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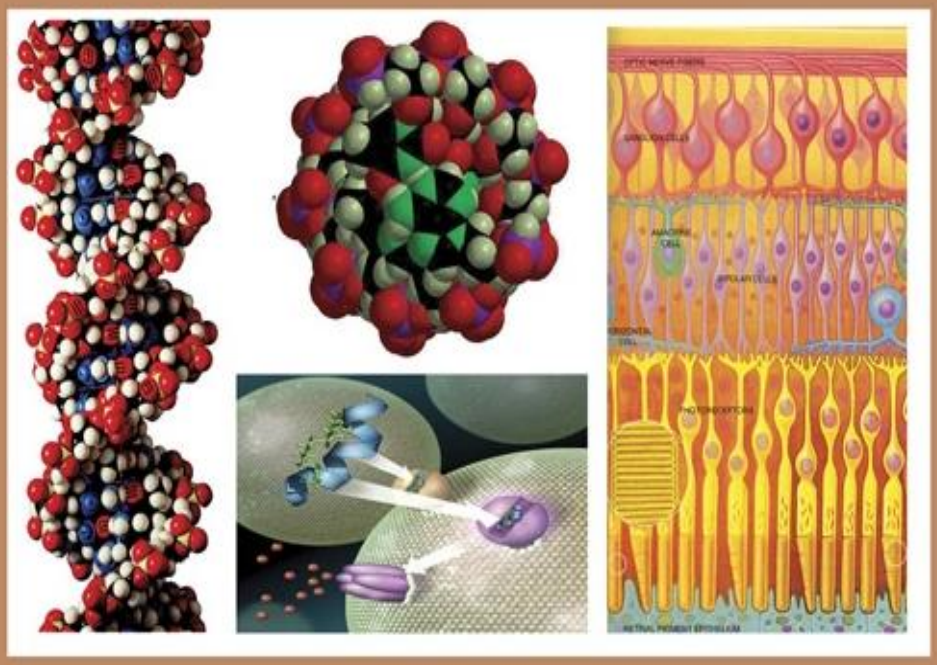

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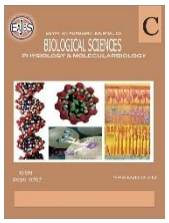

Antioxidant Effect of *Sepia pharaonis* Ink Extract and Ellagic Acid on Oxidative Stress Induced by Cyclophosphamide in male Albino Rats

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ABSTRACT

Objective(s): Present investigations aimed to compare the anticancer and antioxidant activities of *Sepia pharaonis* ink extract (SIE) and Ellagic acid (EA) to cyclophosphamide (CP).

Materials and Methods: Male Wistar Albino rats were grouped into five groups, each with ten rats. Control group (Group I) received (2 ml/kg 0.9% NaCl b.w. i.p.). Group II served as CP group was received (200 mg/kg CP b.w. i.p.). Rats of groups III, IV and V received (200 mg/kg CP b.w. i.p.) and treated with SIE (200 mg/kg b.w.), EA (60 mg/kg) and SIE (200 mg/kg b.w.) + EA (60 mg/kg), respectively. GSH, CAT, SOD, NO, and MDA levels were measured in all groups' liver and renal tissues. Histopathological examinations of the liver and kidney were performed.

Results: In the liver and renal tissue homogenate of CP-treated animals, there was a highly significant reduction in GSH, CAT, and SOD, as well as a striking elevation of MDA and NO. In all treated animals with SIE, EA, and SIE+EA, however, there was a substantial decrease in liver and renal MDA and NO levels with a moderate increase in GSH, CAT, and SOD activities. After CP treatment, liver and kidney tissues showed extensive necrosis, but CP+ SIE+ EA treatment revealed regeneration of some hepatocytes and kidney cells.

Conclusion: SIE and EA were both successful in reducing oxidative stress caused by CP. The effect and antitumor properties of SIE and EA as antioxidants were supported by histopathological examination in this study.

INTRODUCTION

The majority of chemotherapeutic drugs used to treat neoplastic cells harm normal living cells in different ways. Cyclophosphamide (CP) is one of these drugs (Ali *et al*., 2010). CP is a chemotherapy drug that is used to treat cancer. It is inactive in *vitro*, but when activated by liver microsomal enzymes in *vivo*, it can successfully kill cells in the proliferative cycle. Cancer cells, as well as some rapidly proliferating normal tissue cells, are killed by CP. Furthermore, CP was discovered to have a number of negative side effects, including a reduction in body resistance, a decrease in immune function, and the induction of oxidative damage (Ran *et al*., 2020). Nephrotoxicity, neurotoxicity, testicular dysfunction, cardiotoxicity, and hepatotoxicity are some of the more relevant side effects (Abdallah *et al*., 2019).

