



Research Article



# Effect of Brown Algae *Cystoseira trinodis* Methanolic Extract on Renal Tissue

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## ABSTRACT

**Background:** *C. trinodis* brown algae of Oman Sea coast is used traditionally as a diuretic in Chabahar, Sistan and Baluchestan province of Iran. But no researches have been conducted on the distractive effects of this alga on the renal tissues until now.

**Methods:** Forty-two adult male mice were divided into 6 groups. Control group received normal saline (E0), group (E1) treated with 5mg/kg methanolic extract (ME) and group (E2) to (E5) received 10, 15, 25 and 50 mg/kg of ME of alga respectively. All animals in 6 groups were treated for 2 weeks (once every other day). Finally, histopathological evaluations were made especially by morphology and photometric method.

**Results:** ME of *C. trinodis* induced histological damage in kidney. Administration of ME in all experimental groups induced severe glomerular congestion, hyaline cast and severe interstitial inflammatory centers in treated groups. All distractive parameter in test groups increased with increasing dose of extract ( $p < 0.05$ ).

**Conclusion:** Results showed that ME of the *C. trinodis* has a nephrotoxic effect on the renal tissues.

## Introduction

The effect of food and nutritional compositions is one of the most important fields in the human health investigations. Extensive distribution of the algae made them to be considered as an important food source in many regions of the world.<sup>1</sup> Valuable compounds and nutrients such as carbohydrate, minerals, proteins and fatty acids are the reason for many of the algae species to be used as food as well as traditional remedies for many diseases.<sup>2-4</sup> Some of the algae compounds have revealed biological and potential therapeutic properties such as anti-inflammatory, antibacterial, antiviral, cytotoxic and antimetabolic activities.<sup>5-7</sup> Among the chemical compounds, polyphenols, polysaccharides, meroterpenoids and terpenoids are the major chemical groups in the brown algae.<sup>8</sup> Also many of these compounds such as carotenoids and natural pigments have valuable roles as the antioxidant in human health.<sup>9</sup> However several chemical compounds such as terpenoids and steroids have been isolated from different species of the *Cystoseira* genus but limited histopathological studies about effects of algae extracts have been reported.<sup>10,11</sup> Although many of the algae are used as food source and alternative health care remedies insufficient studies on adverse effects of them

clearly necessitate more investigations about them. Chemical compounds induce different injuries in many tissues. Among of them, acute kidney injury (AKI) is a considerable life threatening injury. For this reason AKI studies are important related to many situations including natural chemical compounds toxicity. Because there is no data available about algae extract effects on renal tissue until now, we considered nephropathological effect of polar extract of one species of *Cystoseira* genus in this research.

## Material and Methods

### Plant material

*C. trinodis* was collected from the Coast of Qeshm Island in the Persian Gulf and was identified by Dr. A. Pasdaran, School of Pharmacy, Guilan University of Medical Sciences, Iran (In Herbarium of School of Pharmacy, Guilan Medical of Sciences, Iran). A voucher specimen (M.a 317) was deposited at the herbarium there.

### Extraction

The fresh seaweed fronds were washed and then air dried. Powdered algae (300 g) were Soxhlet-extracted with 2.5 L of n-hexane, dichloromethane and methanol

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for a period of 72 h. The crude extract was filtered, concentrated at 40°C using a rotary vacuum evaporator, and dried in an oven at 40°C for 4-5 h (yield 9.5% on a dry-weight basis). The methanolic extract (2g) was subjected for next step and used for injections in the experiment.

### Animal

The male adult mice were obtained from Animal house of Guilan University of Medical Sciences. Animals were divided into 6 groups (each including seven mice) received 5,10,15,25 and 50 mg of the methanolic extract of algae and a control group. Animals were kept in six cages, at 25°C with 60–75% relative humidity and a 12h light/12h dark cycle, with standard mice diet and water. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication no. 85–23, revised 1996) also related guidelines on the ethical use and care of laboratory animals issued by School of Medicine, Guilan University of Medical Science (1930506209).

### Animals experimental design

Experimental design was based on Sato et al method with some modifications.<sup>12,13</sup> Forty-two mice were randomly divided into the six groups (n=7) as follows: Group (E0): normal control group (E0; untreated), Group (E1): received 5 mg of methanolic extract kg<sup>-1</sup> body weight, Group (E2): received 10 mg, Group (E3): received 15 mg, Group (E4): received 25 mg and Group (E5) received 50 mg. The extract was administered three times a week for two weeks by intraperitoneal injection.

