



PROTECTIVE EFFECT OF POLYSACCHARIDE ISOLATED FROM *Codium elongatum* AGAINST CCl_4 INDUCED HEPATOTOXICITY IN MALE WISTAR RATS

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ABSTRACT

Codium elongatum is a green marine algae belonging to the family Codiaceae. It is found in the intertidal zone of marine water bodies attached to the substratum. Some researchers suggest the presence of alkaloid, flavonoids, amino acids, carbohydrate and polysaccharides in *C. elongatum*. This study is the first report on the protective effect of water extract and isolated polysaccharide from *C. elongatum* against CCl_4 -induced hepatotoxicity. Liver marker enzymes such as ALT, AST and total bilirubin were measured to evaluate the hepatoprotective effect. The results were also supported by the histological study of liver excised from rats used in the experiment. Further, it can be concluded that polysaccharides from *C. elongatum* might have significant hepatoprotective activity

KEYWORDS

Codium elongatum, Codiaceae, hepatoprotective, marine algae.

INTRODUCTION

Hepatotoxicity is damage caused to the liver by oxidative stress, drugs and xenobiotics. Hepatic damage can result in the distortion of various metabolic functions that are regulated by the liver^[1]. Carbon tetrachloride is a selective hepatotoxic xenobiotic that is metabolised by bioactivation of phase I cytochrome P450. This leads to the formation of highly reactive metabolites viz. trichloro- methyl free radical ($\text{CCl}_3\cdot$) and trichloromethyl peroxy radical ($\text{CCl}_3\text{O}_2\cdot$). These free radicals initiate membrane lipid peroxidation by decreasing the activity of the antioxidant enzyme in the liver^[2]. These free radicals reduce glutathione (GSH) dependent antioxidant enzymes and GSH of phase II detoxification enzymes, which is a significant indicator in acute and chronic hepatic injury^[3-5].

Presently, hepatotoxicity is the most proliferating pathology around the globe depicting up to 83% of all cases^[6]. Synthetic drugs that are used in the treatment of hepatic disorder also possess many side effects. These facts rekindled the global interest in complementary and alternative medicine for more safe options. We thus evaluated the hepatoprotective potential of water extract and isolated polysaccharide (ISP) from *Codium elongatum* on CCl_4 -induced hepatotoxicity in Wistar male rats. This study is the first to report the in - vivo hepatotoxic activity of *C. elongatum*.

MATERIALS AND METHODS

Collection And Authentication Of Sample

C. elongatum was collected from the Arabian Sea, Gulf of Kutch (the Marine National Park, Jamnagar) in the month of January and authenticated by CSIR- National Botanical Research Institute (NBRI), Lucknow. The specimen was deposited in the algal herbarium of CSIR-NBRI with serial no. MARIN-01.

Extraction

Algae were cleaned with water and dried in shade for 3-4 weeks. Dried algae were powdered coarsely and weighed 100 gm for extraction. Successive solvent extraction was carried out with solvents in ascending order of polarity viz. chloroform, ethyl acetate, ethanol and water. Each filtrate was concentrated using a rotary evaporator^[7].

Isolation

Algae (200 grams) was treated with 400 ml of 85% ethanol. After depigmentation algae were removed from the solvent and air dried. Then it was treated with 0.2 N HCl (1:10 w/v) at 60°C for 2 hours by stirring in the water bath and left overnight. The mixture was vacuum filtered and algae were re-extracted with the same solvent for 1 hour. The extracts were combined and concentrated under reduced pressure at a temperature not exceeding 40°C. Three volume of ethanol was added

to precipitate the polysaccharide. The precipitated polysaccharide was filtered and washed twice with 85% ethanol for 30 minutes each. The desalted polysaccharide was lyophilized. Finally, the isolated polysaccharide (ISP) was stored at 4°C^[8-11].

