Amelioration of Cyclophosphamide-Induced Hepatotoxicity by the Brown Seaweed *Turbenaria ornata*

Ayman M. Mahmoud¹*, Omnia E. Hussein¹ and Shymaa A. Ramadan²

¹Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Egypt
²Physiology Department, Faculty of Medicine, Beni-Suef University, Egypt

Abstract: The present study was designed to investigate the possible protective effects of the brown seaweed, *Turbenaria ornata*, against cyclophosphamide (CP)-induced hepatotoxicity in rats. The biochemical results showed that administration of CP induced hepatic damage associated with a significant increase in the serum marker enzymes aspartate and alanine transaminases (AST, ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). In addition, CP induced oxidative stress in the liver as evident from the increased lipid peroxidation (LPO), declined glutathione (GSH) content and glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities. Moreover, administration of CP was associated with a significant decrease in serum adiponectin level and an increase in serum tumor necrosis factor alpha (TNF-α). Concomitant administration of *Turbenaria ornata* extract efficiently alleviated the altered biochemical parameters. In conclusion, *Turbenaria ornata* extract showed a marked hepatoprotective effect against CP-induced hepatotoxicity through alleviation of the declined serum adiponectin in addition to its antioxidant and anti-inflammatory efficacies.

Keywords: Cyclophosphamide, hepatotoxicity, adiponectin, oxidative stress, Seaweeds.

1. INTRODUCTION

Cyclophosphamide (CP), a nitrogen mustard alkylating agent, is widely used in the treatment of variety of human malignancies and disorders like breast cancer, carcinoma of the lung [1], systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis [2,3].

However, use of CP is often restricted because of its wide adverse side effects and toxicity that includes nausea, vomiting, alopecia, mucosal ulceration, pulmonary fibrosis, hemopoetic suppression, nephrotoxicity, urotoxicity, cardiotoxicity and hepatic toxicity [4-7]. Biotransformation of CP mediates through involvement of cyt p450 mixed function oxidases with the formation of metabolites phosphoramide mustard and acrolein which are highly toxic [8,9]. Through this pathway, CP has potential to generate superfluous reactive oxygen species (ROS) [10,11]. In addition, experimental evidence suggests that oxidative stress is responsible for CP hepatotoxicity [12-14].

Adiponectin is a protein produced and secreted almost exclusively by adipocytes [15]. First described over a decade ago, the interest in the biology of adiponectin was spurred by the discovery of measurable concentrations in plasma, its structural resemblance to complement factor C1q and the consistent finding of decreased levels in obesity [16].

In humans, adiponectin is an insulin-sensitizing, vascular-protective, anti-inflammatory protein [17,18] associated with a more favourable lipoprotein subclass profile [19]. To date, most of our understanding of adiponectin has been in its association with metabolic and cardiovascular health. Hypoadiponectinemia is associated with obesity, insulin resistance and type 2 diabetes [18], as well as atherosclerosis, hypertension and coronary artery disease [20]. The hepatoprotective and antifibrogenic effects of adiponectin have been demonstrated in animal models by numerous pharmacological, genetic, gain- and loss-of-function studies [21,22].

Over the past several decades, seaweeds have been reported to possess biological activity of potential medicinal value [23]. It was reported that seaweeds are rich source of bioactive compounds, such as terpenoids, phlorotanins, fucoidans, sterols and glycolipids, and the extracts or isolated pure components from seaweeds posses a wide range of pharmacological properties such as anticancer, antibacterial, antifungal, anti-viral, anti-inflammatory, anticoagulant, antioxidant, hypoglycaemic, hypolipidemic, antimelanogenic, anti-bone loss, hepatoprotective and neuroprotective activities [24,25]. Earlier reports indicated that the extracts of brown seaweeds belonging to *Turbinaria spp.* were found to have antioxidant and anti-inflammatory activities [26,27].

Reports regarding the protective effect and clinical significance of brown algae against CP-induced hepatotoxicity in rats are scanty in scientific literature.
Therefore, the current study was designed to investigate the ameliorative potential of *Turbenaria ornata* extract in CP-induced hepatotoxicity in rats with an emphasis on adiponectin, proinflammatory cytokines and CP-mediated oxidative stress.