CP hepatotoxicity appears to be caused by oxidative stress, according to experimental proof (Manda and Bhatia, 2003 and Selvakumar *et al*., 2005). Cyclophosphamide metabolites are the main cause of hepatotoxicity. Furthermore, CP administration resulted in oxidative stress in the liver, as evidenced by increased lipid peroxidation (LPO), decreased glutathione (GSH) content, and increased superoxide dismutase (SOD) activity (Sugumar *et al*., 2007).

The imbalance between oxygen free radicals and antioxidants is known as oxidative stress (Bhimaraj and Tang, 2012). Reactive oxygen species (ROS) are commonly found in oxygen-free radicals (Valko *et al*., 2006).

Chemotherapy remains the most prevalent form of cancer treatment, despite the fact that the benefits outweigh the drawbacks. Until now, developing antineoplastic agents that combine efficacy, safety and patient convenience has posed a significant challenge. As a result, there is a lot of interest in combining anticancer drugs with natural products in order to maximize effectiveness while lowering systemic toxicity by using lower drug doses (Ismael *et al*., 2008).

Freshwater and marine products have recently gained popularity as a nutraceutical and functional foods, as well as a source of material for drug development and particular health foods (Koyama *et al*., 2006).

According to new evidence, marine natural products, particularly secondary metabolites from marine organisms, are much more likely than terrestrial sources to yield anticancer drugs (Hong *et al*., 2009). Animals in nature have their own protective reaction against predators, freshwater and marine mollusks have shells as well, though many of them are not completely protected by shells. Shelled and shell-less mollusks both use chemical defenses extensively. Caldwell (2005) theorized that cephalopod ink contains compounds capable of disrupting predators' chemical senses, but evidence for this theory is lacking.

The ink gland cells in the mantle cavity of cephalopods degenerate and shed their contents into the ink sac, which acts as a reservoir for the exhausted material. Cuttlefish use the ejection of dark ink from the sac as a defensive mechanism to avoid threats and hazards (Liu *et al*., 2011). Squid ink is a multifunctional marine bioactive material that facilitates the production of thromboxane, kills cancer cells, and increases the number of leukocytes (Fahmy and Soliman, 2013). It also has antioxidants (Liu *et al*., 2011), anti-radiation, anti-retrovirus, and anti-bacterial properties (Nithya *et al*., 2011 and Vennila *et al*., 2011).

Ellagic acid is a natural phenol found in a variety of fruits and vegetables. Its antioxidant and anti-proliferative properties provide substantial health benefits. Ellagic acid has been shown to protect against cyclophosphamide-induced pulmonary, testicular, and nephrotoxicity (Türk *et al*., 2010; Rehman *et al*., 2012 and Saba *et al*., 2013).

Ellagic acid is a strong chemo-preventive and radical scavenger (Atesssahin *et al*., 2007 and Yuce *et al*., 2007). Plants high in EA include raspberries, strawberries, walnuts, longan seed, mango kernel (Soong and Barlow, 2004 and 2006), and pomegranate (Sudheesh and Vijayalakshmi, 2005). The hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage. It comprises four hydroxyl groups and two lactone groups (Pari and Sivasankari, 2008).

The purpose of this study was to assess the antioxidant effects of *Sepia* ink extract SIE and ellagic acid EA in the liver and kidney of rats treated with cyclophosphamide CP.

MATERAILS AND METHODS

Chemicals and Reagents:

Cyclophosphamide and ellagic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Bio-diagnostic Company (Giza, Egypt) supplied all of the biochemical

parameter kits.

***Sepia pharaonis* Sampling Collection:**

Sepia pharaonis Ehrenberg, 1831 samples were collected from Hurghada shore (Red Sea). The samples were kept in an icebox and transported to the lab within a few hours of being caught.

Preparation of *S. pharaonis* Ink Extract SIE:

To acquire ink sacs, the gathered samples were cleaned with distilled water and then dissected. The ink was diluted with an equal amount of distilled water and thoroughly mixed. Using a Lyophilizer, the admixture was concentrated and lyophilized to a black residue (LABCONCO, shell freeze system, USA).

