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RESEARCH ARTICLE

Ameliorative effect of ink extract and polysaccharide of *Sepia officinalis* on hepatotoxicity, renal toxicity and hematological disorders in adult male albino rats treated with cyclophosphamide

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Abstract

We aimed to investigate ameliorative effects of the crude extract (SIE) and polysaccharide (SIP) of the ink of Sepia officinalis, on some biochemical and hematological disorders induced by cyclophosphamide (CP). Forty adult male albino Wistar rats were divided into five groups (n= 8 each). In the control group, rats were administered orally with 0.9% isotonic saline solution at a dose (5 ml/kg b.w.). All the other groups were i.p. injected with a single dose of CP (200mg/kg b.w.) only for one time. Then the third group was treated with oral administration of (SIP) (80mg/kg b.w.) daily for 60 days, the fourth group was treated with oral administration of (SIE) (200mg/kg b.w.) daily for 60 days and the fifth group was treated with oral administration of (SIP, 80mg/kg b.w. + SIE, 200mg/kg b.w.) daily for 60 days. All the animals were slaughtered by the end of the experiment for collecting the blood samples for hematological and biochemical assays. The biochemical results indicated that administration of CP was associated with a significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine and uric acid. Moreover, a significant decrease in the levels of albumin and total protein were recorded. In addition, hematological disorders including a significant suppression on the numbers of RBCs, WBCs and PLTs, with a remarkable reduction in hemoglobin contents (Hb) and a significant drop in PCV values. Concomitant administration of SIE and SIP alleviated the altered biochemical and hematological parameters.

Keywords: Cyclophosphamide; Hematology; Hepatotoxicity; Renal toxicity; Sepia ink

1. Introduction

Cyclophosphamide (CP), also known as endoxan, is an alkylating agent as it causes alkylation to DNA which leads to cell apoptosis. It is extensively used to treat a wide range of cancers, as an immunosuppressive agent following organ transplants (Anderson and Bishop, 1995) and for treating many diseases like breast cancer (Zhang *et al*., 2006), systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis (Perini *et al*., 2007). The biotransformation of CP is mediated by the participation of **CYP_{450}** mixed- function oxidases, and the highly toxic metabolites phosphoramide mustard and acrolein are produced. In this way, CP may produce excess reactive oxygen species, which are responsible for inducing oxidative stress (Sudharsan *et al*., 2006).

Previously, CP therapy has been shown to have harmful toxic effects on the liver (Snover *et al.*, 1989). The CP-induced oxidative stress is the main cause of hepatotoxicity in CP-treated animals (Selvakuma *et al.*, 2005). Moreover, Cuce *et al*. (2015), proved that CP caused signs of hepatic degeneration during their histopathological study on the liver of Swiss albino mice. Besides that, Bokolo and Adikwu,

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(2018) observed that CP administration was showed higher levels above normal the serum levels of creatinine, urea and uric acid. This observation is a common feature of CP associated nephrotoxicity (Estakhri *et al.*, 2013).

Additionally, CP has been implicated in short-term damage of the bone marrow which results in abnormally low numbers of erythrocytes, leukocytes and platelets regarded as bone marrow suppression or myelosuppression (Friberg *et al*., 2002).

Nowadays, most pharmaceutical products contain synthetic antioxidants. Regrettably, the synthetic antioxidants used in the industry were indicated to have carcinogenic effects on human tissues, thus fueling an intense search for natural, available and more efficient antioxidants (Amorati *et al*., 2013). In comparison with synthetic products, natural bioactive substances have minimal side effects. However, most pharmaceutical products were obtained from terrestrial life, it is the marine world that may provide next generation of medicines for the pharmaceutical industry (Brita Nicy *et al.*, 2016). Nowadays, cephalopods are considered among the various marine organisms from which, several bioactive compounds have been extracted, purified and characterized (Haefner, 2003).

Sepia ink extract (SIE), used in our study, was crude and has several kinds of vital and essential components, such as melanin, proteins, lipids, glycosaminoglycans (Liu *et al.,* 2011) and possesses antioxidant activities (Fahmy and Soliman, 2012).