### 2. MATERIALS AND METHODS

#### 2.1. Chemicals

Cyclophosphamide (Endoxan) was supplied as vials from Baxter Oncology (Düsseldorf, Germany). All other chemicals were obtained from standard commercial supplies.

#### 2.2. Collection of Algal Samples

The studied seaweed was collected from Red Sea, between Quseir and Marsa-Alam, Egypt. The samples were washed three times with sea water followed by tap water to remove the salt, sand, and epiphytes attached to the surface.

#### 2.3. Extract Preparation

Collected algae were air-dried in shade and separately pulverized to a fine powder. The powdered materials were extracted by maceration in 80% aqueous ethanol until exhaustion at room temperature. 100 g of each sample was soaked in 1000 ml of solvent with sonication at room temperature for 24 hours. After filtration, the filtrate was concentrated under reduced pressure in a rotary evaporator and lyophilisation. The crude extracts were weighted and stored at -20 °C till used.

#### 2.4. Experimental Animals

White male albino rats weighting about 130-150 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages (4 rats/cage) and maintained under controlled room temperature (22±2 °C) and humidity (55±5%) with 12 h light and 12 h dark cycle and were fed a standard diet of known composition, and water *ad libitum*. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in the Guide for the Care and Use of Laboratory Animals [28].

#### 2.5. Experimental Design

The experimental animals were divided into three groups, each group comprising six rats designated as follows:

- **Group 1 (N):** Control rats received normal saline.
- **Group 2 (CP):** Rats received a single intraperitoneal dose of CP (200 mg/Kg b.wt.).
- **Group 3 (CP + T):** Rats received a single dose of CP followed by oral supplementation with *Turbenaria ornata* extract (100 mg/Kg b.wt.) for 10 consecutive days.

After 10 days experimental period, the animals were anaesthetized with diethyl ether and killed by cervical decapitation. Liver tissues were immediately excised and rinsed in ice-cold saline then homogenized for the subsequent assays. Blood samples were collected and serum was separated for analysis of marker enzymes, TNF-α and adiponectin.

#### 2.6. Biochemical Assays

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed according to the method of Schumann and Klauke [29] using reagent kit purchased from Biosystems (Spain). Serum lactate dehydrogenase (LDH) was determined according to the method of Teitz [30] using reagent kit purchased from Human (Germany) and alkaline phosphatase (ALP) was assayed according to method of the International Federation of Clinical Chemistry (IFCC) [31] using BioSystems (Spain) commercial kit.

Serum levels of adiponectin and TNF-α were determined by specific ELISA kits according to the manufacturer’s instructions (R&D Systems, USA). The concentration of both cytokines was determined spectrophotometrically at 450 nm. Standard plots were constructed by using standard cytokines and the concentrations for unknown samples were calculated from the standard plot.

Lipid peroxidation, reduced glutathione (GSH) content, and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured in liver homogenate according to the methods of Preuss *et al.* [32], Beutler *et al.* [33], Marklund and Marklund [34] and Kar and Mishra [35], respectively.

#### 2.7. Statistical Analysis

Statistical analysis was performed using SPSS v.16. Results were articulated as mean ± standard error (SE) of the mean and all statistical comparisons were made by means of one-way ANOVA test followed by Duncan’s multiple range test post hoc analysis. A P value <0.05 was considered significant.
Table 1: Effect of *T. ornata* on Serum AST, ALT, LDH and ALP Activities of Normal and CP-Administered Rats

<table>
<thead>
<tr>
<th></th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>LDH (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>76.13 ± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.07 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210.97 ± 5.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.77 ± 4.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CP</td>
<td>174.01 ± 4.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.41 ± 4.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>562.73 ± 10.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>202.59 ± 3.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CP + T</td>
<td>122.27 ± 5.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.19 ± 2.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>324.94 ± 13.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>123.15 ± 3.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Prob. | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

Means which share the same superscript symbol(s) are not significantly different.

3. RESULTS

Intraperitoneal injection of CP resulted in marked impairment of liver functions as reflected by significant (P<0.001) increase in the levels of serum ALT, AST, LDH and ALP activities as compared with normal control rats. On the other hand, concomitant oral administration of *T. ornata* efficiently alleviated the altered serum marker enzymes (Table 1).

