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**ORIGINAL ARTICLE**

# *Padina boergessenii* ameliorates carbon tetrachloride induced nephrotoxicity in Wistar rats

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**Abstract** This research investigates the ameliorative effect of brown alga *Padina boergessenii* against CCl<sub>4</sub>-induced nephrotoxicity in rats. Twenty-four Wistar albino rats were divided into four groups as follows: control group (saline), CCl<sub>4</sub> group, CCl<sub>4</sub> + *P. boergessenii* extract group and *P. boergessenii* extract alone group. At the end of the experimental period, the animals are sacrificed and tissue samples (kidney) were collected. CCl<sub>4</sub> at a dose of 2 mL/kg, administered subcutaneously, produced significantly decreased activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT). Histopathological examinations showed massive centrilobular necrosis and fat accumulation in CCl<sub>4</sub>-induced animals. In the protective test, especially in animals pretreated with *P. boergessenii* (150 mg/kg) extract, there was a significant increase in antioxidant enzyme levels although there was no sign of abnormality in the kidney of rats pretreated with extract as evidenced by histopathological sections against CCl<sub>4</sub>-induced decrease of lipid peroxidation (TBARS). The ameliorative effect may be due to the presence of various bioactive compounds in alga. These results suggest that an extract of *P. boergessenii* is able to significantly alleviate the nephrotoxicity induced by CCl<sub>4</sub> in Wistar rats.

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**1. Introduction**

Carbon tetrachloride (CCl<sub>4</sub>), a lipid soluble, heavy, and non-flammable liquid is most widely used for experimental induction of hepato (Reinke et al., 1988; Salazar-Montes et al., 2000; Karthikeyan et al., 2010b) and nephrotoxicity (Sylvia Zhao et al., 1997). CCl<sub>4</sub> is an organic compound widely used as a dry cleaning solvent until it was recognized as a potent carcinogen (Kovacic and Jacintho, 2001). The toxic effects of CCl<sub>4</sub> on liver have been extensively studied and it has been attributed to the production of free radical metabolites, with the cytochrome

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P450s involved in the metabolism rapidly destroyed during the metabolic process. There is extensive evidence to implicate free radicals in the development of degenerative diseases and progressive decline in the immune system (Pike and Chandra, 1995). Additionally, CCl<sub>4</sub> is classified as a probable human carcinogen, based on the development of hepatocellular carcinomas in rats, mice and hamsters (Salazar-Montes et al., 2000).

Acute and chronic renal damage are also very common pathophysiological disturbances caused by CCl<sub>4</sub> (Ogeturka et al., 2005). Administration of CCl<sub>4</sub> causes an increase in lipid peroxidation products (Daniels et al., 1995; Abraham et al., 1999; Donder et al., 1999) and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney. The trichloromethyl and trichloromethyl peroxy radicals are reported to enhance lipid peroxidation and protein oxidation, resulting in wide spread membrane damage and decrease in the activity of enzymes protecting lipid peroxidation in the kidney (Dogukan et al., 2003).

The anti-oxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and indirectly glutathione reductase. The role of these enzymes is well known and has been investigated extensively both *in vivo* and *in vitro* in model systems. The nephrotoxic effect of CCl<sub>4</sub> is associated with free radical formation (Feral et al., 2003) which leads to lipid peroxidation and breakdown of the membrane structure. Characteristic renal injuries from carbon tetrachloride exposure are nephritis, nephrosis, and renal failure. Kidney failure is one of the leading causes of death in CCl<sub>4</sub> intoxication. The consequence of oxidative damage is serious, and in many cases apparent in increased activities of enzymes involved in oxygen detoxification. The negative effects of free radicals, oxidative stress may well be overcome by antioxidants (Larson, 1995).

Seaweeds received significant attention for their potential as natural antioxidants (Kumar et al., 2008; Monsuang et al., 2009). In specific, brown algae are rich sources of bioactive compounds like polyphenols, carotenoids and polysaccharides. Recently, we studied the hepatoprotective effect of *P. boergesenii* against CCl<sub>4</sub> induced liver damage in Wistar rats (Karthikeyan et al., 2010b). In addition, we have evaluated the antioxidant activities of brown alga *P. boergesenii* with ferric nitrilotriacetate induced renal damage in circulation of rats (Karthikeyan et al., 2010a) in two different extracts. These results have encouraged us to pay more attention to this particular species of the family Phaeophyceae. The present study was designed to evaluate the putative antioxidant action of brown alga *P. boergesenii* extract in an experimental model of CCl<sub>4</sub>-induced nephrotoxicity in Wistar rats.