Additionally, Sepia ink polysaccharides (SIP), used in this work, is a newly found marine glycosaminoglycon, that have been proved to have antioxidant capabilities and chemotherapy-protective activities. Also, it has effective scavenging on hydroxyl radicals as well as total reducing power that are collectively known as antioxidant ability (Luo and Liu, 2013).

Therefore, the main purpose of this study was to use experimental adult albino male rats to assess the different adverse effects of CP and to report the possible mechanisms that could explain these adverse effects, on the bases of the hematological and biochemical (including assessments of liver and kidney functions) variations. Additionally, this study aimed to investigate the ameliorative effects of SIE, SIP and (SIE+SIP, individually) as antioxidants, on side effects caused by CP.

2. Materials and methods

Drugs and chemicals Cyclophosphamide (CP) was supplied as vials from Baxter Oncology (Düsseldorf, Germany).

2.1. Sepia ink extraction (SIE)

The fresh live sepia (*Sepia officinalis*) was purchased directly from a fishmonger and rapidly transferred to the laboratory where they were dissected to harvest fresh ink sacs and the ink was collected in a mortar then it was diluted with an equal volume of phosphate buffered saline (PBS, pH 6.8) and ground sufficiently followed by ultra-sonication. The mixture was collected in centrifugation tubes and centrifuged at a speed of 15,000 g for 20 min. at 4°C. Then the resulted supernatant was collected at once, freeze-dried and stored at -80°C. The sample should be dissolved in normal saline and diluted to the appropriate concentration, before its administration to animals (Zhong *et al.,* 2009).

2.2. Extraction of sepia ink polysaccharide (SIP)

The collected ink, thawed at 4°C and was suspended with pH 6.7 PBS, and was then ground and subjected to ultra-sonication. Then the ink solution produced from ultra-sonication was stored at 4°C for 24 hr. then was centrifuged

at 14000 g for 1 hr. at 4°C. The supernatant was treated with 1% papain in PBS (pH 6.7) at 60 °C for 24 hr., and then was mixed with a 1/4 volume liquid mixture of chloroform and n-butanol (v/v, 4/1) followed by stirring for 30 min on a magnetic stirrer plate. After centrifugation at 5000 g for 15 min, the supernatant was re-digested two times with papain. SIP was precipitated in the resulting supernatant with four volumes of absolute alcohol, and then it was subjected to air-drying in a vacuum. Before using the powder SIP it was stored at 4°C (Le *et al.*, 2015).

2.3. Experimental Animals

40 adult male Albino Wister rats were included in this study which were housed in the Animal House, Faculty of Science, South Valley University, Qena, Egypt. Their body weights were ranged from 210 to 230 gm. The rats were under a controlled environment (23±2oC, 55% relative humidity and a 12-h light/dark cycle) as they were housed in wire-mesh cages and treated according to the guidelines of the Animal House of South Valley University, Qena, where standard commercial pellets, which were used as food, and water was provided ad libitum. Other conditions pertaining to the health of the animals were maintained during the entire course of the study. All experimental protocols were performed in accordance with the local institutional guidelines and approved by the Animal Ethical Committee, South Valley University, Qena, Egypt.

2.4. Experimental Design

Animals were divided randomly into 5 groups, (n=8 each).

Group 1 (Con group): This group was administered orally with 0.9% isotonic saline solution at a dose (5 ml/kg b.w.) and served as a control group.

Group 2 (CP200 group): orally administered normal saline0.9% and i.p. injected with single dose of CP in normal saline (200mg/kg b.w.) only for one time.

Group 3 (CP200+SI80): i.p. injected with a single dose of CP (200mg/kg b.w.) in normal saline and orally administered SIP (80mg/kg b.w.) once a day for 60 days.

Group 4 (CP200+SI200): i.p. injected with a single dose of CP (200mg/kg b.w.) and orally administered SIE (200mg/kg b.w.) once a day for 60 days.

Group 5 (CP200+SI80+SI200): i.p. injected with a single dose of CP (200mg/kg b.w.) and orally administered with (SIP, 80mg/kg b.w. + SIE, 200mg/kg b.w.) once a day for 60 days.