### Tissue collection and histopathology

The right kidney tissues were excised from the sacrificed animals according to conventional methods.<sup>14</sup> The kidneys were rinsed with normal saline and fixed into 10% neutral buffered formalin for histopathological examinations. The fixed tissues were cut into small sizes and put in a labeled tissue cassette for dehydration processing (LEICA, ASP 300, Nussloch, Germany). After dehydration with a series of different ethanol concentrations (70%, 95%, and 100%) (5 times), the tissues were cleaned twice with xylene before being embedded in paraffin molds (LEICA, EG 1160, Nussloch, Germany). Each cooled paraffin block was sliced to 5 µm thicknesses using a microtome (LEICA, RM2155, Nussloch, Germany). Each section was floated on a 45°C water bath and picked up using a glass microscope slide (LEICA, HI 1210, Nussloch, Germany). The sections were then dried at 58°C on a heater for 15 min to melt the wax and to secure the tissue firmly on the glass slide. For the hematoxylin and eosin (H&E) staining, the sections were routinely deparaffinised using xylene and rehydrated through a series of descending alcohol concentration and water

mixtures (100%, 90%, and 70%) then slides were examined under light microscope (Olympus BX51, Tokyo, Japan).

### Lesion scoring for kidney

The morphological changes related to hyaline cast, tubules with swelling cells, interstitial tissue inflammation centers and glomerular congestion were counted in twenty fields of each tissue section of the kidneys (five sections in each kidney samples were stained) using an image analyzer (Olympus BX51, Tokyo, Japan) at 400x magnifications. The degree of injuries was counted as the percentage mean of lesions in twenty different fields (zigzag manner) in each section.<sup>15</sup>

### Statistical Analysis

Study results are presented as mean and standard deviation (SD). Nonparametric tests (Kruskal Wallis, Bonferroni test) were performed as statistical evaluation and P<0.05 was considered significant.

