HEPATOPROTECTIVE EFFECTS OF BROWN ALGAE PADINA TETRASTROMATICA AGAINST CARBON TETRACHLORIDE INDUCED HEPATOXICITY

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ABSTRACT

The main objective of this study was to investigate the antioxidant and hepatoprotective effects of brown algae Padina tetrastromatica against carbon tetrachloride using animal model mice. Carbon tetrachloride is an organic liquid similar to chloroform. The hepatoprotective effect was assessed by monitoring the serum marker enzymes, antioxidants like thiobarbituric acid, superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase, and protein. The animal groups were divided into four includes Group I Normal mice, Group II Mice treated with Olive oil, Group III treated with Carbon tetrachloride along with olive oil (1:1 (v/v) mixture of CCl₄ and olive oil), Group IV treated with both Carbon tetrachloride and of Padina tetrastromatica extract. The seaweed extract was administered three hours after the administration of carbon tetrachloride. In order to assess the hepatic damage in liver, the activities of Thiobarbituric acid, Glutathione, and Superoxide dismutase, Catalase and Glutathione peroxide were estimated in animal model. Padina tetrastromatica extract produced significant (P<.001) hepatoprotective effects by decreasing the activity of serum enzymes and bilirubin. Based on the histopathological and biochemical parameters, it was suggested that Padina tetrastromatica extract could perhaps protect liver cells from carbon tetrachloride induced liver damage by its antioxidant effect of hepatocytes thus decreasing the harmful effects of toxic metabolites of carbon tetrachloride.

KEY WORDS : Padina tetrastromatica, Hepatoprotective activity, Anti-oxidant activity, liver marker enzymes.

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INTRODUCTION

Carbon tetrachloride (Carbon tetrachloride) is a xenobiotic found from the water sources mainly as industrial wastes as its primary use in manufacture of chlorofluorocarbons, dry cleaning fluids, fire extinguishing agents, etc. This produces hepatotoxicity in human beings and animals. Human body is protected from various injurious substances and toxic metabolic products by the liver, which has been absorbed from intestinal tract. Xenobiotics are often reported to cause potential hepatic damage. Recently, the rates of human diseases and organs toxicity have been increased as a result of exposing to multi environmental factors. These factors include heavy metals and toxic chemicals in addition to different microbial infections. CCl₄ is a selective hepatotoxic chemical agent. CCl₄-induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation. Exposure to toxic chemicals, environmental pollutants and drugs can cause cellular injuries through metabolic activation of reactive oxygen species (ROS). Generally seaweeds are rich sources of various bioactive compounds, including polyphenols, carotenoids and polysaccharide with different physiological effects which play an important role in preventing lipid peroxidation. However, the seaweeds extracts and fractions have been considered to be a rich source of anti-oxidants and different types of antioxidants have been isolated from various species of sea weeds. Antioxidant reduces the risk for chronic disease including cancer and heart disease. Antioxidant like vitamin C, vitamin E, carotenoids, phenolic acids, phytate and phytoestrogen compound such as gallates have strong antioxidant activity. Various antioxidants are present in sea weeds, for example polysaccharides, dietary fibres, minerals, proteins, amino acids, vitamins, polyphenols and carotenoids. The brown algae Padina tetrastromatica is ubiquitous along the coastal region of India. The plant is brown to yellowish brown in colour, thallus is fan shaped, foliaceous 5-55cm long and 1-3cm wide, irregularly branched into dichotomously fan shaped segments with entire margins. Apical margins are involute and the growth of the thallus occurs by means of a marginal meristem. Hairs are scattered all over the surface of the thallus reproductive structures occur as marginal sori, rich in calcium deposits. The pharmacological screening had revealed its methanolic extract to have spasmogenic, anti-fertility, anti-hepatotoxicity, hypotensive and anti-amoebic properties. Methyl palmitate was found to be present in the largest quantity (36.6%) in P. tetrastromatica. It was found to contain highest percentage of arachidic acid. It exhibits significant activity against many bacterial strains and it has anti-bacterial activity. P. tetrastromatica acts as a antifouling agent and posses anti-hepatitis B virus activity. P. tetrastromatica were found to have biosorption efficiency. It was found as the best biosorbent for chromium removal from commercial tannery effluent. In addition to their nutritional value seaweeds are claimed to exhibit antitumour, antiviral, anti-inflammatory, antibacterial, hypoglycemic, hypocholesterolemic and hypotensive activities. It also contains a phycocolloid fucoidan which is a sulphated polysaccharide and trace amounts of alginate as carboxylated polysaccharide. Sulfated polysaccharide present in P. tetrastromatica was shown to have anti-inflammatory properties. In order to evaluate the antioxidant activity of P. tetrastromatica, this study was formulated to investigate the antioxidant activity and effects of P. tetrastromatica on liver function markers in the serum as well as on hepatoprotective activity on the mice liver showing carbon tetrachloride (CCl₄) induced hepatotoxicity.

