

# Brown Seaweed *Sargassum Cinereum* Extract Ameliorates the Hepato and Nephrotoxicity induced by Cyclophosphamide in Male Albino Rats: Hematological Biochemical and histobiochemical examinations

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## Abstract

**Background:** Cyclophosphamide (CP) is a selective antibiotic used clinically as an anticancer drug. Treatment with CP exhibits a number of side effects. Here in this study, we explored the toxic effects of CP and the ameliorating effects of *Sargassum cinereum* in albino rats. **Material and methods:** Eighteen rats divided into 3 groups (6 rats each) control group, CP injected group in a dose of 200 mg/kg b. w and CP injected plus oral administration of *S. cinereum* group in a dose of 180 mg/kg b. w for 20 consecutive days. Results showed cyclophosphamides induced hematological, biochemical, oxidative and histological changes. **Results:** Hematological analyses revealed a significant decrease in the RBCs, WBCs' count and Hb concentration. Biochemical analyses revealed increase in the liver enzymes activity including ALT, AST and ALP, while albumin and total proteins were significantly decreased. Creatinine, urea, and uric acid levels as well as lipid profiles including cholesterol and triglycerides were increased significantly. Moreover, Oxidative stress was active that evidenced by a significant decrease in SOD, GSH and CAT levels, while a significant increase was recorded in the MDA level. For histopathology necrosis and inflammations were observed in the hepatic and kidney tissues. Treatment with brown seaweed *S. cinereum* significantly ameliorated all of the above mentioned hematological, biochemical, oxidative damages and histopathological changes induced by CP injection. **Conclusion:** Cyclophosphamide induced its toxicity through the activation of the oxidative system in the tissues, while the ameliorative effect of brown seaweeds (*S. cinereum*) achieved through activation of the antioxidant system.

**Keywords:** Biochemicals, Cyclophosphamides, Hepatitis, Histopathological, Oxidative stress, *S. cinereum*.

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## INTRODUCTION

Cyclophosphamide (CP) and the associated nitrogen mustard-derived alkylating agents are widely used in the treatment of a variety of human malignancies and disorders like breast cancer, carcinoma of the lung [1], systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis [2,3]. Unfortunately, the use of this drug is accompanied by a number of side effects and toxicity that includes nausea, vomiting, alopecia [4], hematopoietic suppression [5], nephrotoxicity, urotoxicity [6] and cardiotoxicity [7]. The highly toxic effect of CP mediated through the activation of cytochrome P450 enzyme which enhance the oxidation process with the synthesis of the toxic products phosphoramidate mustard and acrolein [8]. In this pathway of CP activation reactive oxygen species were have potentially generated [9]. Oxidative stress is responsible for CP induced hepatotoxicity [10]. The

report presented by Al zeni [11] revealed that CP treatment is associated with disrupt the liver function, evidenced by increases in AST, ALT, g-GT and CPK levels. Cyclophosphamides injection enhanced the oxidative stress system in the liver that marked by increased in the lipid peroxidation (LPO) and decreased the glutathione (GSH) content, glutathione peroxidase (GPx) as well as the superoxide dismutase (SOD) activities [12].

Several studies have shown that CP can be nephrotoxic, both in humans and in animal experimental models as it induced glomerular and tubular dysfunction [13,14]. Besides fibrosis, necrosis of urinary bladder and hemorrhagic cystitis [15,16].

Egypt country characterized by long seashores such as in the Mediterranean and the Red Sea. These shores have a national wealth of marine organisms,

especially algae. The large brown algae often play important roles in the physical and biological structure of the local marine environment [17]. Seaweeds are naturally renewable sources. They are used as food, feed, and fertilizers in different regions in the world. Many studies and trials were done on seaweeds to isolate some life-saving drugs or biologically active compounds all over the world [18]. *Sargassum* sp., is one of the marine macroalgae a member of the class Phaeophyceae, family Sargassaceae and order Fucales and widely spread in tropical and temperate oceans. The brown seaweed *Sargassum Cinereum* is the great source of the biologically active sulfate and fucose compounds which they containing polysaccharide that considered as commercial antibiotics [19]. *S. cinereum* Ethanolic extract has medical uses as such as antibacterial, antifungal, antioxidative and radical scavenging activities [20]. This study was designated to investigate the toxic effects of the CP on the rats with a focus on the ameliorating effects of the *S. cinereum* against the CP toxicity.