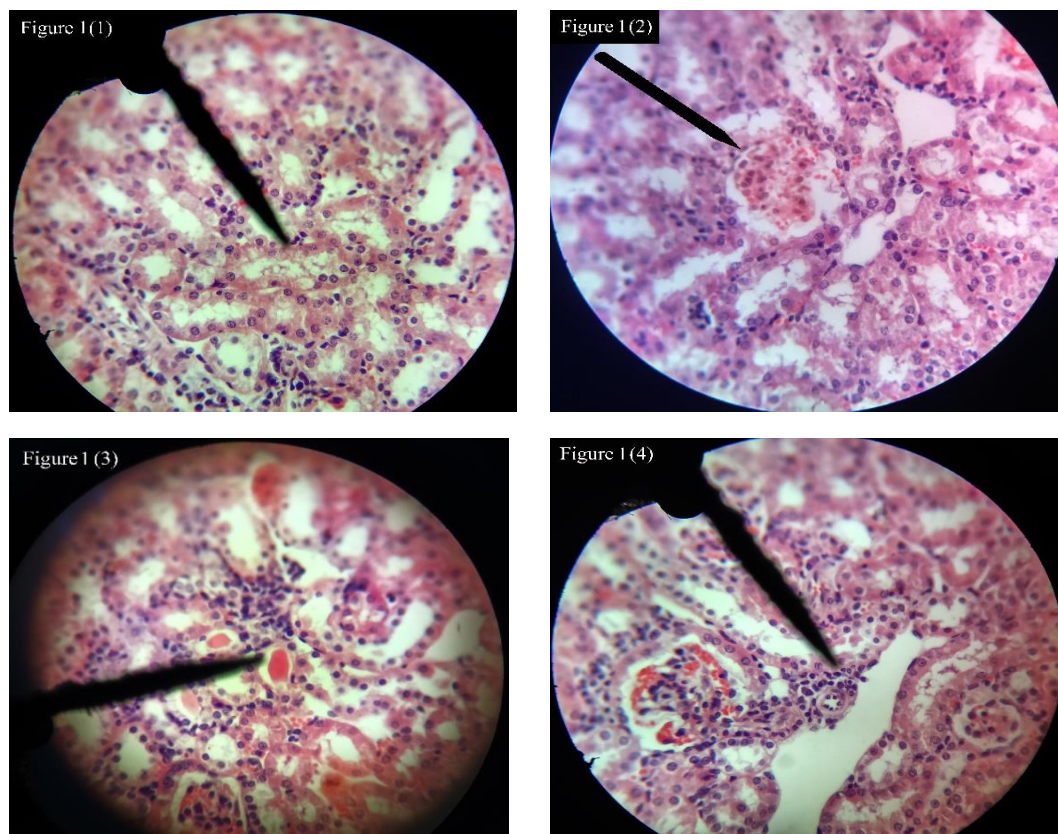
### Results

#### Effects on the renal tissue

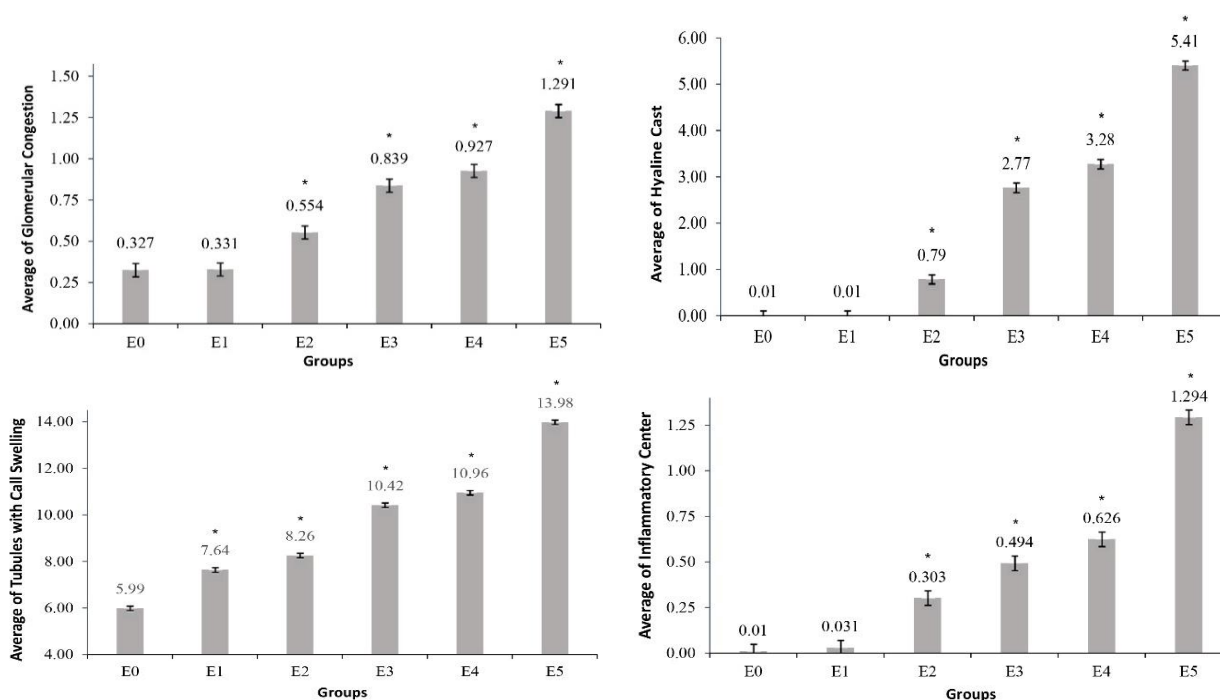
The effects of *C. trinodis* methanolic extract have been shown in (Figure 1). In the control group normal architecture of the renal corpuscle and renal tubules observed. In contrast, the kidneys which received the highest dose revealed acute tubular cell swelling Figure 1(1), severe glomerular congestion Figure 1(2), hyaline cast in the tubules Figure 1(3), and severe interstitial inflammatory centers Figure 1(4). All the glomerular congestion changes were observed in the renal tissues which have received methanolic extract of *C. trinodis* that found to be increased significantly (P<0.05). The congested glomeruli number of test groups increased with increasing dose of extract (Figures 2) and (Table 1). The percentage number of swelling tubular cells in kidney sections of E<sub>5</sub> group mice is significant (P<0.05) (Figures 2, Table 1). Most of hyaline tubular casts were found in group E<sub>5</sub> (P<0.05) in comparison to E<sub>1</sub> group that showed the lowest amount of hyaline casts (Figure 2, Table 1). Inflammatory centers in groups E<sub>5</sub> and E<sub>4</sub> as compared to other groups had the significant highest value (P<0.05) and was observed with lowest number in group E<sub>1</sub> (Figure 2, Table 1). Mean damage in all of the experimental groups and the control group are summarized in Figure 2.