MATERIALS AND METHODS

(i) Seaweed Collection
P. tetrastromatica (Dictyotaceae) was collected during the month of January (2011) in Mandabam sea coast, Ramanathapuram District, Tamil Nadu, India. The plant materials
were cleaned with distilled water and shade dried at room temperature and authenticated (No: BSI/SC/5/23/09/10/Tech-1718) by the Botanical survey of India, Coimbatore, Tamil Nadu.

(ii) Preparation of Sea weed extract
The shade dried sea weed were powdered separately in an electrical blender and stored at 50°C until further use. 100gms of sea weed powder was taken, mixed with 500 ml of distilled water and stirred magnetically in separate containers overnight at room temperature. The residue was removed by filtration and the aqueous extract was concentrated under vacuum to get 20% solid yield. The sea weed extract was tested for hepatoprotective effect in the albino mice at the selected optimum dosage of 250 mg/kg body weight and administered orally.

(iii) Animals
Wister albino mice were used to study the hepatoprotective activity of the P. tetrastromatica extract. The Institution Animal Ethics Committee has approved the animal study for this research work. The experiments were designed and conducted in accordance with the institutional guidelines (No. 1011/C/06/CPCSEA). The animals were kept at 27 ± 2 ºC, relative humidity 44–56% and light and dark cycles of 10 and 14 h respectively, for a week before and during the experiments. Animals were provided with standard diet (Lipton, India) and water. The food was withdrawn 18–24 h before starting the experiment. All experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

(iv) Experimental protocol
Thirty mice (25-30 g) were randomly divided into groups and each group consists of 6 mice. The first group received 1ml/kg of saline (control) orally for 30 days. Group II was treated with Olive oil (1ml/kg body wt .ip) every 72 hours with three successive doses and Group III treated with 30% carbon tetrachloride in olive oil (1ml/kg body wt. ip) every 72 hours with three successive doses. Carbon tetrachloride induced toxicity animals (Group IV) were administered with aqueous extract of P. tetrastromatica (250 mg/kg body wt) orally by using intra gastric tubes for 30 days. At the end of the experimental period the animals were fasted overnight and then scarified by cervical decapitation. Blood was collected without any anti-coagulant for serum separation. Liver was excised immediately and washed in ice-cold saline. A portion was homogenized in 0.1 M Tris-HCl buffer. pH 7.4 at 4°C in a homogenizer using Teflon pestle at 600 rpm for 3 minutes. The ensuing supernatant fraction was analyzed for enzymatic and non-enzymatic biochemical assays.

(v) Determination of Serum Marker Enzymes
Serum hepatic marker enzymes namely, Aspartate transaminase (AST), Alanine transaminase (ALT), total and direct bilirubin and alkaline phosphatase (ALP) were measured using assay kits (Span Diagnostic, Surat). The extent of hepatocyte necrosis was determined with these activities as markers.

(vi) Evaluation of antioxidant status
Dissected out liver samples were washed immediately with ice-cold saline to remove excess blood on it. Liver tissue was homogenized in cold PBS (50 mM, pH 7) at a concentration of 10% (w/v). The homogenate was then centrifuged at 5000 × g for 10 minutes to obtain the supernatant, which was used for the assay of reduced glutathione, superoxide dismutase, catalase(CAT), glutathione peroxidase, thiobarbutric acid, and estimation of GSH. Total cholesterol was estimated. Tissue triglycerides were estimated by the method of Foster and Dunn. Free fatty acids were estimated by the method of Falholt et al. Phospholipids were estimated by the method of Zilversmit et al. The total cholesterol, triglycerides, free fatty acids and serum phospholipids were expressed as mg/dl.
(vii) Statistical analysis
Results are expressed as mean ± S.E.M. Using ANOVA method, experimental significance (P<0.001) were analyzed.