## MATERIALS AND METHODS

### Experimental Animals

Eighteen (18) male White Albino rats 3-6 weeks age, (150-180 g) weight. The animals were purchased from the animal house, Faculty of Medicine, Assuit University, Egypt. Rats were housed in individual cages under a 12-h light/dark cycle. The room temperature and relative humidity were maintained at  $23 \pm 1^\circ\text{C}$  and 52 %, respectively. Rats were left for two weeks before starting the experiment for the acclimatization. All animals were housed under the clean environmental conditions and given the basal diet and water ad-libitum. All protocols were approved by the Institutional Review Board for Animal Experiments of South Valley University, Egypt.

### Cyclophosphamides

Cyclophosphamide (Endoxan) was supplied as vials from Baxter Oncology (Düsseldorf, Germany).

### Collection of Algal Samples

The brown algae *S. cinereum* was collected from Hurghada city, Red Sea. The samples were cleaned with fresh water then distilled water. The samples dried under sunrise and then crushed to form a fine powder.

### Ethanolic extract of *cinereum* preparation

The powdered seaweed (100 g) rinsed in 300 ml of 95% ethanol for 3 days at room temperature then poured out and filtered and repeated 2 times. The filtrates were evaporated at  $40^\circ\text{C}$  under reduced pressure using a rotary evaporator to produce ethanolic crude extract [21].

### Experimental Design

The experimental animals were randomly divided into three equal groups each of 6 animals.

Group 1: The rats were intraperitoneally injected with 0.9% isotonic saline solution at a dose of 5 ml/kg b.w. Group 2: The rats were intraperitoneally (i.p) injected with a single dose of cyclophosphamide (CP) 200 mg/kg b. w. Group 3: The rats intraperitoneally injected with a single dose of cyclophosphamide (CP) 200 mg/kg b. w plus oral administration of *Sargassum cinereum* extract at dose of 180 mg/kg b. w for consecutive 20 days.

At the end of experiment, all groups were sacrificed. Whole blood is collected by heart puncture and divided into 2 parts. The first one was put on heparinized tubes for hematological analyses. The other part was collected without anticoagulant for obtaining serum and kept frozen at  $-20^\circ\text{C}$  until used for biochemical analyses. Liver and kidney was removed aseptically and washed with phosphate buffered saline (PBS). Liver tissues were divided into two portions. First portion of liver tissue was blotted, homogenized using a Branson sonifier (250, VWR Scientific) and stored at  $-80^\circ\text{C}$  until used for determination of hepatic oxidative system (GSH, CAT, SOD, and MDA). The other portion both with kidney portion were kept in 10% buffered formaldehyde for histopathological investigations.

### Hematological analysis

The heparinized blood was used for assessment the RBCs' count, Hb concentration and WBCs' count using automated blood counter (Urit, China).

### Biochemical Assays

#### Liver functions

Serum Alanine aminotransferase (ALT), aspartate aminotransferase, (AST) and alkaline phosphatase (ALP), Albumin and Total proteins were estimated using the available kits purchased from (spectrum Diagnostics Co. Oubor city, Egypt.) and analysed according to the manufacture procedures.

#### Kidney functions

Creatinine, urea and uric acid were estimated using the available kits purchased from (spectrum Diagnostics Co. Oubor city, Egypt), and analysed according to the manufacture rules.

#### Liver Oxidative stress determination

Different oxidative system markers Malondialdehyde(MDA), reduced glutathione(GSH), Catalase(CAT) and super oxide dismutase (SOD), were estimated in the liver homogenates using available kits purchased from (Bio-diagnostic Co. Giza, Egypt) and analyzed according to the manufacture rules.

#### Histopathology

Collected liver and kidney tissues were rapidly fixed in 10% neutral buffered formalin, passed in ascending series of ethanol, embedded in paraffin for

sectioning (4–5  $\mu\text{m}$ ), then mounted on glass slides. Tissues sections were stained with Haematoxylin and Eosin (H&E) for histopathological findings.

### Liver

The liver tissues sections were scored according to Zhang [22] respectively histological activity index/scoring system, for the assessment of the following aspects 1) portal/ periportal activity with or without bridging necrosis grade (0-10), 2) fibrosis grade (0-4). intralobular necrosis grade (0-4) 3) portal inflammation grade (0-4). The sum of the histological activity index of each lesion was multiplied by 2 to give more weight to this value.

### Kidneys

The kidney tissues sections were scored according to Zhang [22] histological activity index/scoring system, for the assessment of the following aspects 1) normal histology grade (0), 2) tubular epithelial cell degeneration, without significant necrosis/apoptosis grade (1), 3) 25%, 50%, 75% and 75% of the tubules showing tubular epithelial cell necrosis/apoptosis grades (2, 3, 4, 5) respectively, Accompanied by other concomitant alterations.