Wistar Albino Rat Samples:

The Animal House of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt, delivered male Wistar Albino rats weighing 180-250g. All animals were kept in standard conditions, with a temperature of (26 ± 3ºC) besides 12:12 h day and night cycle. The study follows the guidelines for the care and use of laboratory animals established by the Faculty of Science's Institutional Animal Ethical Committee, as well as the ethical guidelines of South Valley University in Egypt

Experimental Design:

The rats were divided into five groups (n = 10/group) randomly:

Group 1: received normal saline (2 mL/kg) and considered control.

Group 2: cyclophosphamide CP (200 mg/kg i.p.) was given as a single dose.

Group 3: was given a single dose of CP (200 mg/kg i.p.) before being given *Sepia* ink extract SIE (200 mg/kg) orally.

Group 4: received a single dose of CP (200 mg/kg) by ip injection, followed by oral administration of ellagic acid EA at a dosage of (60 mg/kg).

Group 5: received a single dose of CP (200 mg/kg) through i.p. injection, followed by treatment with SIE (200 mg/kg b.w.) and EA (60 mg/kg).

After CP injection, treatments with (SIE, EA, and SIE+EA) were given for 30 days. Animals were slaughtered at the end of the experiment; liver and kidneys were dissected and washed with physiological saline solution, dried, weighed, and homogenized in phosphate buffer (pH7.4), and kept frozen at -80 ºC until biochemical analysis. Each group's liver and kidney specimens were immediately fixed in 10% formalin for histopathological examination after dissection.

Biochemical Analysis:

Reduced glutathione GSH was measured in the liver and renal tissue homogenate by Beutler *et al*. (1963), catalase CAT by Aebi (1984), superoxide dismutase SOD by Nishikimi *et al*. (1972), nitrite NO by Montgomery and Dymock (1961), and malondialdehyde MDA by Ohkawa *et al*. (1979). The biochemical analysis was performed according to the manufacturer's instructions using Bio-diagnostic assay kits (Giza, Egypt).

Histopathological Examination:

Liver and kidney specimens from all groups were dissected and fixed in a 10% neutral buffered formalin solution before being dehydrated in increasing degrees of alcohol. After clearing in xylene, the specimens were embedded in paraffin wax. Sections of 4-5 μm were prepared using a microtome and stained with hematoxylin and eosin (H&E) before being examined using a light microscope according to Bancroft and Gamble (2002).

Statistical Analysis:

The variability degree of results was expressed as means ± standard deviation of means (Mean±SD). ANOVA, unpaired t-test (prism program), and the least significant difference were used to test the difference between therapies. Results were considered statistically significant when $P \leq (0.05)$.

RESULTS

Oxidative Stress Biomarkers in Liver and Kidney Tissues:

Measurement of MDA as an indicator of lipid peroxidation revealed that CP highly significantly increased lipid peroxidation in liver and kidney tissues as estimated by

increased MDA satisfied which existing in Figs 1& 2 and recorded a highly significant increase at ($p<0.01$) in MDA and NO levels, with a noticeable reduction in SOD, GSH and CAT activities in liver and kidney tissues of rats treated with CP when compared with control animals.

However, when compared to the CP-treated (SIE, EA and SIE+EA) groups, the investigated compounds were able to significantly decrease MDA when compared to similar levels in CP group. Daily treatment with SIE resulted in a highly significant decrease ($p<0.01$) in MDA and NO levels, as well as a highly significant increase ($p<0.01$) in SOD, GSH, and CAT activities, but it did not achieve normal animals.

EA treatment for 30 days appearances a highly increase ($p<0.01$) in SOD, GSH and CAT activities and verified a highly significant reduction ($p<0.01$) in MDA and NO levels when compared with CP animals, though significant improvements ($p<0.05$) were documented almost near to normal values when compared with control animals.

These findings revealed that SIE+EA treatment resulted in a highly significant ($p<0.01$) increase in SOD, GSH, and CAT activities in liver and kidney tissues. In contrast, when compared to corresponding activities in CP-treated animals, showed a highly significant decrease in MDA and NO activities ($p<0.01$). These findings were almost nearly reachable to control levels.