## 2. Materials and methods

### 2.1. Chemicals

Reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase, thiobarbituric acid (TBA), CCl<sub>4</sub> and diethyl ether were purchased from Sigma–Aldrich. All other chemicals and solvents of analytical grade were obtained from S.D. Fine chemicals, Chennai, India.

### 2.2. Collection of seaweeds

In the present study the seaweed *P. boergesenii* belonging to the class Phaeophyceae, of the family Dictyotaceae, was collected

from Vaan island, Gulf of Mannar Biosphere in the intertidal region (Lat 11°29'N; Long 79°46'E) during April and May 2007. The algal species were hand picked and washed thoroughly with seawater to remove all the impurities like sand particles and epiphytes. Morphologically distinct thallus of algae was placed separately in new polythene bags and these were kept in an icebox containing slush ice and transported to the laboratory. Finally, the seaweeds were rinsed carefully in freshwater and shade dried then the samples were pulverized into powder (size 2 mm) using a grinder before extraction. The brown alga *P. boergesenii* was identified by its taxonomical features.

### 2.3. Preparation of seaweed extract

In general, the principle of soxhlet extraction is an extraction method by using chemical solvents (Lim et al., 2002). The 5 g of *P. boergesenii* grinded powder was extracted with 100 mL of diethyl ether (non-polar solvent) using a soxhlet extractor for 8 h at 35 °C. The extraction was repeated many times to obtain a sizable quantity of extract. The extracts were then concentrated under reduced pressure and the resultant residues were stored in a dark at 4 °C until further use. For the investigation of *in vivo* study diethyl ether residues were dissolved in 0.3% carboxyl methyl cellulose as a vehicle.

### 2.4. Animal and housing

The male albino rats of the Wistar strain (4–6 weeks old), weighing 125–150 g, were obtained from Central Animal House, Faculty of Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, India. Animal experiments were approved by the Institutional Ethical Committee Regulations. The Central animal house registration number is 160/1999/CPCSEA. The rats were fed with pellet diet and water *ad libitum*. During experimentation they were housed in the respective polypropylene cages. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with alternating 12 h light/dark cycles.

### 2.5. Experimental induction of nephrotoxicity

All the rats were allowed to acclimatize for 1 week prior to experimentation. The treatment regimen for brown alga *P. boergesenii* extract was based on the preliminary *in vitro* studies carried out in our laboratory (Karthikeyan et al., 2010b). CCl<sub>4</sub> dose was selected according to Janbaz et al. (2004). A total of 24 rats were used the animals were randomized and had free access to standard laboratory feed. The rats are grouped into experimental and control rats ( $n = 6$  in each group). Group I served as saline-treated negative control and was administered with saline (0.85% NaCl) alone. Group II subcutaneously injected with CCl<sub>4</sub> (2 mL/kg body weight) every day for 1 month. Group III were pretreated with diethyl ether extract with dose of 150 mg/kg body weight orally with the help of gavage tube before 1 h of CCl<sub>4</sub> administration. Group IV rats were orally administered with *P. boergesenii* diethyl ether extract (150 mg/kg body weight) alone for 1 month. At 24 h after the last treatment of extracts or pellet feed animals on the 30th day from groups II and III received an *i.p.* injection of CCl<sub>4</sub> (2 mL/kg body weight). All 24 animals were sacrificed by cervical dislocation within a period of 1 and 12 h after treatment with CCl<sub>4</sub> or saline.

## 2.6. Evaluation of kidney damage

Kidney of all rats was excised immediately after collection of the blood. The right and left tissue specimens were washed twice with a cold saline solution placed into glass bottles and labeled. The right kidneys were used for biochemical estimations and the left kidney tissue specimens were used for histopathological examination.

## 2.7. Preparation of tissue samples

The tissue sample (kidney) was rinsed in physiological saline, and homogenized. The homogenate (25%, w/v) was in ice-cold 0.25 M sucrose, then the homogenate was deproteinized with an equal volume of ice-cold (10%, w/v) trichloroacetic acid. The ensuing supernatant fraction was analyzed for enzymatic and non-enzymatic biochemical assays.