2.5. Blood samples Collection

At the end of the experiment, twenty-four hours after treatment with the last dose, all rats from different groups were sacrificed and blood samples were collected from the retro-orbital veins then the blood was divided into two portions one portion was taken in EDTA containing tubes from every animal. This blood was used for the examination of complete blood picture (red blood cells count (RBCs), leukocytes count (WBCs), platelets count (PLTs), total hemoglobin (Hb) and hematocrit (PCV %) assays). The later portion of the collected blood was left in plain clean tubes at room temperature to clot then after an hour; it was subjected to centrifugation at 3000 rpm for 30 minutes for separating the serum. The clarified serum was collected in labeled epindorff's tubes and stored at - 80 °C until used for subsequent biochemical analyses.

2.6. Haematological investigations

By using automated hematology analyzing machine, all the hematological parameters (RBCs, WBCs, PLTs, Hb and PCV) were determined.

2.7. Biochemical analysis

Serum ALT, AST, Albumin, Total protein, creatinine, urea and uric acid levels were assayed (using commercial kits obtained from biodiagnostics, Egypt) with a spectrophotometer (Chem-7, Erba Diagnostics Mannheim GmbH, Germany).

Statistical analysis

All data were analyzed using one-way ANOVA analysis of variance (prism computer program); the variability degree of results was expressed as Means ± Standard Deviation of means (Mean ±S. D). And the least significant difference (L.S.D) was used to test the difference between treatments. Results were considered statistically significant when $P < (0.05)$.

Table 1. Mean ± S.D. of RBCs, WBCs, PLTs, Hb conc. and PCV value among the studied groups.

Parameters	Control-group (Mean ± S.D)	CP-group (Mean ± S.D)	CP+SIP-group (Mean ± S.D)	CP+SIE-group (Mean ± S.D)	CP+SIP+SIE-group (Mean ± S.D)
RBCs count ($x10^6$ / mm^3)	7.680 ± 0.19	3.190^{-a} ± 0.240	6.72^{+b} ± 0.260	7.170^{+b} ± 0.210	7.460^{+b} ± 0.310
WBCs count (x 10^3 /mm^3)	11.23 ± 0.53	4.42^{-a} ± 0..33	9.74^{+b} ± .61	11.16^{+b} ± 0.28	12.92^{+a+b} ± 0.74
Platelets count (x 10^3 mm^3)	647 ± 31.25	255.28^{-a} ± 19.63	517^{+b} ± 12.22	583.60^{+b} ±27.32	612.03^{+b} ± 22.34
Hb conc. (g/dL)	13.81± 0.350	5.67^{-a} ± 0.330	9.93^{+b} ± 0.510	11.84^{+b} ± 0.480	13.14^{+b} ± 0.660
PCV (%)	39.43 ± 1.140	16.86^{-a} ± 0.93	31.89^{+b} ± 1.75	38.53^{+b} ± 1.22	40.07^{+b} ± 1.39

Results are expressed as mean ± S.D. of 8 animals for each group.

+a = significant increased compared with control at p<0.05

-a = significant decreased compared with control at p<0.05

+b =significant increased compared with CP200 at p<0.05

-b = significant decreased compared with CP200 at p<0.05

Table 2. Mean ± S.D of serum levels of ALT, AST, Albumin and Total protein among the studied groups.

Parameters	Control-group (Mean ± S.D)	CP-group (Mean ± S.D)	CP+SIP-group (Mean ± S.D)	CP+SIE-group (Mean ± S.D)	CP+SIP+SIE-group (Mean ± S.D)
ALT (Units/ml)	41.93 ± 2.63	82.3^{+a}± 3.7	51.00^{-b}±3.2	46.82^{-b}± 6.03	44.55^{-b}± 1.55
AST (Units/ml)	141.52 ± 3.8	194.2^{+a}± 2.83	156.12^{-b}± 4.73	149.27^{-b}± 5.21	145.20^{-b}± 4.17
Albumin (g/dL)	3.97 ± 0.13	2.04^{-a}± 0.04	3.28^{+b}± 0.06	3.54^{+b}± 0.09	3.86^{+b}± 0.07
Total protein (g/dL)	4.934 ± 0.229	2.706^{-a} ± 0.246	4.1578^{+b} ± 0.731	4.4597^{+b} ± 0.26	4.6823^{+b} ± 0.321

Results are expressed as mean ± S.D. of 8 animals for each group.