Regarding serum TNF-α, CP induced a significant (P<0.001) increase in serum TNF-α level as compared with normal control rats. The administration of *T. ornata* showed marked improvement of serum TNF-α concentration (Figure 1).

Serum adiponectin level exhibited an opposite pattern; it was significantly (P<0.001) decreased in the CP group as compared to normal ones and was significantly increased as a result of treatment with *T. ornata* as illustrated in Figure 2.

In the same regard, CP induced a remarkable elevation (P<0.001) in LPO, assayed as malondialdehyde.
dehye (MDA). Oral administration of *T. ornata* extract produced a notable decrease in the elevated MDA levels (Figure 3).

Figure 3: Effect of *T. ornata* on liver lipid peroxidation of normal and CP-administered rats.

Figure 4 showed the effect of *T. ornata* supplementation on the level of hepatic tissue GSH content. Following CP injection, GSH was significantly (P<0.01) declined in CP-injected rats as compared to

Figure 4: Effect of *T. ornata* on liver GSH of normal and CP-administered rats.

Figure 5: Effect of *T. ornata* on liver GPx of normal and CP-administered rats.
normal ones. *T. ornata* administrations significantly ameliorated the altered GSH content.

The activities of GPx and SOD in hepatic tissue were represented in Figures 5 and 6, respectively. CP group of rats revealed a noticeable reduction in hepatic GPx (P<0.01) and SOD (P<0.001) activities in comparison with control rats. Conversely, treatment with *T. ornata* potentially alleviated the declined antioxidant enzyme activities.

4. DISCUSSION

Cyclophosphamide, one of the most widely drugs in chemotherapy, is a cytotoxic alkylating drug with a high therapeutic index and is effective against a variety of cancers. Although CP is effective for the treatment of cancer, it induces a wide range of adverse side effects and toxicity, such as nausea, vomiting, and hematopoietic toxicity, that restrict the use of this drug in clinic [1]. The pathogenetic pathways may include oxidative damage, release of some inflammatory endocoids such as cytokines and nitric oxide as well as poly (adenosine diphosphate-ribose) polymerase activation [36]. Therefore, it is necessary to search for agents that can reduce the harmful side effects of CP.

CP-induced hepatotoxicity involves cellular injury as a consequence of which, the cytosolic enzymes (AST, ALT, ALP and LDH) leach out leading to their raised levels in the blood [37]. Hepatotoxicity of CP was observed in our study as shown by elevation in the levels of the marker enzymes in the serum and this elevation indicates hepatic damage as recorded by Ansari *et al.* [38]. Administration of *T. ornata* extract obviously protected against CP-induced hepatotoxicity as shown by the amelioration of serum level of these marker enzymes.

The importance of the contribution of the immune system to drug-induced hepatotoxicity has been well recognized over the past years. Several hepatotoxicants and nephrotoxicants have been shown to induce an inflammatory response, which participated in the organ injury [39-41]. The current study revealed a significant increase in serum TNF-α of CP-administered rats when compared to normal control rats. Consistently, the proinflammatory cytokine, TNF-α, has been implicated in the pathogenesis of CP-induced haemorrhagic cystitis [42]. In addition, Malley and Vizzard [43] have documented increased cytokine expression after CP-induced cystitis.

Moreover, it is believed that during drug-induced toxicity, the initial insult by the toxicant results in tissue damage, which leads to generation of inflammatory mediators by the injured cells as well as by immune cells. Subsequently, these inflammatory mediators induce migration and infiltration of leukocytes into the injured organs and aggravate the primary injury induced by the toxicant [44]. For liver and kidney, the pro-inflammatory cytokine TNF-α is the main orchestrator of this inflammatory response and in several cases has been shown to aggravate the toxicant-induced pathophysiological responses [45]. *T. ornata* notably alleviated the altered serum TNF-α and this may be attributed to its anti-inflammatory effect. Our findings are in agreement with the study of Ananthi *et al.* [46] who revealed that the anti-inflammatory activity of *T. ornata* could be due to its potential antioxidant and free radical scavenging activity.
Adiponectin is made almost exclusively by adipose tissue [47], although in culture other cell types such as hepatocytes can be induced to produce it [48]. Adiponectin can bind to collagens [49], and is structurally similar to complement component C1q [50]. Adiponectin has anti-inflammatory and vasoprotective properties [50]. For example, adiponectin blocked TNF-α induced adherence of monocytes to endothelial cells by attenuating surface expression of the adhesion molecules vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin [51]. Moreover, adiponectin increased the production of tissue inhibitor of metalloproteinase-1 by human macrophages through an interleukin-10–dependent mechanism [52]. Furthermore, the anti-inflammatory effect of adiponectin has been shown to be at least partially mediated by the suppression of pro-inflammatory cytokines, especially TNF-α, from various cell types, including monocytes and macrophages [18,53,54]. Thus by increasing serum adiponectin levels, *T. ornata* provided a protection against CP-induced hepatotoxicity via its anti-inflammatory effect.