## 2.8. Vitamin E in kidney

The level of vitamin E were determined by the method of reduction in  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by the tocopherols with the formation of a red complex of the  $\text{Fe}^{2+}$  with  $\alpha,\alpha'$ -dipyridyl (Emmerie and Engel, 1938a,b). The transmissions were measured by calorimeter and it is expressed in terms of  $\mu\text{g}/\text{mg}$  protein.

## 2.9. Estimation of thiobarbituric acid reactive substances (TBARS)

The level of TBARS was estimated in kidney by measuring malondialdehyde and TBARS reactivity with thiobarbituric acid (TBA) to generate a pink coloured chromophore, which was read at 535 nm (Nichans and Samuelson, 1972). The transmissions were measured by calorimeter and it is expressed in terms of  $\text{mM}/100$  g wet tissue.

## 2.10. Estimation of reduced glutathione

Reduced glutathione was estimated in kidney by the method of Ellman (1959). The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 mL of buffer. The amount of glutathione was expressed as  $\text{mg}/100$  g tissue.

## 2.11. Superoxide dismutase

Superoxide dismutase (SOD) activity was determined by the method of reaction between superoxide radicals with nitroblue tetrazolium (NBT) in the presence of NADH and produced formazan blue (Kakkar et al., 1984). SOD removes the superoxide radicals and inhibits the formation of formazan blue. The intensity of colour is inversely proportional to the activity of the enzyme. One unit of the enzyme activity is defined as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min under the assay conditions and expressed as specific activity in units/mg protein.

## 2.12. Estimation of catalase

The catalase (CAT) activities were determined by the method of conversion of dichromate in acetic acid to perchromic acid and then to chromic acetate in the presence of  $\text{H}_2\text{O}_2$  when subjected to heat (Claiborne, 1985). Thus formed chromic acetate was

measured at 620 nm and the activities were expressed as  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  consumed units/mL and in liver as units/mg protein.

## 2.13. Estimation of glutathione peroxidase (GSH-Px)

Glutathione peroxidase was estimated by the reaction of the sample with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH were measured by the method of Rotruck et al. (1973). The activities were expressed as  $\mu\text{g}$  of GSH consumed units/mL and in liver as units/mg protein.

## 2.14. Histopathological examination

Dissected kidneys were fixed in 10% neutral formalin solution for at least 24 h, then embedded in paraffin wax (melting point 56–58 °C), sectioned to 4 mm thickness and stained with Mayer's hematoxylin and eosin (H&E) for histopathological examination under light microscope and sectioned (Drury and Wallington, 1980). All sections were evaluated for the degree of tubular and glomerular injury, vascular congestion, interstitial mononuclear inflammatory cell infiltration and fibrosis by an experienced observer.

## 2.15. Statistical analysis

The results were analyzed among the experimental group of animals and statistical significance ( $*p < 0.05$ ,  $**p < 0.01$ ) between controls and treated groups were evaluated using multivariate analysis of variance (ANOVA) followed by Duncan's multiple range test.

## 3. Results and discussion

The aim of the present study was to investigate the protective effects of brown alga *P. boergessenii* against carbon tetrachloride induced nephrotoxicity in Wistar rats. In  $\text{CCl}_4$  toxicity, the rat's kidney weight was increased during oxidative damage as compared to the control group. The rats pretreated with extract (150 mg/kg body weight) almost restored the kidney weight against toxicant-induced toxicity when compared to the control group (Table 1). Treatment of rats with extract alone (150 mg/kg body weight) did not show any significant changes. The ameliorative effect of *P. boergessenii* against  $\text{CCl}_4$  was studied by the following antioxidant assays.

### 3.1. Effect of vitamin-E levels in *P. boergessenii* extract on $\text{CCl}_4$ -induced nephrotoxicity

Table 1 shows the level of vitamin-E in kidney. There was a significant decrease ( $p < 0.01$ ) in the relative vitamin-E level in  $\text{CCl}_4$ -induced rats, when compared to the control group. Pretreatment of rats with *P. boergessenii* extract (150 mg/kg) for 30 consecutive day's significantly increased ( $p < 0.01$ ) the vitamin-E level, when compared to rats induced with  $\text{CCl}_4$  alone. However, there is no significant effect on rats administered with extract alone.