+a = significant increased compared with control at p<0.05

-a = significant decreased compared with control at p<0.05

+b =significant increased compared with CP200 at p<0.05

-b = significant decreased compared with CP200 at p<0.05

Table 3. Mean ± S.D of serum levels of Creatinine, Urea and Uric acid among the studied groups.

Parameters	Control-group (Mean ± S.D)	CP-group (Mean ± S.D)	CP+SIP-group (Mean ± S.D)	CP+SIE-group (Mean ± S.D)	CP+SIP+SIE-group (Mean ± S.D)
Creatinine (mg/dL)	0.523± 0.019	0.8672^{+a} ± 0.028	0.6782^{-b}± 0.022	0.6266^{-b}± 0.017	0.5722^{-b}± 0.016
Urea (mg/dL)	37.9 ± 2.18	68.39^{+a}± 3.27	45.23^{-b}± 2.46	40.15^{-b}± 2.45	38.351^{-b}± 2.78
Uric acid (mg/dL)	3.0587± 0.32	5.5762^{+a} ± 0.17	3.6875^{-b}± 0.33	3.3562^{-b} ± 0.21	3.2087^{-b}± 0.19

Results are expressed as mean ± S.D. of 8 animals for each group.

+a = significant increased compared with control at p<0.05

-a = significant decreased compared with control at p<0.05

+b =significant increased compared with CP200 at p<0.05

-b = significant decreased compared with CP200 at p<0.05

3. Results

3.1. Hematological parameters among the studied groups

The mean ± SD values of the RBCs, WBCs, Platelets, Hb content and PCV in the CP group (3.190 × 106/mm3 ± 0.240, 4.42×103/mm3 ± 0.33, 255.28 × 103/mm3 ± 19.63, 5.67 g/dL ± 0.330 and 16.86% ± 0.93, respectively) were significantly lower than those in the control group (7.680 × 106/mm3 ± 0.19, 11.23 ×103/mm3 ± 0.53, 647 × 103/mm3 ± 31.25, 13.81 g/dL ± 0.350 and 39.43 % ± 1.140, respectively), with p<0.05 for all (Table1). And the mean ± SD values of the RBCs, WBCs, Platelets, Hb content and PCV in the CP+SIP group (6.72 × 106/mm3 ± 0.260, 9.74 × 103/mm3 ± 0.61, 517 × 103/mm3 ± 12.22, 9.93 g/dL ± 0.510 and 31.89% ± 1.75, respectively), CP+SIE group (7.170 × 106/mm3 ± 0.210, 11.16×103/mm3 ± 0.28, 583.60 × 103/mm3 ± 27.32, 11.84 g/dL ± 0.480 and 38.53% ± 1.22, respectively) and CP+SIP+SIE group (7.460 × 106/mm3 ± 0.310, 12.92 × 103/mm3 ± 0.74, 612.03 × 103/mm3 ± 22.34, 13.14 g/dL ± 0.660 and 40.07% ± 1.39, respectively) were significantly higher than those in the CP group (p<0.05 for all) (Table1). Also, the mean ± SD value of the WBCs in the CP+SIP+SIE (12.92 × 103/mm3 ± 0.74) was significantly higher than this in the control group (11.23 × 103/mm3 ± 0.53). Besides that the mean ± SD value of the PCV in the CP+SIP+SIE (40.07% ± 1.39) was non significantly higher than this in the control group (39.43 % ± 1.140) (Table1).