## STATISTICAL ANALYSIS

The results are expressed as mean  $\pm$  S.D. The means comparisons were made by using one-way analysis of variance (ANOVA) using Graph Pad Prism 03n software, where appropriate. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Hematological analyses

Intraperitoneal injection of Cyclophosphamide (CP) induced a significant decrease in the RBCs' count, WBCs, count and Hb concentration in group 2 and group 3 in comparison to group 1 (normal animals). On the other hand, treatment with Sargassum Cinereum induced a significant increase in previous parameters when compared with group 2 as shown in Fig (1).

### Liver functions

Injection of CP induced a significant increase in the liver enzymes activities (ALT, AST and ALP) associated with a significant decrease in total protein and albumin levels in group 2 and group 3 when in comparison with normal animals. Furthermore, treatment with *S. cinereum*, induced improvement in the enzymes activities and increased in the total protein and albumin levels in comparison with CP injected group according to Fig( 2 ).

### Kidney functions

In the CP injected animals there is a significant increase in the kidney function tests( creatinine, urea

and uric acid) levels of group 2 and 3 when compared with normal animals. Treatment with *S. cinereum* induced a significant decrease in these parameters in group 3 when compared with CP injected group Fig (3).

### Lipid profiles

Fig.4, described the effect of CP injection on the serum lipid profiles as it induced significant increase in cholesterol and triglycerides levels with decrease in HDL- cholesterol in all groups in comparison to the normal group. Treatment with *S. cinereum*, exhibited a significant decrease in cholesterol and triglycerides levels, while HDL- cholesterol level was increased significantly when compared with the CP injected group.

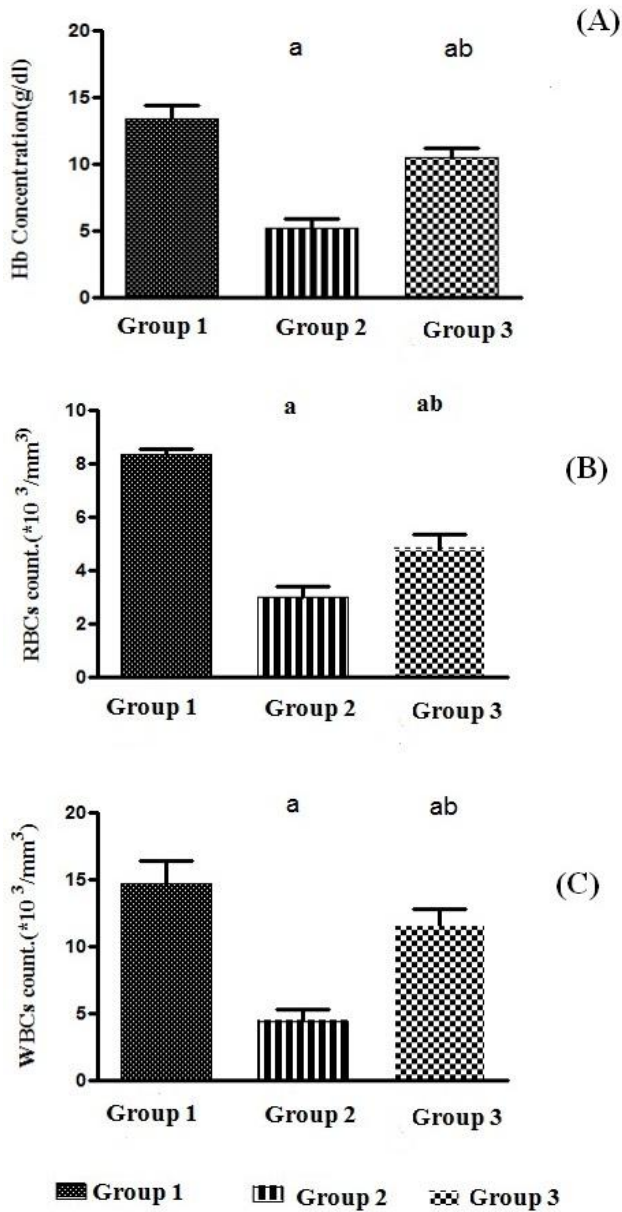
### Liver homogenate biochemical oxidative stress

As shown in Fig (5), injection of CP decreased the CAT, GSH and SOD concentration associated with a significant increase in MDA concentration in all groups when compared with group 1. In contrast, treatment with *S. cinereum* induced a significant increase in CAT, GSH and SOD activities and decrease in MDA concentration in group 3 when compared with group 2.