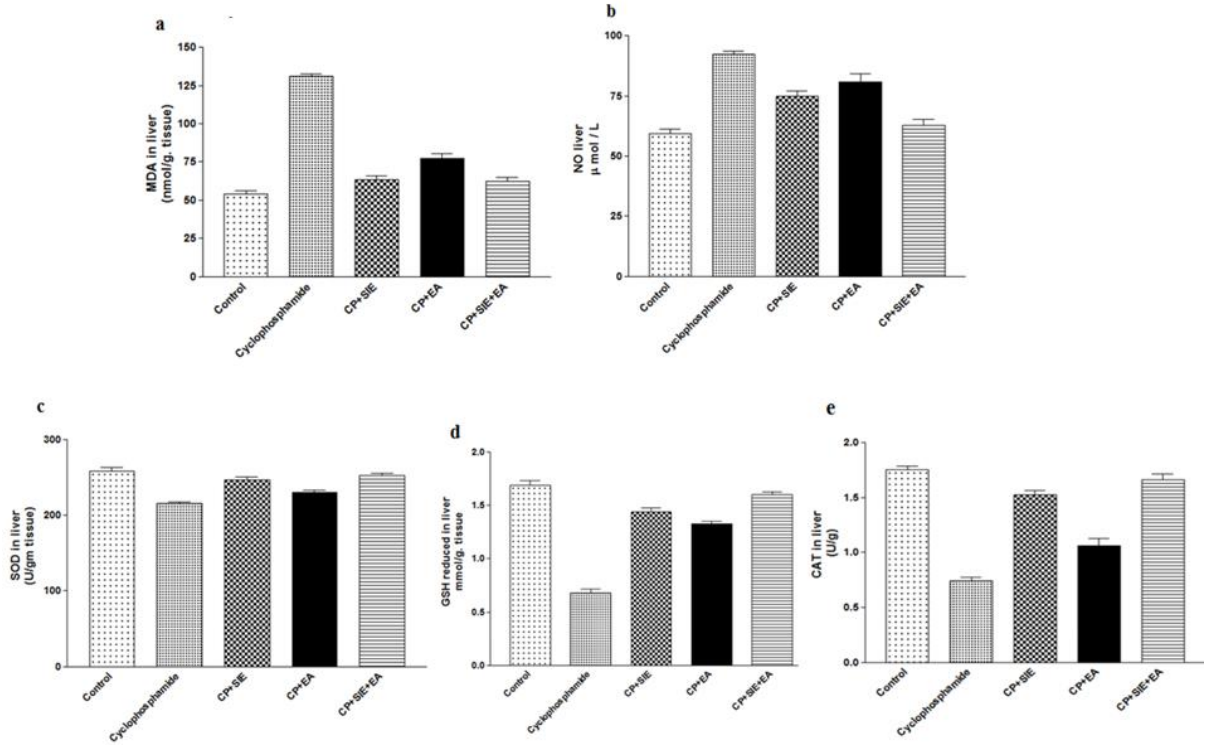

Fig. 1: Therapeutic effects of *Sepia pharaonis* ink extract SIE (200mg/kg), ellagic acid EA (60mg/kg) and SIE (200mg/kg)+ EA (60mg/kg) on the liver antioxidant enzymes induced by cyclophosphamide CP (200mg/kg). (a) MDA (nmol/gm tissue), (b) NO (µmol/L), (c) SOD (U/gm tissue), (d) GSH reduced in liver (mmol/gm tissue) and (e) CAT (U/g).