CCl_4 Induced Hepatotoxicity

Animal Treatment

Twenty-four Wistar male albino rats (n = 4) were divided into six different groups. Group I and II served as control groups and were treated with vehicle only (olive oil and water respectively). Group IV served as standard (Liv 52, 56 mg/kg). Group V and VI are test I (water extract, 500mg/kg) and test II (ISP, 250 mg/kg). Above groups were treated for 5 days. Group III-VI were treated with CCl_4 in olive oil (1:1, 1 ml/kg) from day 2 to day 5. All doses were administered orally (p.o.). On day 6, all animals were sacrificed by cervical dislocation under the effect of 2% ethyl ether. The liver was excised and rinsed with phosphate buffer saline followed by EDTA (7mM). Blood was collected by cardiac puncture was centrifuged at $1500 \times g$ for 10 min, at 4°C to obtain serum^[12].

Assessment Of Liver Marker Enzyme

Blood serum was used to analyse various liver marker enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST) and total bilirubin by using standard diagnostic kits (Truechemie, India).

Histological Studies

Histological slides were prepared from liver tissue by using a microtome. Slides were stained with hematoxylin and eosin and washed with ethanol and xylene. The slides were observed under the microscope. Any architectural change in liver tissue is taken into consideration^[13-14].

Statistical Studies

The data were analysed using a one-way analysis of variance (ANOVA), followed by Tukey's test. The mean $\pm$ S.E.M. was calculated for each data set. The P value < 0.0001 was considered as significant

RESULTS

Effect Of Serum Marker Enzymes

There was a notable increase in mean plasma ALT, AST and total bilirubin levels in CCl_4 -intoxicated rats ($p < 0.0001$) as compared with the control group (Table 1). Liv.52, water extract and ISP reduces the activity of serum enzymes. These outcomes suggest the probability of water extract and ISP from *C. elongatum* to protect the liver against the harmful effect of CCl_4 .

Table 1. AST, ALT And Total Bilirubin Level In The Liver Of Experimental Rats

	AST (IU/L)	ALT (IU/L)	Total Bilirubin (mg/dl)
Group 1 (olive oil control)	280.35±18	72.15±5.5	0.08±0.008
Group 2 (water control)	253.4±0.19	68.4±0.10	0.06±0.014
Group 3 (CCl ₄)	640.3±21.8a**	530.3±25.2	0.32±0.01a****
Group 4 (Liv. 52)	475.5±15.5a,b***	359.8±10.1a,b****	0.17±0.01a***,b****
Group 5 (water extract)	469.8±34a,b**	370.2±20a,b*	0.21±0.01a,b***
Group 6 (ISP)	433±17.6a,b**	282.6±28.2a,b****	0.20±0.01a,b***

Results were presented as Mean±S.D. (n=4); Tukey's multiple comparison test:***p<0.001, ****p<0.0001 a refers to comparison with olive oil control and b refers to comparison with toxic control.

Histological Examination

The hepatoprotective effect of water extract and ISP from *C.elongatum* was supported by histological examination. These results suggest that CCl₄-induced hepatotoxicity was reduced by water extract and ISP significantly. As shown in Fig. 1(a) and 1(b) liver tissue of olive oil and water control groups showed no abnormal condition in the portal vein and central vein. The liver section exhibits hepatic cells with a nucleus and well-integrated cytoplasm. Whereas the liver tissue of the CCl₄ treated group in Fig. 1(c) showed moderate diffuse necrosis, moderate vacuolization, accumulated lipid surrounding the central vein and mild inflammatory cell infiltration of hepatocytes. Tissue damage in Liv 52-treated group was much lighter as compared with the pathological changes found in the CCl₄-treated group as shown in Fig. 1(d). Histopathological damage induced by CCl₄ was improved by water extract and ISP as evident from Fig. 1(e) and 1(f). This study suggests that *C.elongatum* can prevent the hepatotoxicity caused by CCl₄ based on histological observations.