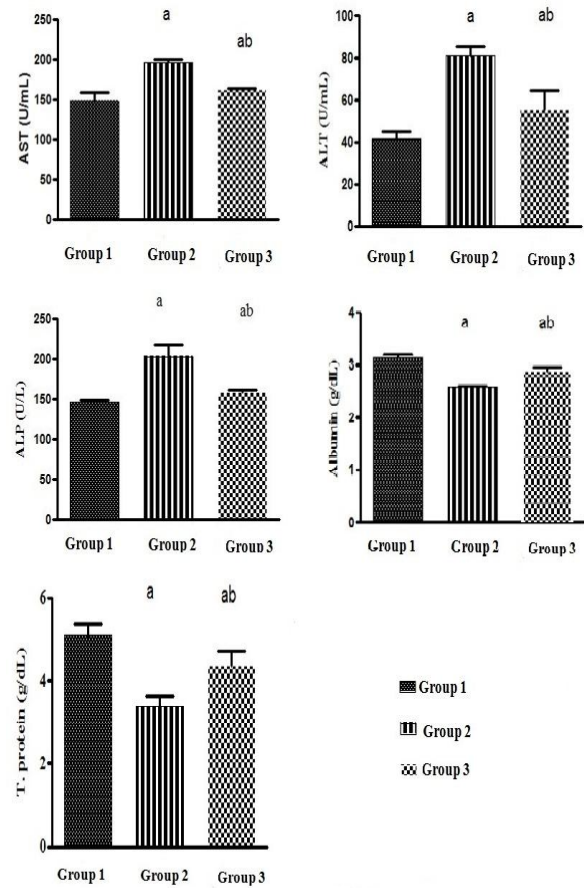
## RESULTS OF HISTOPATHOLOGY

Histopathological injuries in the hepatic and renal tissues were induced due to CP injection. In Liver tissues as shown in Fig (6 a, b and c). The histopathological changes include periportal necrosis plus bridging necrosis. In the other hand treatment with *S. cinereum* caused moderate necrosis involves <50 % of the circumference of most portal tracts without bridging necrosis. Furthermore, CP injection induced moderate inflammation characterized by inflammatory cells infiltration in 2/3 of portal tracts while treatment with *S. cinereum* induced mild inflammatory responses characterized by few inflammatory cells in <1/3 of portal tracts. Moreover, CP toxicity exhibited fibrous portal expansion while no fibrosis was recorded in *S. cinereum* treated group. In the kidney, CP injection induced renal injury that manifested by severe necrosis in both renal tubules and renal glomeruli leading to their atrophy. Some inflammatory cells infiltrated around the severely injured tissue. Recovery in the renal tissue was achieved after *S.cinereum* administration, which characterized by decrease the necrotic tissues and inflammatory cells infiltration as shown in Fig( a, b, and c). Histology index score (HIS).

Injection of CP induced liver tissues damages confirmed by increase the total of histology index score, which recovered by oral administration of *S. cinereum* and evidenced by decrease in the total of histology index score as shown in Fig (4d).



**Fig-1:** Toxic effect of cyclophosphamides and the ameliorating effect of *Sorgasum .Cinereum* on RBCs, WBCs' count and Hb concentration. P<0.01 compared with control values. Bars represent means ± SD (N=6).



**Fig-2:** Serum liver enzymes activity (ALT, AST, ALP, Albumin and Total protein) in cyclophosphamides injected group and orally administered *Sorgasum Cinereum* group. P<0.01 compared with control values. Bars represent means ± SD (N=6)