RESULTS

1. Activity levels of ALT, ASP, ALP and Bilirubin
In group III, animals were injected with Carbon tetrachloride to induce hepatotoxicity, the levels of ALT, AST, ALP and bilirubin were found to have been significantly (P<0.001) elevated by 71.42%, 41.59%, 81.23% and 134.21% respectively when compared to levels in normal groups. The animals which received seaweed extract orally showed elevated levels of these marker enzymes were found to have significantly (P<0.001) decreased by 31.48%, 25.22%, 40.35% and 42.69% when compared to the levels in Carbon tetrachloride induced mice. The activity levels of cellular marker enzymes are shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters in the serum</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (IU/l/min/mg protein)</td>
<td>AST (IU/l/min/mg protein)</td>
</tr>
<tr>
<td>Normal</td>
<td>31.5±2.22</td>
<td>119±3.68</td>
</tr>
<tr>
<td>Olive oil Control</td>
<td>32.16±1.47</td>
<td>123±1.37</td>
</tr>
<tr>
<td>% of change (Normal Vs Olive oil)</td>
<td>+2.09 NS</td>
<td>+3.3 NS</td>
</tr>
<tr>
<td>Carbon tetrachloride Control</td>
<td>54±2.04</td>
<td>168.5±6.02</td>
</tr>
<tr>
<td>% of change (Normal Vs Carbon tetrachloride)</td>
<td>+71.42 P&lt;0.001</td>
<td>+41.59 P&lt;0.001</td>
</tr>
<tr>
<td>Seaweed Extract</td>
<td>37±1.72</td>
<td>126±4.84</td>
</tr>
<tr>
<td>% of changes ( Carbon tetrachloride Vs seaweed extract fed)</td>
<td>-31.48 P&lt;0.001</td>
<td>-25.22 P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 1
Effect of P. tetrastromatica extract on Carbon tetrachloride induced hepatotoxicity. To induce the hepatotoxicity effect in liver, olive oil was administered along with CCl₄.

Values are mean of six individual observations in each group±S.D. ‘P’ denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the CCl₄ induced liver damaged groups. NS denotes Not significant.

2. Levels of antioxidants defense viz., TBARS, SOD, CAT, GPx, and GST in the liver tissue
The activity levels of antioxidant defense enzymes were examined in normal, olive oil; Carbon tetrachloride and sea weed extract treated groups. The levels of TBARS in Group-II were found to have been elevated by 1.51%, which was not significant when compared to levels in normal groups. In Group III animals which were injected with 30% Carbon tetrachloride intraperitoneally, the levels of TBARS was found significantly (p<0.001) elevated by 119.69% when compared to the levels in normal (Table: 2). After administration of sea weed extract the elevated levels of TBARS found to have reduced significantly (p<0.001) by 48.27% (Group IV) when compared to levels in Carbon tetrachloride induced liver damaged groups. The antioxidant enzymes include SOD, CAT, GPx and GST were observed in normal, Olive oil, Carbon tetrachloride and sea weed extract treated animals. In Group II, the olive oil induced animals, antioxidant defense enzymes were not significantly decreased when compared to the
level in normal animals. In Group III, animals were induced liver damage by Carbon tetrachloride administering intraperitonelly. In this group the antioxidant defense enzymes were found to have significantly (P<0.001) decreased by 54.71%, 43.51%, 43.22% and 50.59% respectively when compared to the levels in normal groups (Group I). After administration of *P. tetrastromatica* extract the decreased levels of antioxidant enzymes were found to have increased significantly (P<0.001) by 90%, 69.20%, 58.15% and 57.53% respectively (Table:2) when compared to levels in the Carbon tetrachloride induced liver damaged groups (Group III).

