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PROTECTIVE EFFECT OF DIOXOLOPYRAN DERIVATIVE 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 dioxolopyran derivative (DOPD) obtained 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. There was a notable increase in mean plasma ALT, AST and total bilirubin levels in CCl_4 -intoxicated rats as compared with the control group. Histopathological damage induced by CCl_4 was improved by water extract and DOPD. The results were supported by the histological study of liver excised from rats used in the experiment. Further, it can be concluded that DOPD derived from *C.elongatum* might have significant hepatoprotective activity.

Keywords: *Codium elongatum;* Codiaceae; hepatoprotective; marine algae, dioxolopyran derivative.

1. 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 (Snehal *et al.,* 2005). 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. trichloromethyl free radical (CCl_3•) and trichloromethyl peroxy radical (CCl_3O_2•). These free radicals initiate membrane lipid peroxidation by decreasing the activity of the antioxidant enzyme in the liver (Kanter *et al*., 2005). 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 (Preethi & Kuttan, 2009; Szymonik-Lesiuk *et al*., 2003; Ichi *et al*., 2009).

Presently, hepatotoxicity is the most proliferating pathology around the globe depicting up to 83% of all cases (Cemek *et al*.,2010). 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 dioxolopyran derivative (DOPD) from *Codium elongatum* on CCl_4-induced hepatotoxicity in male sistra rats. This study is the first to report the *in – vivo* hepatotoxic activity of *C.elongatum.*

2. Materials and methods

2.1 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.

2.2 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 (Okunade 2002).

2.3 Isolation

Algae (200 grams) was treated with 400 ml of 85% ethanol. After de-pigmentation algae was removed from the solvent and air dried. Then it was treated with 0.2 N HCl (1:10 w/v) at 60^oC for 2 hours by stirring in the water bath and left overnight. The mixture was vacuum filtered and algae was re-extracted with the same solvent for 1 hour. The extracts were combined and concentrated under reduced pressure at a temperature not exceeding 40^oC. Three volume of ethanol was added to precipitate the DOPD. The precipitated DOPD was filtered and washed twice with 85% ethanol for 30 minutes each. The desalted DOPD was lyophilized. Finally, the isolated DOPD was stored at 4^0C (Manila *et al*., 2009; El-Rafie *et al*., 2013; Barros *et al*., 2013; Hernández-Garibay *et al*., 2011).

2.4 CCl_4 induced hepatotoxicity

2.4.1 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 (DOPD, 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 × g for 10 min, at 4°C to obtain serum. (Sahreen *et al.*, 2011).

2.4.2 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).

2.4.3 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 (Khan *et al*., 2012; Boyd *et al*., 2020).

2.5 Statistical studies

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

3. Results

3.1 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 DOPD reduces the activity of serum enzymes. These outcomes suggest the probability of water extract and DOPD from *C. elongatum* to protect the liver against the harmful effect of CCl_4.

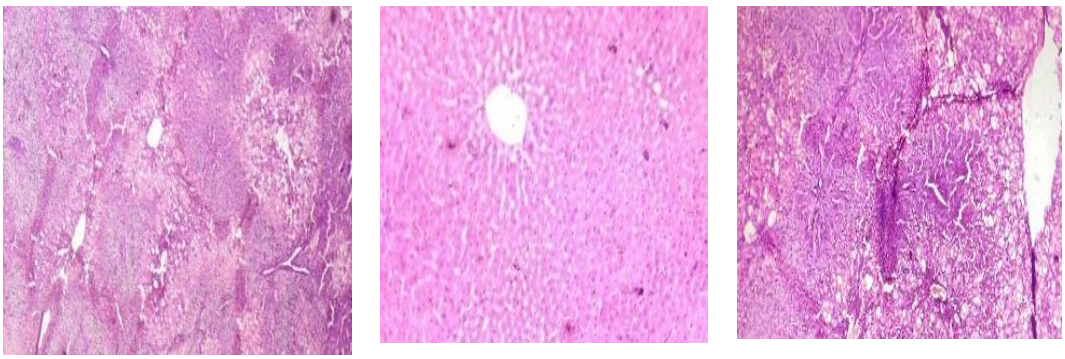
Table 1. AST, ALT and total bilirubin level in the liver of experimental rats.