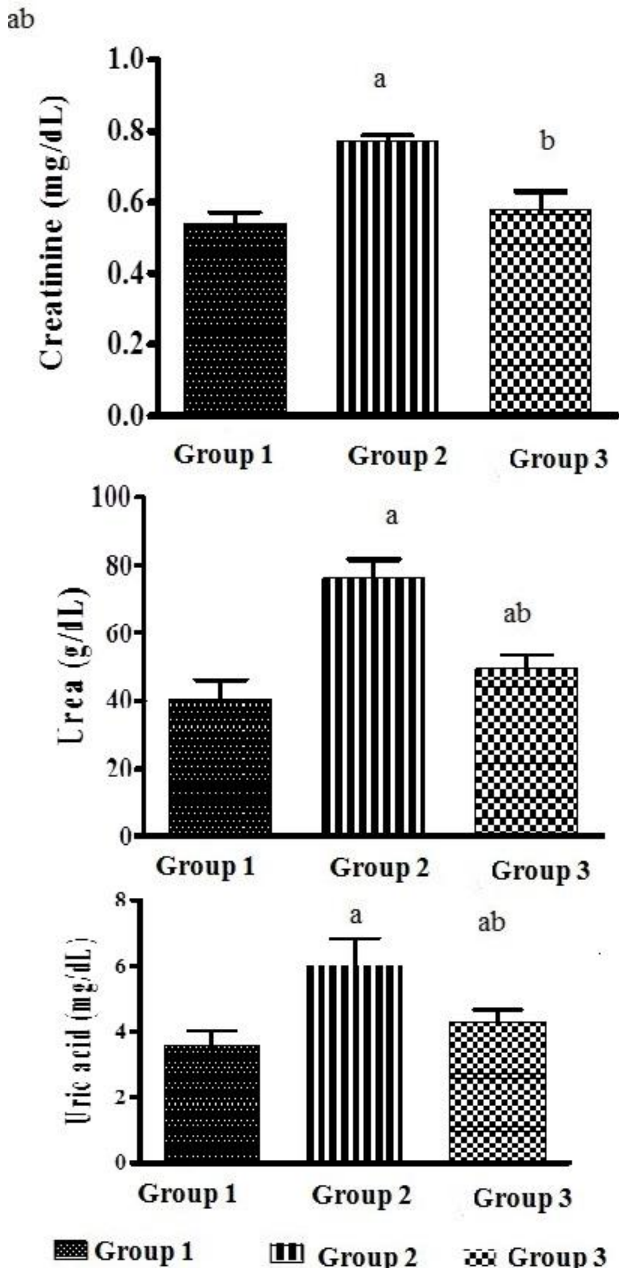


Fig-3: Kidney function markers (creatinine, urea and uric acid) in cyclophosphamides injected group and orally administered *Sorgasum Cinereum* group. P<0.01 compared with control values. Bars represent means ± SD (N=6).