### Table 2

**Effect of *P. tetrastromatica* extract on the levels of antioxidant enzymes in liver**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters in the Liver</th>
<th>TBARS (nm/100g tissue)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GST (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>0.66±0.02</td>
<td>2.65±0.18</td>
<td>124.5±2.58</td>
<td>10.48±0.21</td>
<td>5.91±0.14</td>
</tr>
<tr>
<td>Olive oil Control</td>
<td></td>
<td>0.67±0.34</td>
<td>2.59±0.10</td>
<td>123±2.73</td>
<td>10.2±0.38</td>
<td>5.71±0.11</td>
</tr>
<tr>
<td>% of change (Normal Vs Olive oil)</td>
<td></td>
<td>+1.51</td>
<td>-2.2</td>
<td>-1.2</td>
<td>-2.6</td>
<td>-0.57</td>
</tr>
<tr>
<td>Carbon tetrachloride control</td>
<td></td>
<td>1.45±0.05</td>
<td>1.20±0.14</td>
<td>70.33±1.94</td>
<td>5.95±0.18</td>
<td>2.92±0.11</td>
</tr>
<tr>
<td>% of change (Normal Vs Carbon tetrachloride)</td>
<td></td>
<td>+119.69 P&lt;0.001</td>
<td>-54.71 P&lt;0.001</td>
<td>-43.51 P&lt;0.001</td>
<td>-43.22 P&lt;0.001</td>
<td>-50.59 P&lt;0.001</td>
</tr>
<tr>
<td>Seaweed extract</td>
<td></td>
<td>0.75±0.02</td>
<td>2.28±0.22</td>
<td>119±2.73</td>
<td>9.41±0.21</td>
<td>4.60±0.14</td>
</tr>
<tr>
<td>% of changes (Carbon tetrachloride Vs seaweed extract)</td>
<td></td>
<td>-48.27 P&lt;0.001</td>
<td>+90 P&lt;0.001</td>
<td>+69.20 P&lt;0.001</td>
<td>+58.15 P&lt;0.001</td>
<td>+57.53 P&lt;0.001</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are mean of six individual observations in each group ± S.D

*P* denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the Carbon tetrachloride induced liver damaged groups.

SOD – *U*<sub>1</sub>: One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.

CAT – *U*<sub>2</sub>: µmoles of hydrogen peroxide consumed per minute.

GPx – *U*<sub>3</sub>: µg of glutathione consumed per minute.

GST – *U*<sub>4</sub>: µmoles of CDNB

### 3. Levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum

Lipid profiles viz., total cholesterol, triglycerides, free fatty acids and phospholipids in serum were estimated in normal, olive oil, Carbon tetrachloride and sea weed treated animals. Group-II animals were induced by olive oil intraperitonelly. In this group the serum lipid profile like Total cholesterol, Triglycerides, Free fatty acid and phospholipids were elevated by 2.97%, 2.7%, 1.9% and 2.5% respectively which was considered as not significant. The level of lipid profile in group III Carbon tetrachloride induced liver damaged animals was found to have significantly (P<0.001) elevated by 46.53%, 87.15%, 105.7% and 48.10% respectively when compared to the levels in normal animals (Table:3). In Group IV toxicity induced animals were treated with the aqueous sea weed extract of *P. tetrastromatica*. The elevated levels of lipid profile enzymes were found to have significantly (P<0.001) decreased when compared to the Carbon tetrachloride induced liver damaged groups (Table:3).
Table 3
Effect of *P. tetrastromatica* extract on serum lipid profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters in the Serum</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Free fatty acids (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>101±2.16</td>
<td>54.5±2.42</td>
<td>52±3.14</td>
<td>79±2.42</td>
</tr>
<tr>
<td>Olive oil Control</td>
<td></td>
<td>104±1.75</td>
<td>56±1.87</td>
<td>53±1.16</td>
<td>81±1.03</td>
</tr>
<tr>
<td>% of change (Normal Vs Olive oil)</td>
<td>+2.97</td>
<td>+2.7</td>
<td>+1.9</td>
<td>+2.5</td>
<td></td>
</tr>
<tr>
<td>Carbon tetrachloride Control</td>
<td></td>
<td>148±2.92</td>
<td>102±4.19</td>
<td>107±3.06</td>
<td>117±2.94</td>
</tr>
<tr>
<td>% of change (Normal Vs Carbon tetrachloride)</td>
<td>+46.53</td>
<td>+87.15</td>
<td>+105.7</td>
<td>+48.10</td>
<td></td>
</tr>
<tr>
<td>Seaweed extract</td>
<td></td>
<td>105±3.27</td>
<td>60±1.41</td>
<td>59.1±4.03</td>
<td>89±1.72</td>
</tr>
<tr>
<td>% of changes (Carbon tetrachloride Vs Seaweed extract)</td>
<td>-29.05</td>
<td>-41.17</td>
<td>-44.76</td>
<td>-23.93</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are mean of six individual observations in each group ± S.D
‘P’ denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the Carbon tetrachloride induced liver damaged groups.