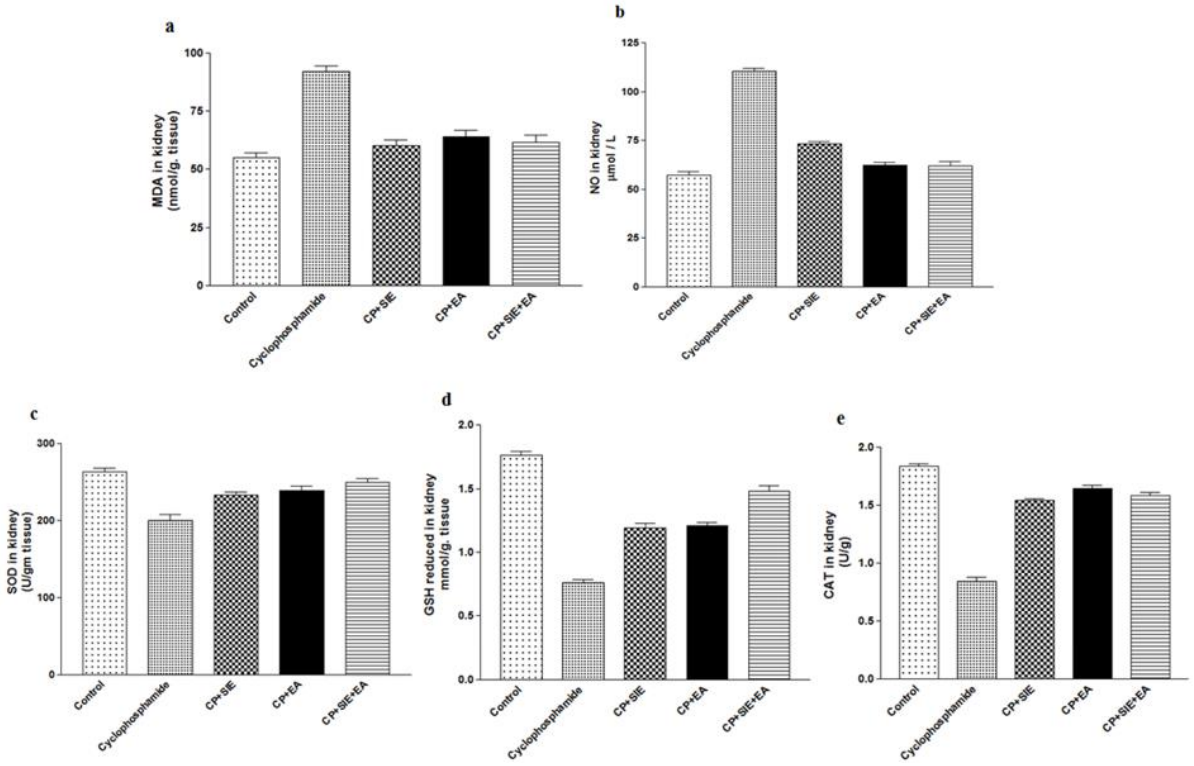

Fig. 2: Therapeutic effects of *Sepia pharaonis* ink extract SIE (200mg/kg), ellagic acid EA (60mg/kg) and SIE (200mg/kg)+ EA (60mg/kg) on the kidney antioxidant enzymes induced by cyclophosphamide CP (200mg/kg). (a) MDA (nmol/gm tissue), (b) NO (µmol/L), (c) SOD (U/gm tissue), (d) GSH reduced in liver (mmol/gm tissue) and (e) CAT (U/g).

Histopathological Examination:

Liver:

Hepatocytes were arranged normally in the liver of control albino rats (Fig. 3 a). On the other hand, the liver of group 2 showed widespread necrosis and destruction of hepatic tissues with loss of architecture (Fig. 3 b), as well as severe congestion and dilatation of the blood vessels (Fig. 3 c). The liver of group 3 showed some hepatocyte regeneration along with a mild degree of blood vessel congestion (Fig. 3 d). The liver of group 4 had congestion of the blood vessels, with some hepatocytes regenerating (Fig. 3 e). The liver of group 5 showed regeneration of some hepatocytes as well as mild blood vessel congestion (Fig. 3 f).

Kidney:

The renal tubules and glomeruli of control Albino rats' kidneys were in a regular arrangement (Fig. 4 a). The kidneys of group 2 showed extensive destruction and lytic necrosis of the renal tubules, as well as a significant amount of inflammation (Figs. 4 b & c). The kidney of group 3 demonstrated inflammatory cell infiltration with dilatation of bowman's space (Fig. 4 d). Inflammatory cells infiltrated the kidney of group 4, causing hypercllularity and glomeruli congestion (Fig. 4 e). The kidneys of group 5 showed glomerular congestion and hypercllularity, as well as a mild degree of renal inflammation (Fig. 4 f).