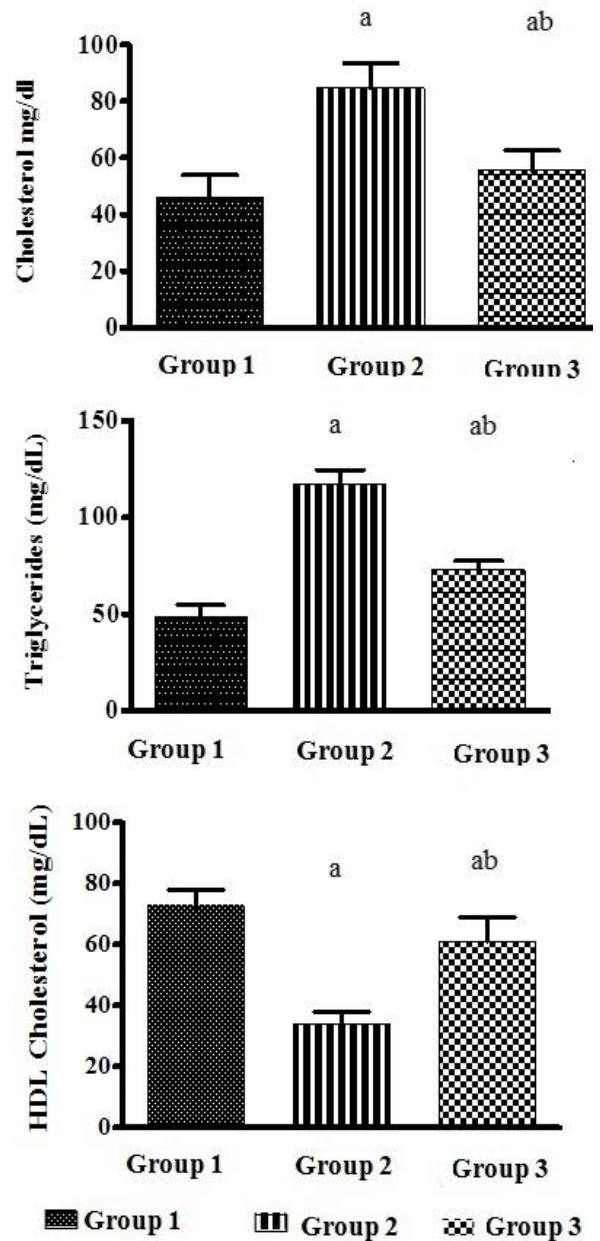
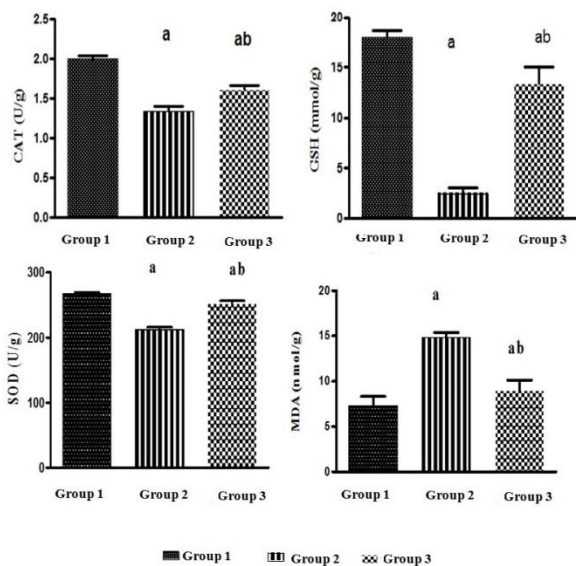
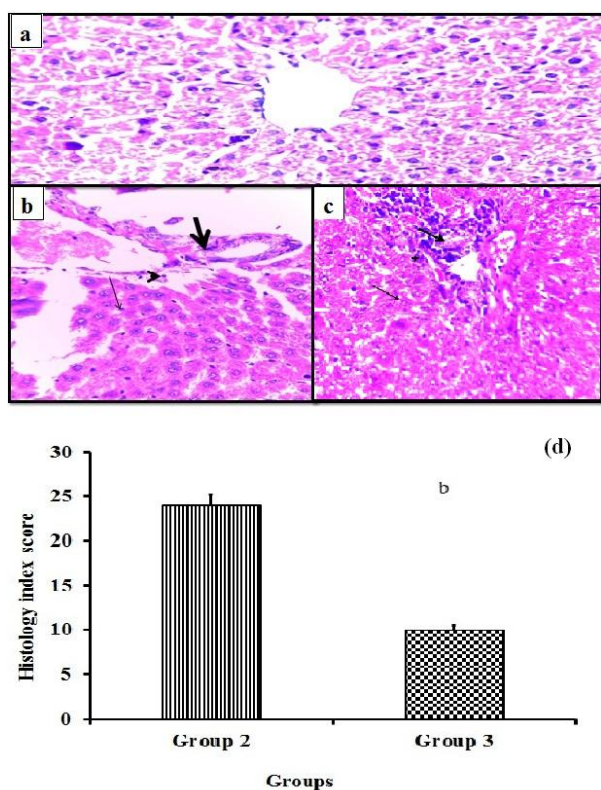


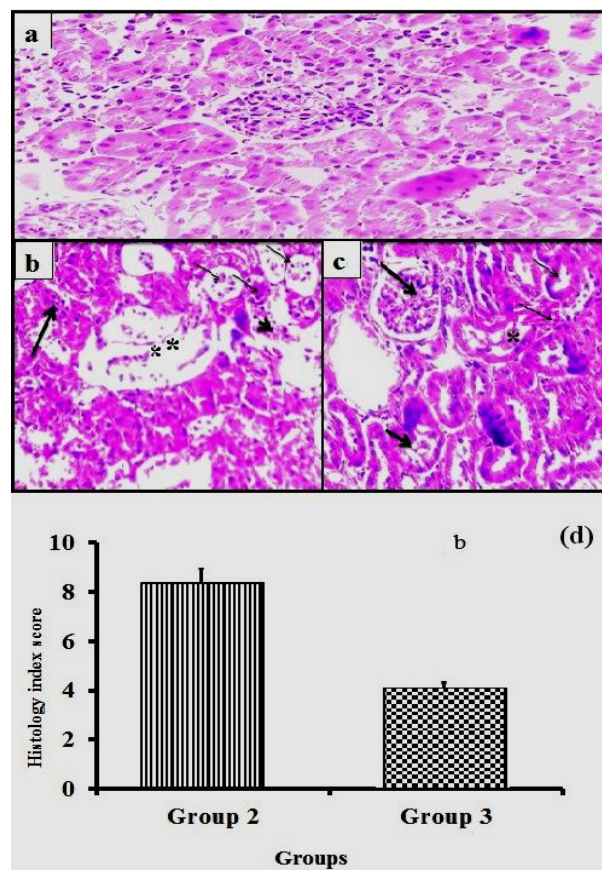
Fig-4: Serum lipid profiles levels (Cholesterol, triglyceride and cholesterol-HDL) in cyclophosphamides injected group and orally administered *Sorgasum Cinereum* group. P<0.01 compared with control values. Bars represent means ± SD (N=6)



**Fig-5:** Effect of cyclophosphamides and the ameliorating effect of *Sorgasum .Cinereum* on the oxidative stress markers in liver homogenates (CAT, SOD, GSH and MDA).  $P < 0.01$  compared with control values. Bars represent means  $\pm$  SD (N=6).