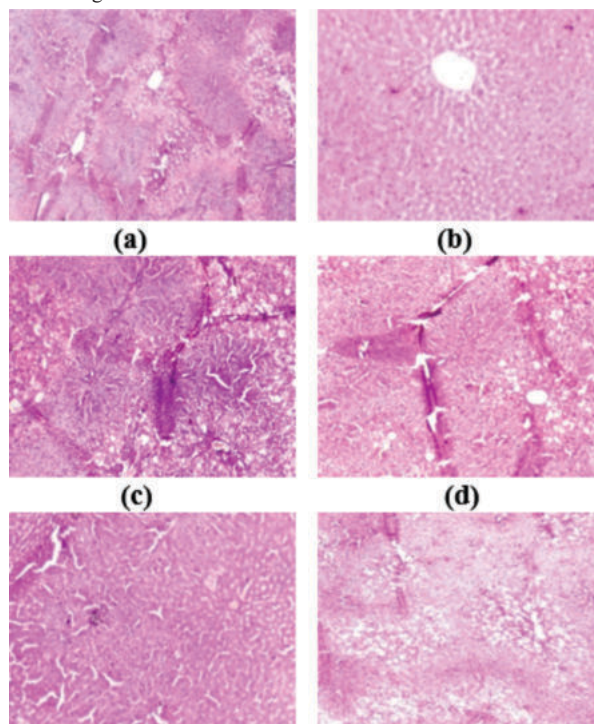


Figure 1. Micrograph of liver of experimental rats (H&E stain). (a) Group I, liver cell of water control group (5 ml/kg) p.o. (b) Group II, liver cell of the olive oil control group (5 ml/kg) p.o. (c) Group III, liver cell of CCl₄ intoxicated group (1 ml/kg) p.o. (d) Group IV, liver cell of the standard group treated with Liv. 52 (56 mg/kg; p.o.) + intoxicated with CCl₄ (e) Group V, liver cell of the standard group treated with water extract of *C.elongatum* (250 mg/kg; p.o.) + intoxicated with CCl₄ (f) Group VI, liver cell of the standard group treated with polysaccharide isolated from *C.elongatum* (500 mg/kg; p.o.) +

intoxicated with CCl₄.

DISCUSSION

Trichloromethyl radical (CCl₃*) and trichloromethyl peroxy radical (CCl₃O₂*) formed by the metabolism of CCl₄ by CYP 450 have the potential to combine with lipids and proteins. Which leads to membrane peroxidation, protein desaturation and eventually liver cell necrosis [15]. In the present study rats intoxicated with CCl₄ represents remarkable hepatic damage as evident by activities of ALT, AST and total bilirubin. Any modification in the activity of these liver marker enzymes will lead to cellular dysfunction and tissue lesion [16-18]. In earlier studies, it was also reported that a reduction in the level of these enzymes indicates the stabilisation of plasma membrane and repair of the damaged liver [19]. The efficiency of a drug to reduce the toxic effect or preserving the normal physiological mechanism of liver cell is the index of its protective effect.

Histological studies also demonstrated the efficacy of water extract and ISP as hepatotoxicity protective agents. Simultaneous treatment of water extract and ISP causes less damage to liver cells as compared to rats treated only with CCl₄. The results of biochemical parameters were supported by the results of the histological study.

CONCLUSIONS

It can be concluded that the alteration in plasma liver marker enzyme due to CCl₄ administration is overturned by water extract and ISP. *C.elongatum* increases the reparative and regenerative capacity of the hepatic cells which may be due to the effect of polysaccharides found in the cell walls of this marine algae.

