-fibrosis, group IV (c-e) showing grade 2-fibrosis, and group V (f-i) showing grade 2-fibrosis. (Note: sections in figures. b, e and i stained with Masson's trichrome and the rest with H&E)

DISCUSSION

The experiment of the present work was designed to study the *in vivo* effect of ethanolic extract of *S. dentifolium* on some biomarker enzymes and histopathological changes of hepatic lesions indicating hepatocellular injury induced by CCl_4 in male albino Wistar rats. Serum enzymes AST and ALT are sensitive markers of liver injury and their elevated levels are indicative of cellular leakages and loss of functional integrity of cell membrane in liver that was initiated by hepatocellular damage caused by drug toxicity and xenobiotics[32,33]. Increased levels of ALT in the serum are usually specific for inflammation of liver cells while levels of AST can be triggered in other conditions apart from hepatocellular damage such as myocardial infarction[34]. In an inflammatory condition, ALT level increases above that of AST, while in gross cellular necrosis, as in CCl_4, the level of AST may rise higher than that of ALT[35]. In the present study, significant increased levels of aminotransferases, with rise in the levels of AST over that of ALT in CCl_4 intoxicated rats (Group II) indicated hepatic damage, which potentiate the release of AST into circulation over that of ALT.

Serum ALP is the prototype of aminotransferases that reflects the pathological alteration in biliary flow, and serum bilirubin is considered as an index of hepatic function[36]. Therefore, any increase in the levels of ALP and bilirubin in the serum indicate hepatobiliary diseases and disturbance of hepatocellular function[34]. Furthermore, the reduced levels of albumin (hypoalbuminemia) are the most frequent in advanced chronic liver disease and can deem as a useful index of severity of cellular dysfunction in chronic liver disease[37]. Pattanayak and Priyashree[38] attributed this reduction to the initial damage in the endoplasmic reticulum which results in decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. The present results confirmed these effects when significant increase ($p< 0.05$) of both ALP and bilirubin levels were observed in CCl_4 intoxicated rats while albumin content decreased significantly ($p< 0.05$). It is obvious that administration of CCl_4 induced sufficient injury to hepatic parenchyma which produced elevation in AST, ALT, ALP and bilirubin levels, in addition to the reduction of albumin content in plasma.

The results of the present study demonstrated that co-administration of ethanol extract of *S. dentifolium* significantly decreased ($p< 0.05$) the serum AST, ALT, ALP and bilirubin which restored all parameters towards normal levels. This indicates that *S. dentifolium* extract preserved the structural integrity of the hepatocellular membrane and improved metabolic processes. Since the antioxidant activity of seaweeds has been documented[39,40], the restorative effect of *S. dentifolium* extract could be attributed to its ability to prevent or decrease the metabolism of CCl_4 into more toxic metabolite. This could minimize the production of free radicals and boost the activities of their scavengers, diminishing produced hepatocellular injury. Also, the observation of the increased levels of albumin proposes the stabilization of endoplasmic reticulum leading to protein synthesis. Comparison between the activity of the extract against the CCl_4 induced toxicity and that of the standard drug, sylimarin, evidenced that the effect of ethanol extract of *S. dentifolium* and sylimarin was almost comparable in all parameters tested with better effect for concomitant administration of *S. dentifolium* and CCl_4. This suggests that *S. dentifolium* extract possess a protective activity against $CC1_4$- induced liver damage in rats.

Hepatocytes make up 70–80% of the cytoplasmic mass of the liver. These cells are involved in protein synthesis, protein storage and transformation of carbohydrates. Other roles for these cells are synthesis of cholesterol, bile salts and phospholipids, as well as detoxification, modification and excretion of exogenous and endogenous substances[41]. Chronic liver disease is characterized by the excessive deposition of collagen and other extracellular matrix (ECM) proteins within the liver. It is thought that activated hepatic stellate cells in the perisinusoidal space are the main contributors to the fibrotic process[42]. Diagnosis of liver fibrosis is based on histological examination of chronic liver damage for lobular architecture, degree of hepatocyte damage, inflammatory infiltration, fibrous deposition, and regeneration and nodular formation. A semi-quantitative estimation of fibrosis may be performed by staining of collagen with Masson's trichrome stain[43]. In the present study, the appearance of two forms, porto- portal and porto-central bridging fibrosis, in rats administrated with $CC1_4$ represents the adaptive response to the disturbance of hepatocytes metabolism induced by potentially toxic stimuli. The intensity of the degenerative and necrotic changes of hepatocytes in rats treated with *S. dentifolium* and sylimarine was mild when compared with that of $CC1_4$-intoxicated rats. Moreover, the improvement of these changes in rats treated with *S. dentifolium* extract concomitant with CCl_4 was more than that in rats treated with *S. dentifolium* extract or sylimarine after 8 weeks of CCl_4 administration. This emphasizes that treatment with *S. dentifolium* considerably prevented the alterations in liver cell structural integrity triggered by CCl_4 and restored the induced histopathological abnormalities.

In summary, it could be concluded that ethanol extract of *S. dentifolium* was able to reduce all the elevated biochemical parameters and had therapeutic and preventive efficiencies in CCl_4 induced hepatotoxicity in rats. Results revealed that the hepatoprotective effects of seaweed *S. dentifolium* may be due to improving the structural integrity of the hepatocyte as a result of their antioxidant activity, which enhance ability to scavenge free radicals and inhibit lipid peroxidation, all of which are capable of hepatocellular injury. Evidently, histopathological examination of liver also supported *S. dentifolium* therapy as it helped in improving liver cell architecture damage caused by CCl_4.

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