4. Histopathological studies

Figure 1

*Light microphotographs of haematoxylin and eosin stained sections of the formalin fixed liver cell of normal mice. (H, hepatocytes; SS, sinusoid).*

Liver cells of Group 1 mice (normal) have hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. These normal hepatocytes were polyhedral in shape with defined cell lining in the liver tissue. The cytoplasm was well preserved with prominent nucleus and nucleolus. Hepatocytes consisted 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.
Figure 2

Light microphotographs of haematoxylin and eosin stains sections of the formalin fixed liver cell of mice treated with carbon tetrachloride revealed extensive fatty changes, characterized by the disruption of the lattice nature of the hepatocyte, damaged hepatic sinusoids and necrosis. Presences of reticular sides are visible and nucleuses of two to three are joined together. (B, ballooning; S, steosis).

Figure 2 shows mice liver damage induced with carbon tetrachloride in olive oil (1 ml/kg body wt). Toxic effects such as liver damage, haemolytic anaemia, oxidative damage to the red blood cells and bleeding tendencies due to over dosage of carbon tetrachloride were noted. Marked distorted hepatocyte structure was observed in the liver of mice induced with carbon tetrachloride along with olive oil. The liver showed severe toxicity characterized by inflammatory cell collection and scattered inflammation. The cell lining of the hepatocytes are not visible after being exposed to high toxicity. Morphologic features that include vacuolization, ballooning degeneration, inflammatory cell infiltrate and mixed micro and macro vesicular steosis were observed. Besides that, the occurrence of focal necrosis, unnatural death, the progression of living tissue around the cell body and dented hepatic sinusoids of cells were observed.
Figure 3 shows the mice liver tissue induced with carbon tetrachloride and treated with *P. tetrastromatica* extract. It is highly known for its medicinal value as an antioxidant agent that prevents free radicals produced by carbon tetrachloride toxicity. Only minimal disruption of the structure of hepatocytes was noted in liver tissue of mice exposed to carbon tetrachloride and *P. tetrastromatica* extract. The liver tissue of mice treated with *P. tetrastromatica* extract displayed cell recovery compared to the mice induced with carbon tetrachloride alone. Hepatocytes were being transformed to normal polyhedral shape with some cell lining observed. Nucleus was recovered and clumping of nucleus was not seen.

**DISCUSSION**

A major consequence of oxidative stress is damage to nucleic acid bases, lipids and proteins, which can severely compromise cell health and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis. A number of studies have also shown that various herbal extracts and plant derived pure chemicals could protect organs against Carbon tetrachloride induced oxidative stress by altering the level of antioxidant enzymes. Endogenous antioxidants in medicinal herbs may play an important role in antioxidative defense against...
oxidative damage, possibly, protecting the biological functions of cells\textsuperscript{19}. In the present study the extract of \textit{Padina tetrastromatica} treated liver cells revealed restoration of the hepatic tissue architecture. In the liver sections of animals which were given Carbon tetrachloride and treated with \textit{Padina tetrastromatica} restoration of the hepatic cells was seen. A few micro and macro-vesicular type of fatty droplets, less vacuole formation, absence of necrosis and less visible changes overall were observed in the sea weed extract treated hepatocyte. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with Carbon tetrachloride\textsuperscript{20}. Carbon tetrachloride an extensively studied liver toxicant and its metabolites such as trichloromethyl peroxyradical are known to be involved in the pathogenesis of the liver damage\textsuperscript{21}. Levels of all marker enzymes increased significantly in Carbon tetrachloride induced animals. Liver marker enzymes such as ALT, AST, ALP and Bilirubin in the serum of Carbon tetrachloride administered mice indicate damage to hepatic cells\textsuperscript{22}. Damage to the cell integrity of the liver by Carbon tetrachloride is seen in the form of an increased in the activity of AST, which is released into circulation after cellular damage. ALP is an ectoenzyme of the hepatocyte plasma membrane. Carbon tetrachloride mediated acute toxicity increased permeability of the hepatocyte membrane and cellular leakage\textsuperscript{23}. The present findings concur with the above report. The increased ALT, AST, ALP and bilirubin activities suggested the possibility of the extract to give protection against liver injury upon Carbon tetrachloride induction.