3.2. The liver function index in the serum of the studied groups

The serum biochemical analysis of the CP group showed a significantly higher mean of ALT and AST levels (82.3 Units/ml ± 3.7 and 194.2 Units/ml ± 2.83, respectively) than the control group (41.93 Units/ml ± 2.63 and 141.52 Units/ml ± 3.8, respectively) (p<0.05 for all). Moreover, the mean in the CP+SIP (51.00 Units/ml ± 3.2 and 156.12 Units/ml ± 4.73, respectively), CP+SIE (46.82 Units/ml ± 6.03 and 149.27 Units/ml ± 5.21, respectively) and CP+SIP+SIE (44.55 Units/ml ± 1.55 and 145.20 Units/ml ± 4.17, respectively), were significantly lower than those in CP group. (p<0.05 for all) (Table2). Meanwhile, the serum biochemical analysis of the CP group showed a significantly lower mean of Albumin and Total protein levels (2.04 g/dL ± 0.04 and 2.706 g/dL ± 0.246, respectively) than in the control group (3.97 g/dL ± 0.13 and 4.934 g/dL ± 0.229,

respectively). Also, their means in the CP+SIP group (3.28 g/dL ± 0.06 and 4.1578 g/dL ± 0.731, respectively), CP+SIE group (3.54 g/dL ±0.09 and 4.4597 g/dL ± 0.26, respectively) and CP+SIP+SIE group (3.86 g/dL ± 0.07 and 4.6823 g/dL ± 0.321, respectively) were significantly higher than those in CP group. ($p<0.05$ for all) (Table2).

3.3. The kidney function index in the serum of the studied groups

The serum biochemical analysis of the CP group showed a significantly higher mean of Creatinine, Urea and Uric acid levels (0.8672 mg/dL ± 0.028, 68.39 mg/dL ± 3.27 and 5.5762 mg/dL ± 0.17, respectively) than in the control group (0.523 mg/dL ± 0.019, 37.9 mg/dL ± 2.18 and 3.0587 mg/dL ± 0.32). Also, their means in the CP+SIP group (0.6782 mg/dL ± 0.022, 45.23mg/dL ± 2.46 and 3.6875 mg/dL ± 0.33, respectively), CP+SIE group (0.6266 mg/dL ± 0.017, 40.15 mg/dL ±2.45 and 3.3562 mg/dL ± 0.21, respectively) and CP+SIP+SIE group (0.5722 mg/dL ± 0.016, 38.351 mg/dL ± 2.78 and 3.2087 mg/dL ± 0.19, respectively) were significantly lower than those in CP group. ($p<0.05$ for all) (Table3).

4. Discussion

The potential harmfulness of chemicals and drugs in humans is a fundamental and essential area of search. CP has been chosen for the current study because it is widely and commonly used drug in chemotherapy. It is an effective drug against various types of cancers with high therapeutic effects. Moreover, it has been widely used as an immunosuppressive drug during organ transplantation (perini *et al*., 2007). Unfortunately, it is known to have several side effects including general cell-damaging effects. This study reports the possible mechanisms by which CP treatment can result in hematological disorders, hepatotoxicity and renal toxicity and explores the possible therapeutic and ameliorative effects of Sepia ink extract (SIE) and its polysaccharide (SIP), either each one separately or both together, after CP administration.

The results of this work showed significant side effects in the hematological disorders, which are caused by CP in the form of a significant reduction in the number of RBCs, WBCs and PLTs, remarkable fall in hemoglobin contents (Hb) and a significant drop in PCV value, These findings are in accordance with (Kennedy *et al*., 2014; Cengiz, 2018).The main site of continued generation and regeneration of blood cells including the cells responsible for immune activity in the blood is the bone marrow. A significant degree of cell proliferation makes bone marrow a sensitizer, especially to cytotoxic drugs including CP (Ukpo *et al.,* 2013). The inefficiency of bone marrow to produce new blood cells and the loss of blood stem cells will lead to leukopenia and thrombocytopenia (Hackett, 2003). The decrease in erythrocyte counts may be due to increasing their destruction or a fall in their production from the bone marrow, where the property of CP is to kill the rapidly proliferating cells in the body without distinguishing between their types, including red blood cells (Chakraborty *et al*., 2009; Vinoy *et al*., 2013).