### Discussion

In some investigations, blue-green and brown algae have shown toxicities in animal models.<sup>16</sup> Many studies indicated that marine algae compounds such as substituted phenols, quinols and unsaturated diterpens (meroditerpens) act as effective antioxidant.<sup>17</sup> The *Cystoseira* genus major chemical compound (meroditerpens and toluquinols) are very potent antioxidant reactive chemical compounds.<sup>18</sup>



**Figure 1.** Photomicrographs of the renal tissues damage in different groups. Renal cortex of normal control mice showed renal corpuscle and tubules with moderate congestion of the cortical blood vessels (400 x). E<sub>5</sub> group's renal cortex showed severe morphological changes such as tubules with cell swelling (Figure 1(1)), glomerular congested (Figure 1(2)), Hyaline cast (Figure 1(3)), Interstitial inflammatory centers (Figure 3(4)) (400x). In all groups that received the extract showed the morphological changes seen in Group E<sub>5</sub>.



**Figure 2.** Mean of injurers (Average) occurred in all of the experimental and the control group. E<sub>0</sub>: normal control group mice, Group (E<sub>1</sub>): mice received 5 mg of methanolic extract kg<sup>-1</sup> body weight, Group (E<sub>2</sub>): mice received 10 mg of methanolic extract kg<sup>-1</sup> body weight, Group (E<sub>3</sub>): mice received 15 mg of methanolic extract kg<sup>-1</sup> body weight, Group (E<sub>4</sub>): mice received 25 mg of methanolic extract kg<sup>-1</sup> body weight, Group (E<sub>5</sub>): mice received 50 mg of methanolic extract kg<sup>-1</sup> body weight. \*P value compared to the control group and indicated in table 1.

**Table 1.** Statistical distribution of the mean counts in twenty fields in each kidney section (five sections in each kidney) experimental and the control groups at the end of study.

Mean and Standard deviation number of count	Groups(n=7)					
	E <sub>0</sub>	E1	E2	E3	E4	E5
M GC	0.327	0.331	0.554	0.839	0.927	1.291
±SD GC	0.339	0.007	0.062	0.043	0.056	0.067
M TCS	5.994	7.643	8.263	10.419	10.959	13.979
±SD TCS	0.138	0.036	0.165	0.055	0.103	0.540
M HC	0.010	0.143	0.793	2.769	3.281	5.406
±SD HC	0.010	0.005	0.053	0.058	0.049	0.056
M IC	0.010	0.031	0.303	0.494	0.626	1.294
±SD IC	0.004	0.004	0.004	0.053	0.064	0.068

M: Mean, ±SD: Standard deviation, GC: Glomerular congestion, TCS: Tubules with Cell Swelling, HC: Hyaline Cast, IC: Inflammatory center. Kruskal-Wallis test. Mean number of GC, TCS, HC and IC in different groups had statistically significant difference (P<0.005).

There has been evidence for a role of excessive amounts of the antioxidant in the pathogenesis of a variety of renal injuries that probably produced by imbalance of oxido reductase systems.<sup>19</sup> Inflammation process induced by reactive chemical compounds but some studies has revealed that antioxidant compounds also showed distractive effect in absence of oxidative agent. Such compounds also showed oxido reductase systems inhibition that probably triggered distractive cycle of renal damage.<sup>20</sup> In this study we observed severe glomerular congestion, hyaline cast in the tubules and severe interstitial inflammatory centers. Glomerular congestion and other histopathology changes have denoted a dose dependent manner. The severity of the injuries was markedly observable in the 50mg/kg of the extract which is parallel to concentrations in other groups. Based on this observation, using brown algae such as *Cystoseira* genus for therapeutic application call for more toxicity studies.<sup>21</sup> We can determine that probably the polar compounds of the methanolic extract of *Cystoseira* species have a nephrotoxic effect on animal model by accumulation of the antioxidant excessive amount.

### Conclusion

According to this research, further studies on chemical compounds of *Cystoseira* species can be useful for finding toxin in these algae genus. This genus has a large biodiversity among other algae but most of them have not yet been investigated for pharmacological and biological activities.

### Conflict of interests

The authors claim that there is no conflict of interest.

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