The levels of TBARS were found to have been significantly elevated in Carbon tetrachloride induced liver damaged animals. This might be due to increased lipid peroxidation and increase of free radicals in hepatic cells. The significant depletion of levels of TBARS in the liver tissue of the seaweed extract administered animal group might be due to reduced lipid peroxidation and or elevation of tissue antioxidant defense enzymes activity levels indicating that the sea weed extract could reduce the generation of free radicals and increase the free radicals scavenging mechanism. The GHS antioxidant system consists of an array of non enzymatic and enzymatic reaction pathways involving the neutralizing of free radical species. Perturbation of the GSH status of a biological system has been reported to lead to serious consequence\textsuperscript{24}. GPx utilizes it for the decomposition of lipid hydroperoxides and other reactive oxygen species (ROS) and Glutathione – S – tansferase (GST) maximizes the conjugation of free radicals and various lipid hydroperoxides to GSH to form water soluble products that can be easily excreted. The decreased activities of SOD and CAT enzymes were recorded after the administration of Carbon tetrachloride \textsuperscript{25}. In our study, decline in the activities of SOD, CAT, GPx and GST levels in Carbon tetrachloride administered mice and recovery to near normal in sea weed extract administered groups revealed that oxidative stress elicited by Carbon tetrachloride intoxication has been nullified due to the antioxidant effect of \textit{P. tetrastromatica}. Many researchers reported the hepatoprotective effect of natural products, they mentioned the degree of protection through biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin (BRN), and total protein (TP)\textsuperscript{26}. An active constituent of \textit{P. tetrastromatica} possesses free radical scavenging activity.

In Carbon tetrachloride induced liver damaged groups, lipid profiles viz., total cholesterol, triglycerides, free fatty acids and phospholipids were found to have significantly increased. After treatment with sea weed extract the increased levels were restored to normal. The decreased serum cholesterol level in the extract administrated animals might be due to increased activity of enzyme CAT involved in esterification of cholesterol in the plasma. The significant decrease in the triglycerides in serum in the sea weed extract administrated animals might be due to decreased accumulation of lipoprotein. This might be due to increased activity of lipoprotein lipase which is involved in the uptake of
triglyceride-rich lipoprotein by extra hepatic tissue. The significant decrease in the free fatty acid accumulation in serum of sea weed extract administrated animals might be due to decreased lipid breakdown which corroborates with results obtained wherein a decreased lipid peroxidation and increased activity levels of antioxidant defense enzyme were recorded. The significant decrease in levels of phospholipids in the serum of sea weed extract administrated mice might be due to decreased peroxidation in the biomembrane of hepatocytes. Histological changes such as steatosis (Fatty changes in hepatocytes), perivenular fibrosis and significant pathomorphological alteration were observed in induced liver damaged groups. These changes can alter the properties of a cell. Morphologic features that include vacuolization, ballooning degeneration, inflammatory cell infiltrate and mixed macro and micro vesicular steatosis were observed in the liver of Carbon tetrachloride induced liver damaged groups. All the changes observed were very much reduced in the animals after the oral administration of aqueous sea weed extract of P. tetrastromatica. Histopathological observations suggested the ability of this extract to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation and increasing the antioxidant defense enzymes. However, the possible mechanism of hepatoprotection is rather speculative at this stage and investigations are underway to isolate and characterize the bioactive compounds from P. tetrastromatica.

CONCLUSION

These findings showed that the seaweed extract P. tetrastromatica is having antioxidant activity with properties of hepatoprotective. Since the seaweed P. tetrastromatica is available in south east coast of India, it is a promising candidate for use as an antioxidant and hepatoprotective agent.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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