**Fig-6:** Histological changes (a,b and c) and in hematoxyline and eosine stained section in liver following CP injection (b) and oral administration of *Sorgasum .Cinereum* (c) in comparison to normal liver histology (a). Hepatotoxicity marked by necrosis in the hepatocytes (thin arrow) and in the portal triade associated with inflammatory cells infiltration (arrow head). Periportal fibrosis (thick arrow) evidenced around the hepatic lobules (b). *Sorgasum .Cinereum* induced restoration in the hepatic tissues evidenced by decrease in the hepatic cells necrosis and inflammatory cells infiltrations (thick arrow). No marked fibrosis was seen around the portal triade (thin arrow). Histology index score (d) showing significant reduction in the histology index score after *S.cinereum* administration.  $P < 0.01$  compared with control values. Bars represent means  $\pm$  SD (N=6)



**Fig-7:** Histological changes (a,b and c) and in hematoxyline and eosine stained section in kidney following CP injection (b) and oral administration of *Sorgasum .Cinereum* (c) in comparison to normal kidney histology (a). Nephrotoxicity marked by necrosis in the glomeruli (thin arrow) and in the tubular epithelium (Thick arrow), associated with inflammatory cells infiltration (arrow head). Renal tubules cystic dilation (Stars) was evidenced (b). *Sorgasum .Cinereum* induced recovery in the renal tissues evidenced by increase glomerular cells and decreases its necrosis (thick arrow) besides few inflammatory cells infiltrated (thick arrow). Histology index score (d) showing significant reduction in the histology index score after *S.cinereum* administration.  $P < 0.01$  compared with control values. Bars represent means  $\pm$  SD (N=6)

## DISCUSSION

The present study was done to explore the toxic effects of Cyclophosphamide on the some hematological and biochemical parameters as well as liver and kidney tissues in albino rats besides to estimation of the ameliorating effects of *S. cinereum* against the CP toxicity.

In the present study injection of CP induced a significant decrease in the RBCs, WBCs' count and Hb concentration causing anemia and leucopenia. Same results obtained by Korkmaz *et al.* [23]. The decrease in the RBCs, WBCs' count and Hb concentration resulted from the direct toxic effect of cyclophosphamides on the bone marrow causing bone marrow suppression and myelotoxicity [24]. Moreover, the disrupted hematopoiesis may be returned to the indirect effect of cyclophosphamides hematopoietic system through the oxidative cells damage by free radical and reactive oxygen species [25]. Oral administration of the *S.*

cinereum induced improvement in the blood parameters levels may be through activation of the antioxidative system.

CP injection exhibited significant hepatotoxic effects signified by the increased in the serum liver's enzymes ALT, AST and ALP activities. Additionally, hepatotoxicity also evidenced by decrease in the liver synthesis function represented by decrease of serum albumin and total proteins levels. The abnormal high level of liver enzymes observed in our study is the consequence of CP-induced liver dysfunction and hepatic cell damage. These observations are compatible with previous findings [26,12]. In accordance, the higher levels of serum liver's enzymes is indicative of the cellular damages and loss of functional integrity of the cell membrane of the hepatocytes [27]. In the hepatocellular lesions, hepatic enzymes are released from the liver into the bloodstream [28,26]. It is well known that liver has the dominant role in the biosynthesis of the plasma proteins [29]. In accordance injury to the hepatocytes impair plasma protein synthesis.

Using of the natural compounds as alternative medicine has been recommended because of relatively low costs and side effects when they compared to modern biochemical medicinal drugs [30]. Administration of *S. cinereum* extract protected the liver against CP-induced hepatotoxicity as it confirmed by lowering of serum level of these enzymes. In same line with the findings presented here, in a vitro study estimated that *S. cinereum* extract has potent radical scavenging activity [20].