	Group 1 (olive oil control)	**Group 2 (water control)**	**Group 3 (CCl_4)**	**Group 4 (Liv. 52)**	**Group 5 (water extract)**	**Group 6 (DOPD)**
AST (IU/L)	280.35±18	253.4±0.19	$640.3±21.8^{a****}$	$475.5±15.5^{a,b****}$	$469.8±34^{a,b****}$	$433±17.6^{a,b****}$
ALT (IU/L)	72.15±5.5	68.4±0.10	530.3±25.2	$359.8±10.1^{a,b****}$	$370.2±20^{a,b****}$	$282.6±28.2^{a,b****}$
Total lBilirubin (mg/dl)	0.08±0.008	0.06±0.014	$0.32±0.01^{a****}$	$0.17±0.01^{a***,b****}$	$0.21±0.01^{a,b****}$	$0.20±0.01^{a,b****}$

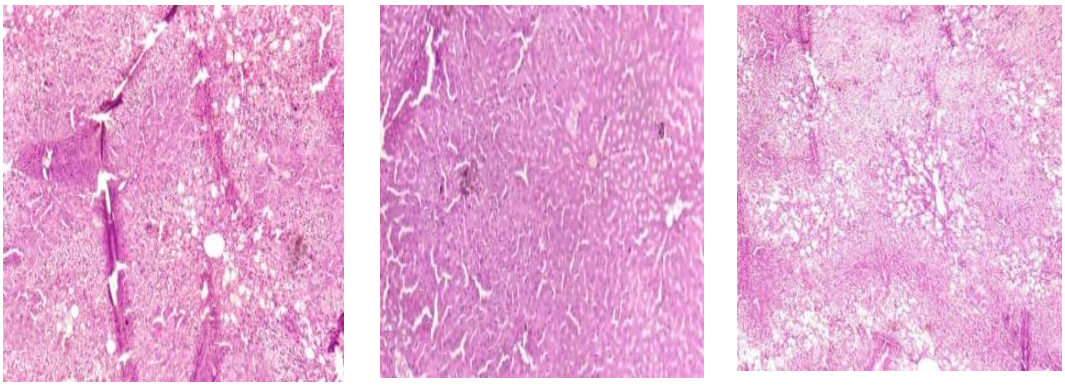
Results were presented as Mean±S.D. (n=4); Tukey's multiple comparison test: ***p<0.001,: ****p<0.0001 a refers to comparison with olive oil control and b refers to comparison with toxic control.

3.2 Histological examination

The hepato protective effect of water extract and DOPD from *C.elongatum* was supported by histological examination. These results suggest that CCl_4-induced hepatotoxicity was reduced by water extract and DOPD 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 CCl_4 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 CCl_4-treated group as shown in Fig. 1(d). Histopathological damage induced by CCl_4 was improved by water extract and DOPD as evident from Fig. 1(e) and 1(f). This study suggests that *C.elongatum* can prevent the hepatotoxicity caused by CCl_4 based on histological observations.



(a) (b) (c)



(d) (e) (f)

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 CCl_4 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 CCl_4 (e) Group V, liver cell of the standard group treated with water extract of *C.elongatum* (250 mg/kg; p.o.) + intoxicated with CCl_4 (f) Group VI, liver cell of the standard group treated with DOPD isolated from *C.elongatum* (500 mg/kg; p.o.) + intoxicated with CCl_4.

4. Discussion

Trichloromethyl radical (CCl_3*) and trichloromethyl peroxyl radical (CCl_3O_2*) formed by the metabolism of CCl_4 by CYP 450 have the potential to combine with lipids and proteins. Which leads to membrane peroxidation, protein desaturation and eventually liver cell necrosis (Brattin *et al*., 1985). In the present study rats intoxicated with CCl_4 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 (Recknagel *et al*., 1989; Weber *et al*., 2003; Lin *et al*., 2008). 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 (Sreelatha *et al*., 2009). 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 DOPD as hepatotoxicity protective agents. Simultaneous treatment of water extract and DOPD causes less damage to liver cells as compared to rats treated only with CCl_4. The results of biochemical parameters were supported by the results of the histological study.

5. Conclusions

It can be concluded that the alteration in plasma liver marker enzyme due to CCl_4 administration is overturned by water extract and DOPD. *C.elongatum* increases the reparative and regenerative capacity of the hepatic cells which may be due to the effect of dioxo;opyran derivative found in the cell walls of this marine algae.

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