REFERENCES

- [1] Snehal, D.F., Uday, R.S., Divakar, G. & Perfect, S. (2008). Hepatoprotective activity of leaves of *Rhododendron arboreum* in CCl₄ induced hepatotoxicity in rats. *Journal of Medicinal Plants Research*, 2(11), 315-320.
- [2] Kanter, M., Coskun, O., & Budancamanak, M. (2005). Hepatoprotective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World journal of gastroenterology: WJG*, 11(42), 6684.
- [3] Preethi, K. C., & Kuttan, R. (2009). Wound healing activity of flower extract of *Calendula officinalis*. *Journal of basic and clinical physiology and pharmacology*, 20(1), 73-80.
- [4] Szymonik-Lesiuk, S., Czechowska, G., Stryjecka-Zimmer, M., Słomka, M., Małdro, A., Celiński, K., & Wielosz, M. (2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *Journal of hepato-biliary-pancreatic surgery*, 10, 309-315.
- [5] Ichi, I., Kamikawa, C., Nakagawa, T., Kobayashi, K., Kataoka, R., Nagata, E., Kitamura, Y., Nakazaki, C., Matsura, T. & Kojo, S. (2009). Neutral sphingomyelinase-induced ceramide accumulation by oxidative stress during carbon tetrachloride intoxication. *Toxicology*, 261(1-2), 33-40.
- [6] Cemek, M., Aymelek, F., Büyükköroğlu, M. E., Karaca, T., Büyükbek, A., & Yılmaz, F. (2010). Protective potential of Royal Jelly against carbon tetrachloride induced-toxicity and changes in the serum sialic acid levels. *Food and chemical toxicology*, 48(10), 2827-2832.
- [7] Okunade, A. L. (2002). *Ageratum conyzoides* L. (asteraceae). *Fitoterapia*, 73(1), 1-16.
- [8] Manilal, A., Sujith, S., Selvin, J., Kiran, G.S. & Shakir, C. (2009). In vivo antiviral activity of polysaccharide from the Indian green alga, *Acrosiphonia orientalis* (J. Agardh): potential implication in shrimp disease management. *World Journal of Fish and Marine Sciences*, 1(4), 278-282.
- [9] El-Rafie, H. M., El-Rafie, M., & Zahran, M. K. (2013). Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. *Carbohydrate polymers*, 96(2), 403-410.
- [10] Barros, F.C., da Silva, D.C., Sombra, V.G., Maciel, J.S., Feitosa, J.P., Freitas, A.L. & De Paula, R.C. (2013). Structural characterization of polysaccharide obtained from red seaweed *Gracilaria caudata* (J. Agardh). *Carbohydrate Polymers*, 92(1), 598-603.
- [11] Hernández-Garibay, E., Zertuche-González, J. A., & Pacheco-Ruiz, I. (2011). Isolation and chemical characterization of algal polysaccharides from the green seaweed *Ulva clathrata* (Roth) C. Agardh. *Journal of Applied Phycology*, 23, 537-542.
- [12] Sahreen, S., Khan, M. R., & Khan, R. A. (2011). Hepatoprotective effects of methanol extract of *Carissa opaca* leaves on CCl₄-induced damage in rat. *BMC complementary and alternative medicine*, 11, 1-9.
- [13] Khan, R. A., Khan, M. R., & Sahreen, S. (2012). CCl₄-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC complementary and alternative medicine*, 12(1), 1-6.
- [14] Boyd, A., Cain, O., Chauhan, A., & Webb, G. J. (2020). Medical liver biopsy: background, indications, procedure and histopathology. *Frontline Gastroenterology*, 11(1), 40-47.
- [15] Brattin, W. J., Glende Jr, E. A., & Recknagel, R. O. (1985). Pathological mechanisms in carbon tetrachloride hepatotoxicity. *Journal of free radicals in biology & medicine*, 1(1), 27-38.
- [16] Recknagel, R. O., Glende Jr, E. A., Dolak, J. A., & Waller, R. L. (1989). Mechanisms of carbon tetrachloride toxicity. *Pharmacology & therapeutics*, 43(1), 139-154.
- [17] Weber, L. W., Boll, M., & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical reviews in toxicology*, 33(2), 105-136.
- [18] Lin, H. M., Tseng, H. C., Wang, C. J., Lin, J. J., Lo, C. W., & Chou, F. P. (2008). Hepatoprotective effects of *Solanum nigrum* Linn extract against CCl₄-induced oxidative damage in rats. *Chemico-Biological Interactions*, 171(3), 283-293.
- [19] Sreelatha, S., Padma, P. R., & Umadevi, M. (2009). Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Food and Chemical Toxicology*, 47(4), 702-708.