CP injection, which resulting in severe damage to the blood-forming tissues in the bone marrow reflects also on the immune system, thereby leading to transient reduction in number of white blood cells (Hickman-Davis *et al*., 2001). These adverse effects are directly related to the mechanisms of action in immunosuppression, and affects cellular humoral immune responses (Raj and Gothandam, 2015). From this point, CP acts as an immunomodulatory agent especially in patients with organ transplant (Lawson *et al*., 2008).The bone marrow suppression in response to CP may suppress the rate of the thrombopoiesis which inhibits the production of megakaryocytes, the precursor cells of blood platelets (Sekhon and Roy, 2006). It is well known that RBCs contains mainly Hb so, from

our present results, the significant reduction of Hb content might be linked to the reduction of RBCs count. Also, our results are in accordance with the findings of Vinoy *et al*. (2013), which suggested that CP suppresses bone marrow ability to produce new ones of blood cells, resulting in lowering of RBCs count which results in decrease of Hb content in blood. In addition, ROS produced by CP induced Hb oxidation and denaturation (Puchala *et al.,* 2004). Leading to its content reduction. Furthermore, the decrease in Hb content may be due to CP induced changes in RBCs membrane emphasize the formation of free radicals. The effect of free radicals in RBCs membrane may contribute to the eventual leak of Hb out of the cell (Hussein *et al.,* 2007). As well as, the present recorded results in this study showed a significant drop in PCV value after administration of CP. This suppression in hematocrite value may be related to the total blood cell depletion in the peripheral blood, especially RBCs, after CP injection. This observed result runs in full agreement with (Zhang *et al*., 2017).

Additionally, CP induced a significant rise in the activities of ALT and AST enzymes; however total protein and albumin recorded a significant fall in CP-injected rats comparing to the control ones. This is consistent with previous studies (Germoush and Mahmoud, 2014). Many blood enzymes are well known to be as indexes for liver dysfunction and damage, and the leaching out of hepatic enzymes such as ALT and AST and their elevation into the systemic circulation is routinely used as reliable biochemical indicators for hepatic cells damage by CP hepatocellular necrosis (Haldar *et al.,* 2011). Above all, our recorded results of this study are in accordance with Kumar *et al*. (2005), who reported that the high levels of serum enzymes activity is a reflection of cellular damage in the liver tissue and alteration of functional membrane integrity of cell membranes in the hepatic cells. It is worth to mention that however the exactly mechanism and causes of liver damage, due to CP injection, are unclear, but the basic and main sites for the microsomal activation of the drugs, including CP, are the liver cells. CP requires chemical and enzymatic activation to release its inactive and active components as it is an inactive prodrug (Ismahil *et al.,* 2011) i.e., converted to its active form in liver by hepatic cytochrome P_{450} (CYP_{450} enzyme system).

Also, the data of this work indicated a significant decrease in the albumin and total proteins in rats injected with CP comparing with those in the control group. These results are in accordance with (Soliman *et al.,* 2014). Following higher doses administration of CP, hepatocyte dysfunction was observed with disturbances in selected protein synthesis (Soliman *et al.,* 2014). It is well known that the most vital and important protein formed by the liver is albumin and it is considered as a very important indicator for liver functions (Singh and Khan, 2013). Also, its levels are considered one of the best indicators to demonstrate hepatic function efficacy (Latimer *et al.,* 2003). Moreover, its serum level may be useful for determining dosage adjustments of drugs in people suffering from severe hepatic failure (Mano *et al.,* 2006). Furthermore, hypoproteinemia was noticed in CP- treated samples as CP affects plasma cells directly and causes inhibition in the protein synthesis, leading to liver injury (Senthilkumar *et al.,* 2006). So, our recorded low levels of serum albumin and total proteins in rats treated with CP ensured hepatotoxicity that induced by CP.

Moreover, the current data indicated that there is a significant increase in the serum creatinine, urea and uric acid in CP-injected group; these data are in agreement with (Bokolo and Adikwu, 2018). ROS are produced with high levels in CP-administered rats, and leading to injury in

the balance between the oxidants and antioxidants of vital organs including kidneys and this mainly causes oxidative stress (Patel, 1987). As well as, this observed elevation in serum creatinine and urea in rats injected with CP, can be explained as a repercussion of worsening kidney function (Geraci *et al.,* 1990) because of the formation of ammonia by the process of the deamination of amino acids throughout the hepatic cells, that is after that converted to urea (Osman and Hamza, 2013). The significant elevation in uric acid in CP-treated group in our current study may be due to fall in the rate of glomerular filtration and renal urate excretion. Also, it has been suggested that the inflammatory factors that caused oxidative stress and apoptosis are essential factors for more serious liver injury, leading to the formation of uric acid (Xie *et al.,* 2013).