### 3.2. Effects of *P. boergesenii* extract on TBARS

As shown in Table 1, is the level of TBARS in kidney. The level of TBARS was significantly increased ( $p < 0.01$ ) in rats treated

**Table 1** Effect of *P. boergesenii* extract on CCl<sub>4</sub> induced changes in kidney weight, TBARS, reduced glutathione and vitamin-E in rat kidney.

Groups	Kidney weight (g/rat)	TBARS (mM/100 g tissue)	Reduced glutathione (mM/100 g tissue)	Vitamin-E (mg/dL)
Control	2.08 ± 0.23	1.45 ± 0.21	117.75 ± 0.18	1.33 ± 0.09
CCl <sub>4</sub>	2.86 ± 0.20*	4.3 ± 0.22*	81.033 ± 0.69*	1.46 ± 0.09*
CCl <sub>4</sub> + extract	2.53 ± 0.19**	2.7 ± 0.32**	101.66 ± 0.14**	0.67 ± 0.04**
Extract	2.11 ± 0.23	1.4 ± 0.22	127.03 ± 0.32	1.14 ± 0.08

The treatment with *P. boergesenii* extract (150 mg/kg bw) was started at 1 h interval before CCl<sub>4</sub> (2 mL/kg body weight) administration for 1 month. Each value represents the mean value ± SEM of six animals.

\* Statistical difference with control group at  $p < 0.01$ .

\*\* Statistical difference with CCl<sub>4</sub> group at  $p < 0.05$ .

with CCl<sub>4</sub>, when compared to the control group. *P. boergesenii* extract at the dose of 150 mg/kg of bw per day significantly ( $p < 0.05$ ) decreased the TBARS level as compared to CCl<sub>4</sub> induced group. There is no significant change in the level of TBARS in rats treated with extract alone. The biochemical mechanisms involved in the development of CCl<sub>4</sub> hepatotoxicity have long been investigated but rare are the data on nephrotoxicity. It is generally believed that lipid peroxidation caused by the carbon trichloromethyl radical, CCl<sub>3</sub>. TBARS, a stable metabolite of the free radical mediated lipid peroxidation cascade, is widely used as marker of lipid peroxidation. CCl<sub>4</sub>-induced nephrotoxicity resulted in a significant increase in TBARS level, which is similar with previous findings by Feral et al. (2003) and Ogeturka et al. (2005). The data of TBARS level demonstrate that oral administration of extracts to rats suppressed ischemia–reperfusion-induced renal lipid peroxidation and tissue injury probably via the alteration of cytochrome P-450.

### 3.3. Effects of *P. boergesenii* extract on reduced glutathione content

Glutathione (GSH) constitutes the first line of defense against free radicals (Ogeturka et al., 2005). As shown in Table 1, the CCl<sub>4</sub>-induced group showed a significant reduction in GSH content ( $p < 0.01$ ) (Feral et al., 2003; Ogeturka et al., 2005). Pretreatment with extract at the dose of 150 mg/kg during CCl<sub>4</sub> administration significantly ( $p < 0.05$ ) protected the GSH depletion. *P. boergesenii* extract can prevent the renal

GSH depletion and it is also an antioxidant that reacts with free radicals. The ameliorative effects may be due to its ability to enhance glutathione production by providing more substrate for reactive intermediates that promote detoxification mechanisms. The protective quality of extract on CCl<sub>4</sub> poisoning may be due to its action on GSH. Recently, our data have shown the antioxidant properties of *P. boergesenii* extract against ferric nitrilotriacetate induced renal oxidative damage (Karthikeyan et al., 2010a) and CCl<sub>4</sub> induced hepatotoxicity in Wistar rats (Karthikeyan et al., 2010b).

### 3.4. Effects of *P. boergesenii* extract on SOD, CAT and GSH-Px

Table 2 shows the effects of *P. boergesenii* extract on antioxidant enzymes in CCl<sub>4</sub>-induced nephrotoxicity in rats. After injection of CCl<sub>4</sub>, the activities of GSH-Px were significantly ( $p < 0.01$ ) decreased as compared to the control group (Feral et al., 2003). Pre-treatment of animals with *P. boergesenii* extract (150 mg/kg) for 4 weeks significantly elevated the expression of GSH-Px as compared to the rats induced with CCl<sub>4</sub> alone ( $p < 0.05$ ).