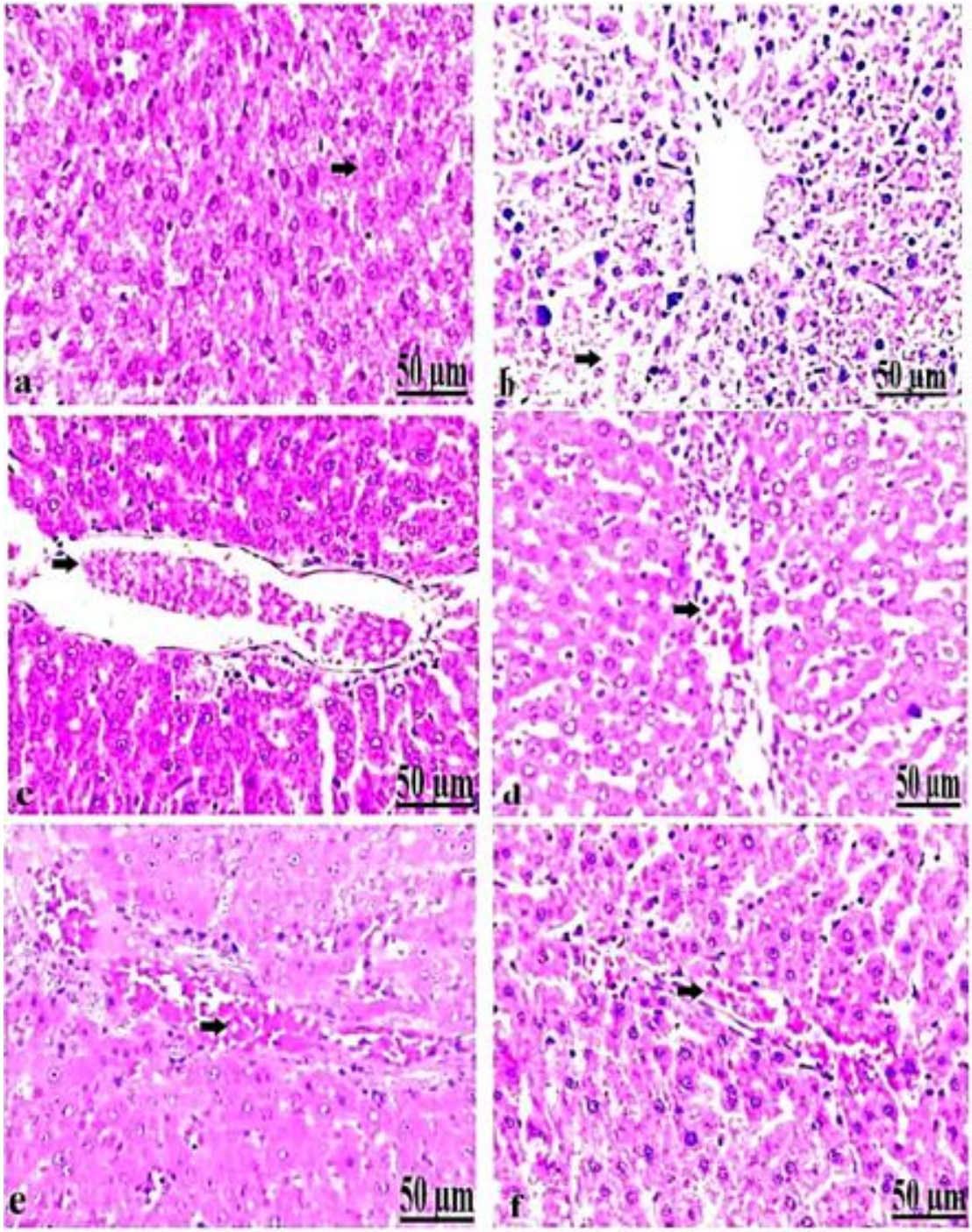

Fig. 3: Histopathological photomicrographs of the liver sections from different treatment groups. (a) Liver of control albino rats showing normally arranged hepatocytes, (b) Liver of group 2 injected with a single dose of CP (200mg/kg) showing extensive necrosis and destruction of hepatic tissues with loss of architecture, (c) Liver of group 2 showing the destruction of hepatic tissues besides severe congestion and dilatation of the blood vessels, (d) Liver of group 3 received CP (200 mg/kg) + *Sepia* ink extract SIE (200 mg/kg body weight) showing regeneration of some hepatocytes with a moderate degree of congestion of the blood vessels, (e) Liver of group 4 received CP (200 mg/kg) + Ellagic acid EA (60 mg/kg) showing congestion of the blood vessels with the regeneration of some hepatocytes, (f) Liver of group 5 received CP (200 mg/kg) + SIE (200 mg/kg body weight) + EA (60 mg/kg) showing regeneration of some hepatocytes with mild congestion of the blood vessels. (H&E., bar=50 µm)**.**