In fact there are no sufficient studies about the effect of SIP on hematological parameters, liver and kidney functions. However, our present findings showed a significant improvement in these parameters, against CP toxicity. SIP, is a marine bioactive substance that is possesses strong scavenging on $OH^{\bullet}$ besides the total reducing power that are together known as antioxidant ability (Luo and Liu, 2013). And by this way SIP can modulate the lipid peroxidation of the blood elements cell membranes, induced by CP. This mainly reflects on a well improvement in the total blood elements. So, we can conclude that this positive therapeutic role of SIP may be due to its antioxidant properties.

In addition, it has been indicated that the bioactive SIP could elevate the antioxidant capabilities of many organs like heart, liver, lungs, and kidneys of samples treated with CP (Liu *et al.,* 2012), which implies that the animal organs can be protected by SIP from injury caused by CP as an anticancer drug. Our current results indicated a significant fall in the serum ALT and AST levels and a pronounced elevation in total protein and albumin in rats treated with SIP in comparison with the CP-treated group, meaning that SIP plays a vital role in treatment and prevention the liver injury and in suppression the enzymes leakage within the plasma membranes of cells caused by CP. Moreover, due to the antioxidant and anti-chemotherapy functions of SIP, it is mainly considered to be a potentially effective, non-toxic, broad spectrumcy to protective agent (Le *et al.,* 2015; Zuo *et al.,* 2015).Previously, it is proved that, SIP has a highly scavenging ability on hydroxyl radical where the $OH^{\bullet}$ is the strongest one among all of free-radicals and has the ability to react with any macromolecule, leading to gene mutations that resulting in tumorigenesis and aging (Luo and Liu, 2013).

Moreover, when we compared the concentration of creatinine, urea and uric acid in the group treated with SIP, after CP administration, with their of animals injected with CP, the enhancement was very clear. Renal damage is one of the dose-limiting side effects of CP, and this is highly relates to oxidative stress that is mainly caused following CP injection. It was reported that, the high generation of reactive oxygen species (ROS) by CP in renal tissues plays an essential role in the pathogenesis of CP-induced renal injury (Abraham and Rabi, 2009). On the other hand, SIP acts as potentially effective, non-toxic free radical scavenger and antioxidant (Le *et al.,* 2015). Also, the toxicity of the chemotherapeutic drug including CP, on the spleen, heart, lung, liver, kidney and intestines could be alleviated by SIP (Tang *et al.*, 2014; Zuo *et al.,* 2014).

Meanwhile, after SIE administration, the levels of the hematological and biochemical parameters previously disrupted by CP were significantly restored in CP treated rats. Increasing evidence to support the idea that the toxicity of CP can be weakened by some natural materials (Selvakumar *et al.,* 2006; Tripathi and Jena, 2008). Furthermore, it was indicated that

this compound is potential cytoprotector that could be applied in clinical therapy for cancer and chemotherapeutic drugs disorders.

SIE is a colloid containing mainly melanin, proteins, carbohydrates and lipids (Liu *et al.,* 2011). In addition, SIE contains considerable amounts of taurine (Derby *et al.,* 2007; Soliman, 2011). Taurine is an amino acid contains sulfur that exhibits antioxidant properties (Das *et al.,* 2009; Li *et al.,* 2009). Moreover, melanin of SIE is a copolymer of eumelanin constituted of approximately 75% of units of 5,6-dihydroxyindole-2-acid carboxylic (DHICA)and 20% of units of 5, 6-dihydroxyindole (DHI) (Katritzky *et al.,* 2002). Also, SIE melanin is an efficient free radical scavenger and antioxidant as it likes SOD and it has the ability to catalyze O^{2-} to H_2O_2, and thus avoid the free radical chain reaction triggered by O^{2-} (Chen *et al.,* 2007). It acts as SOD due to the presence of DHI which catalyzing the disproportionation of $O^{2\bullet-}$ to H_2O_2 and O_2 (Meyskens *et al.,* 2001). Also, SIE melanin can absorb cationic metal ions such as iron and copper in vivo that can dramatically affect the redox state of the polymer by promoting the production of the highly reactive $HO^{\bullet}$ in a Fenton type reaction (Fisher, 2003).