Table 2 illustrates the superoxide dismutase and catalase status in control and experimental group of rats. However, pretreatment of rats with *P. boergesenii* extract could restore the antioxidant capacity exhausted by CCl<sub>4</sub>. Administration of CCl<sub>4</sub> results in oxidative damage to the lipids and proteins of the kidney in rats (Abraham et al., 1999). In the present study, the defense antioxidant enzymes were significantly

**Table 2** Effect of *P. boergesenii* extract on CCl<sub>4</sub> induced changes in SOD, CAT and GSH-Px in kidney with histopathological inference.

Groups	Superoxide dismutase (SOD) (U/mg protein)	Catalase (CAT) (U/mg protein)	Glutathione peroxidase (GSH-Px) (U/mg protein)	Histopathology inference
Control	16.21 ± 0.87	58.5 ± 1.17	7.03 ± 0.55	Normal
CCl <sub>4</sub>	8.50 ± 0.70*	31.08 ± 3.08#	5.21 ± 0.31*	Glomerular changes and tubular dilation
CCl <sub>4</sub> + extract	13.07 ± 0.88**	48.48 ± 3.25###	6.02 ± 0.47**	Absence of tubular dilation
Extract	15.28 ± 0.85	57.7 ± 1.87	7.23 ± 0.32	Normal

The treatment with *P. boergesenii* extract (150 mg/kg bw) was started at 1 h interval before CCl<sub>4</sub> (2 mL/kg body weight) administration for 1 month. Each value represents the mean value ± SEM of six animals.

\* Statistical difference with control group at  $p < 0.01$ .

\*\* Statistical difference with CCl<sub>4</sub> group at  $p < 0.05$ .

# Statistical difference with control group at  $p < 0.05$ .

### Statistical difference with CCl<sub>4</sub> group at  $p < 0.05$ .

altered by induction of CCl<sub>4</sub>. There was a significant decrease in SOD, GSH-Px ( $p < 0.01$ ) and CAT activities ( $p < 0.05$ ) (Ozen et al., 2004). The significant increase in the level of antioxidant enzymes probably occurred as a result of the rats pretreated with extracts. As reported earlier by Rush et al. (1984), the decreased activity of GSH-Px is due to changes in the level of glutathione content. Similarly, the decreased activity of SOD in CCl<sub>4</sub>-induced rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes. Overall biochemical investigations, the pretreatment of *P. boergessenii* extract prior to CCl<sub>4</sub> intoxication could not only renovate the changes in lipid peroxidation and reduce GSH content, but also protected the antioxidant machineries of the rat kidney as revealed from the enhanced levels of SOD, GSH-Px ( $p < 0.05$ ) and CAT ( $p < 0.05$ ) activities.