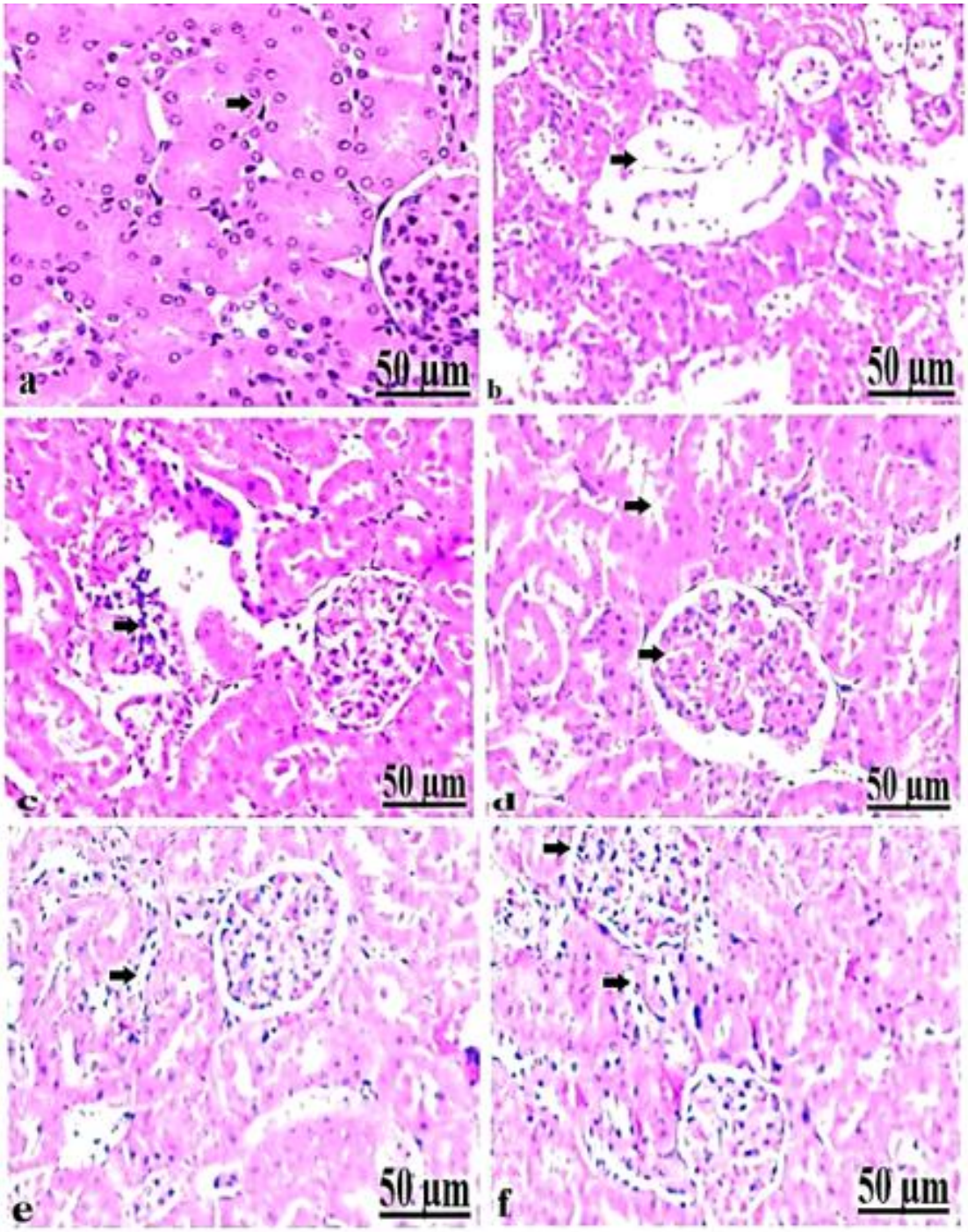

Fig. 4: Histopathological photomicrographs of the kidney sections from different treatment groups. (a) Kidney of control albino rats showing a normal arrangement of the renal tubules and glomeruli, (b & c) Kidney of group 2 injected with a single dose of CP (200mg/kg) showing severe destruction and lytic necrosis of the renal tubules in addition to a remarkable degree of inflammation, (d) Kidney of group 3 received CP (200 mg/kg) + *Sepia* ink extract SIE (200 mg/kg body weight) showing inflammatory cells infiltration with dilatation of bowman's space, (e) Kidney of group 4 received CP (200 mg/kg) + Ellagic acid EA (60 mg/kg) showing inflammatory cells infiltration with hypercllularity and congestion of the glomeruli, (f) Liver of group 5 received CP (200 mg/kg) + SIE (200 mg/kg body weight) + EA (60 mg/kg) showing congestion and hypercllularity of the glomeruli, besides the mild degree of renal inflammations (f). (H&E., bar=50 µm).