Our present study demonstrated that, SIE introduced to rats treated with CP, caused hematological changes in the blood of these rats, this characterized by a well improvement of the total number of RBCs, WBCs and PLTs, with a remarkable improvement in haemoglobin content (Hb) and in PCV value. These recorded findings are in accordance with (Soliman *et al.,* 2015). It is worth to mention that, it was reported that SIE has the ability to promote a lot of cytokines, like colony stimulating factor (CSF) (Xie and He, 2001). The proliferation and differentiation of hemopoietic stem cells are carried out and occurred mainly under the stimulation of CSF. So, this improvement in the hematological parameters after the treatment with SIE may be due to the induction of the bone marrow for producing different blood elements (RBCs, WBCs and PLTs) under the stimulation of CSF. Also, the induction of Hb following treating with SIE may be as a result of enhancement of level of iron by SIE that leading to advancement of the hematological functions (Soliman *et al.,* 2015). As the sepia ink melanin is an effective source of iron supplement for treatment of iron deficiency anemia (IDA) in rats and might be exploited as a new iron fortifier (Wang *et al.,* 2014). Besides that, the PCV (hematocrit) measures the volume of red blood cells compare to the total blood volume. On this basis, any change in the erythrocyte number affects on PCV and Hb. This point gives us a clear and well explanation for the observed improvement in PCV in rats treated with SIE comparable with results of CP injected ones.

Additionally, based on what was mentioned before, Acrolein, a metabolite of CP causes a significant oxidative stress. On the other hand, SIE possesses antioxidant prosperities related to its chemical components. And according to Chew and Park, (2004), the plasma membranes of blood cells are well known to have a high percentage of polyunsaturated fatty acids (PUFAs) so they are very sensitive to oxidative stress and free radicals. So, finally, we can relate this high improvement in the total blood elements in SIE treated rats compared to CP ones, to its antioxidant prosperities.

Above all, the results in our current study indicated a significant reduction in the serum ALT and AST activities in the SIE treated rats compared with the CP i.p. injected rats, indicating that SIE tend to treat and stop the liver damage as well as reducing the enzymes leakage throughout hepatic cellular membranes. It is worth to mention that, SIE treatment caused a general suppression in the activities of serum ALT and AST comparing to bile duct ligation rat groups (Saleh *et al.,* 2015). Also, the data of the present work indicated a pronounced

increase in the total protein and albumin in the SIE treated animals compared with ones treated with CP, meaning that SIE tend to treat and inhibit the liver injury. The same SIE ameliorative effect was observed in serum albumin after its disturbance by bile duct ligation (Saleh *et al.,* 2015). Moreover, this elevation may be due to the motivation and stimulation of proteins and albumin synthesis that helps to accelerate the renewal and restoration process, thus affording preservation to the hepatic cells (Murali *et al.,* 2012). Based on the antioxidant properties of SIE which related to its chemical components, we suggest this positive therapeutic effect of SIE may be due to its high ability in scavenging free radicals and to its antioxidant properties.

In this work there is a pronounced decrease in serum creatinine, urea and uric acid values in SIE treated group after the experimental period. This SIE ameliorative effect is in accordance with Soliman *et al.* (2014), who showed the positive therapeutic effect SIE against the renal injury caused by ligation of bile duct. We suggest that this reduction in serum urea, creatinine and uric acid may be due to decreased oxidative stress or increased elimination of hepatotoxicants from the body by SIE. Also, SIE plays a critical role in free radical scavenging and acts as a vital antioxidant based on its chemical components.

Conclusions

In conclusion, our outcomes showed that CP causes harmful effects on the hematological parameters as well as liver and kidney functions. On the other hand, the treatment with SIP and SIE orally, either each one separately or both together, individually, expressed positive antioxidant and ameliorative effects against all these side effects caused by CP, so SIP and SIE, especially both together, should be recommended during chemotherapeutic courses as natural, nontoxic and available marine products.

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