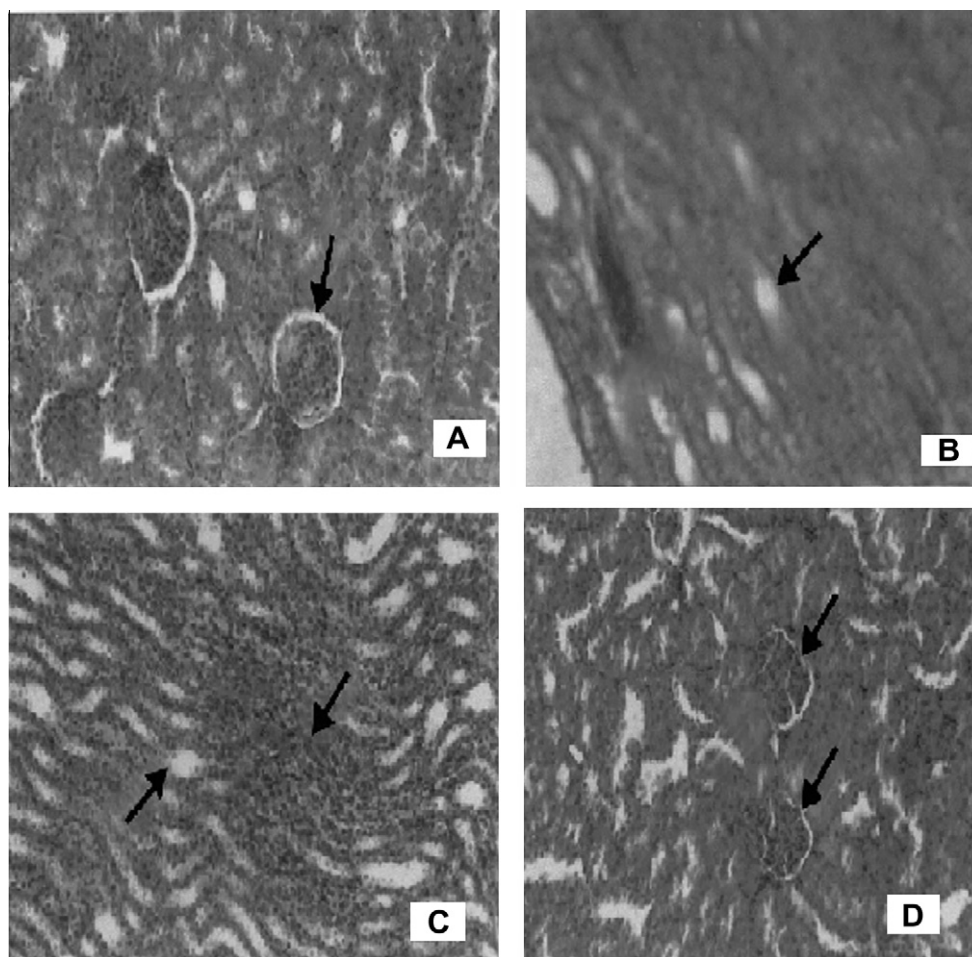
### 3.5. Histopathological changes

The tissue sections stained with H&E were examined for the extent of kidney damage. Morphologic damage was cortical and ranged from none (control and *P. boergessenii* extract group) to moderate (CCl<sub>4</sub> + *P. boergessenii* extract group) to severe (CCl<sub>4</sub> group). However, extensive cortical damage and focal glomerular necrosis were observed in CCl<sub>4</sub> chal-

lenged group (Doi et al., 1991). In addition, there are morphological changes in cortical tubules, such as dilated and flattened epithelial cells (Fig. 1). Interstitial inflammatory cell infiltration was also seen in the CCl<sub>4</sub> group (Feral et al., 2003; Ogeturka et al., 2005). Where as, the group of rats pretreated with extract reversed the CCl<sub>4</sub>-induced nephrotoxicity; it shows the glomeruli were normal and sparse tubular changes were observed. In this group, the affected tubules showed vacuolization, dilation, and a few cell detachments.

Results suggest that the extract possesses protective action against CCl<sub>4</sub> induced nephrotoxicity. This has been confirmed by antioxidant assays and histopathological observations. In our earlier report, the protective nature of *P. boergessenii* was confirmed by damage in the structural integrity of cell membrane in liver and leakage of transaminases from the cytoplasm into the blood circulation (Karthikeyan et al., 2007).

In conclusion, *P. boergessenii* extract effectively protect the kidney from oxidative damage against CCl<sub>4</sub> through free radical scavenging and avoiding the oxidation of detoxifying enzymes. Moreover, not only CCl<sub>4</sub>-induced hepatotoxicity in our earlier findings (Karthikeyan et al., 2010b) but also nephrotoxicity was effectively alleviated by the *P. boergessenii*-pretreatment showed in these study experiments. Therefore,



**Figure 1** Histopathology of kidney. (A) Normal glomeruli. (B) CCl<sub>4</sub> toxicity shows focal hyaline cast. (C) Extract treated group show hemorrhage and cloudy swelling. (D) Extract alone show normal glomeruli and tubules.

the brown alga *P. boergeseni* is beneficial in reducing free radical damage. While it is unclear to what extent each mechanism may contribute, all these reactions may provide a fundamental protective milieu for kidney in coping against CCl<sub>4</sub> induced nephrotoxicity, by favoring the removal of oxidants as well as by increasing the amount of anti-oxidants from *P. boergeseni* extract. At this stage we identified and characterized the presence of polyphenols in *P. boergeseni* (data not shown) and still the investigations are underway for structural elucidation of bioactive compounds.

In summary, the results of this study suggest that *P. boergeseni* could prevent renal damage by improving the lipid peroxidation products through the scavenging activity of free radicals induced by CCl<sub>4</sub>. Further, the improvement of antioxidant enzymes may be another possible mechanism of action of brown alga *P. boergeseni*.

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