Our results showed significantly increased lipid peroxidation in the liver of CP treated rats when compared to normal ones. Bhatia *et al.* [55] and Kouidhi *et al.* [56] revealed that CP-induced hepatotoxicity involves induction of oxidative stress due to excessive formation of ROS which causes lipid peroxidation of the cellular membrane. Oral administration of *T. ornata* extract prevented the CP-induced lipid peroxidation which could be attributed to the free radical scavenging activity as well as suppressing oxidative stress. The inhibition in lipid peroxidation may be due to the presence of polyphenolic antioxidants that were reported to disrupt free-radical chain reaction by donating proton to fatty acid radicals to terminate chain reactions, may have roles to inhibit lipid peroxidation [57]. Phenolic compounds are considered to exhibit radical scavenging properties [58]. Several studies demonstrated a significant correlation between the phenolic content and the antioxidant activity in seaweed extracts [59]. Earlier studies revealed that ethanolic and dichloromethane fractions are the major fractions of seaweeds harboring the principle antioxidative components [60]. There are other reports which suggests that extracts of brown seaweeds belonging to *Turbinaria* spp. are anticipated to be very good inhibitors of lipid peroxidation [61].

On the other hand, the current study revealed a significant depletion of GSH following CP administration. The depletion of GSH level could be attributed to the direct conjugation of CP metabolites with GSH, thereby reducing its cellular level leading to induction of oxidative stress [62]. In addition, Srivastava and Shivanandappa [63] demonstrated that GSH depletion leads to lowered cellular defense against free radical induced cellular injury resulting in necrotic cell death. The alleviation of the GSH content following *T. ornata* administration plays a part of the mechanism of hepatoprotection against CP-induced toxicity.

Consistently, the activities of the antioxidant enzymes, SOD and GPx, in the liver were significantly reduced by CP treatment. The current study is in agreement with Rajasekaran *et al.* [64] who reported that CP-induced toxicity is associated with oxidative stress caused by the reduction in the antioxidant enzymes. Collectively, our results show that the antioxidant status in the liver was markedly lower after CP administration, which is in agreement with previous reports [65]. Earlier reports suggest that natural antioxidants protect against CP-induced hepatotoxicity [66-67]. On the other hand, treatment of rats with *T. ornata* potentially ameliorated the declined antioxidant enzyme activities. The beneficial effects of *T. ornata* may be attributed to its rich contents of polyphenols, carotenoids and polysaccharides [68], and it is an excellent source of vitamins such as A, B1, B12, C, D, E, riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as Ca, P, Na and K [69]. In addition to potential role of polyphenols, Lewin [70] have stated that, the lipids of algae comprise photosynthetic pigments-chlorophylls, carotenes and other compounds while carotenoids are powerful antioxidants.

Overall, results of the present study suggests that concurrent administration of *T. ornata* protected against CP-induced hepatotoxicity by mechanisms related to their ability to decrease lipid peroxidation and potentializing the antioxidant defense system. In addition, *T. ornata* hepatoprotective effect may be attributed to its anti-inflammatory activity that is partially mediated through alleviating adiponectin production.

**ACKNOWLEDGMENT**

The authors wish to thank Khaled NM ElSayed for his assistance in collection and authentication of *T. ornata*.
REFERENCES


[26] http://dx.doi.org/10.1038/jcb.2009.170


Amelioration of Cyclophosphamide-Induced Hepatotoxicity


Received on 02-09-2013 Accepted on 19-09-2013 Published on 30-11-2013

DOI: http://dx.doi.org/10.14205/2310-4007.2013.01.01.2

© 2013 Mahmoud et al.; Licensee Pharma Professional Services. This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.