Furthermore, the toxicity by CP affected the kidney function and induced a significant increase in the serum urea, uric acid and creatinine following CP administration. It may be associated with the harmful effects on the hepatic tissues leads to a stage with characters of hepatorenal syndrome [31] the hepatorenal syndrome manifested by reduction in creatinine clearance and low glomerular filtration rate [32], in association with increase in the uric acid level. In the present study following to CP administration increase in the uric acid level may be due to a reduction in the glomerular filtration rate and renal urate excretion [33]. In accordance, production of reactive oxygen species (ROS) by CP toxicity in the kidney tissues playing a critical role in the pathogenesis of renal injury [34] On the other hand, there is an improvement in the kidney functions after treatment with *S. cinereum* extract which may attributed to its potent free radical scavenging activity.

Increase in the cholesterol and triglycerides levels with decrease in HDL-cholesterol level in the cyclophosphamide-treated group indicates that cyclophosphamide has the ability to change cholesterol profile which may be returned to oxidation of the

cholesterol. The rise in ROS generated by CP cause cellular cholesterol accumulation by increasing cholesterol biosynthesis and its esterification due to decreasing cholesteryl ester hydrolysis and reduction in the cholesterol efflux [35,36]. The animals treated with the seaweed extract prevented the elevation of total cholesterol and triglycerides. the natural protection of the lipids by the seaweed may be return to the presence some natural such as sulfated polysaccharide that may retard the intestinal absorption of cholesterol or speed up the cholesterol excretion [37]. Rats given *S. cinereum* exhibited improvement in the levels of HDL, which may be due to the ability of the extract to hurry the deactivation of the free radical species produced during cyclophosphamide administration.

For better understand of the hepatotoxic effect of the cyclophosphamides oxidative stress markers were detected. Oxidative stress is one of the mechanisms responsible for the liver damage and diseases progression. Comatition of the free radical and oxidative compounds minimizing its deteriorative effects was done by antioxidant [38]. Membrane lipids are more susceptible to free-radical damage. Lipids after reacting with free radicals undergo lipid peroxidation [39]. During cyclophosphamide injection, the production of free radicals may interrupt the oxidative-antioxidative balance present in the normal physiological status leading to oxidative stress. this may happen especially when the antioxidative system is not able to meet the overproduction of the reactive oxygen species [40]. Moreover, the cyclophosphamide metabolites such as acrolein. Acrolein is an  $\alpha$ ,  $\beta$ -unsaturated aldehyde, that produces oxidative stress and increase in the lipid peroxidation and the formation of intracellular ROS such as superoxide anion radicals, hydroxyl radicals, and singlet oxygen. These reactive oxygen and nitrogen species injures the cellular lipid, proteins, and DNA [41].

In our study, we find that intraperitoneal injection of CP-induced oxidative stress evidenced by significant decreases in the antioxidant enzymes such as SOD, GSH and CAT; and increased MDA production [12,42], the changes in the oxidative stress markers may be attributed to the inactivation of the cellular antioxidants by the lipid peroxides and ROS produced during CP intoxication. Treatment of the rats with *S. cinereum* enhanced the declined antioxidant enzyme activities. The restoration of the anti-oxidative power of the liver tissues returned to improvement of the anti-oxidant defense system due to free radical scavenging/anti-oxidant properties of the phytochemical contents present in *S. cinereum*. Other reports which suggest that extracts of brown seaweeds belonging to *Sargassum* spp. are anticipated to be very good inhibitors of lipid peroxidation [43, 44].

CP has great pathological effects on the liver and kidney. Intraperitoneal injection of cyclophosphamides induced both hepatic and renal

toxicity manifested by many histopathological changes includes necrosis in the hepatocytes and renal epithelium associated with inflammatory cells infiltration in both organs.

The histological changes observed in the hepatic cells and blood vessels were due to the direct effect of the drug on the cells structures or may attributed to the free radicals production which caused cell membrane integrity of the hepatocytes [12]. Histological injuries in the hepatocytes give the explanation of the biochemical results obtained in this study which represented by increase of the liver enzymes activity and the oxidative stress markers.

In accordance, the histopathological changes in the kidney following CP injection also were due to the direct effect of the toxins on the renal tuular epithelium increasing the cellular permeability of the glomeruli leading to decreased glomerular filtration rate and kidney function impairment. Recovery of the renal tissues histology was achieved by oral administration of seaweed. This improvement and recovery may be due to the multi-beneficial properties of *S cinerarium* extracts such as antioxidant and anti-inflammatory [45].

## CONCLUSION

In conclusion, these results provide new evidences that toxic effect of CP is possibly mediated through depression the antioxidant system and subsequent cellular damage while the protective effect of *S.cinereum* mediated through the activation of this system and subsequent suppression of inflammatory and oxidative stress.

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