DISCUSSION

The aim of this study was to determine the toxicity of CP and the potential curative effects of SIE and EA on liver and kidney antioxidant enzyme activities, as well as the changes in the histopathological status of liver and kidney tissues.

Chemotherapy is now the primary treatment option for many cancers. Multidrug resistance (MDR) to chemotherapeutic drugs, on the other hand, is a major impediment to the successful treatment of malignant tumors (Tao *et al*., 2010). As a result, the development of novel chemotherapeutic agents will be critical in the treatment of cancer patients who are refractory or reverting.

CP is one of the most widely used anticancer drugs, but its use is limited due to its toxic side effects. The results of this study show that CP causes tissue oxidative stress, which is reflected by a substantial increase in MDA and NO levels, as well as inhibition of GSH, CAT, and SOD enzyme activities. These findings are consistent with those of Gu *et al*. (2017). It's worth noting that the toxic properties of CP are the result of its metabolism, which results in the formation of highly reactive compounds, such as free radicals (Huitema *et al*., 2000). When CP is metabolized in the liver by Cytochrome P450 3A4 (CYP3A4) into the reactive aldehydes chloroacetaldehyde and dichloroethyl CP, reactive oxygen species (ROS) are produced (Huitema *et al*., 2000 and Wahlang *et al*., 2015).

ROS are chemically active and can react with nearly every component of a cell. Lipid peroxidation, which causes an increase in the production of hydroxyl radicals, a particularly energetic reactive oxygen species, is one of the main changes that occur in the components of a cell after CP injection (Manda and Bhatia, 2003). Also, because of the link between oxidative stress and cancer, antioxidant supplementation is thought to be beneficial in preventing carcinogenesis (Terry *et al*., 2000 and Fahmy and Soliman, 2013). Furthermore, the use of antioxidants to reduce inflammation has been investigated in relation to the risk of carcinogenesis (Fahmy and Soliman, 2013).

In cultured rat hepatocytes and various kidney cells, CP increases superoxide anion and H_2O_2 formation and induces oxidative stress (Al-Malki, 2014). Various enzymes leak into the circulatory fluid when cell membranes are damaged. Furthermore, during inflammation, reactive oxygen species and lysosomal enzymes are released by activated neutrophils, macrophages, and granulocytes under the influence of an oxidant, which can attack normal tissues.

Stankiewicz *et al*. (2002), on the other hand, found that CP induces changes in antioxidant status, and the findings of this research suggest that antioxidant abilities in the liver are also altered after CP administration. To begin with, antioxidant enzymes such as GSH, CAT, and SOD have shown a decrease in activity. The decrease in their activities following CP administration is most likely due to damage to these enzymes' structures, which is caused by reactive oxygen species that oxidatively alter protein structures (Davies and Goldberg, 1987 and Bilto *et al*., 2012).

Antioxidant defense systems include enzymes like SOD, GSH, and CAT (Ighodaro and Akinloye, 2018). MDA is a byproduct of lipid peroxidation and a marker of oxidative stress (Gaweł *et al*., 2004 and Jiang *et al*., 2009). The administration of CP induces a decrease in GSH concentration in the current study. The reduction of glutathione in tissues is an energetic frequency, which can lead to impairment of the cellular resistance against reactive oxygen species and may product peroxidative (Kasem, 2019) injury This may be caused by the oxidation of glutathione by reactive oxygen species (ROS) or the formation of conjugates with drug metabolites in the hepatocytes (Stankiewicz and Skrzydlewska, 2003). When the free thiol group of gluthatione reacts with the CP metabolite acrolein, conjugation occurs (Uchida *et al*., 1998). This conjugation also shows the toxic properties of less toxic

compounds, such as free aldehyde (Ramu *et al*., 1995).

The most cytotoxic aldehydes and acrolein can react with the thiol groups of various cell components, causing GSH levels to drop and protein thiol groups to disappear quickly (Esterbauer *et al*., 1991). SOD is the first antioxidant enzyme to deal with oxiradicals, which can speed up superoxide anion dismutation into hydrogen peroxide. CAT is a hemi-protein found in peroxisomes that catalyzes the removal of hydrogen peroxide produced during the SOD-catalyzed reaction (Basu *et al*., 2015). Thus, SOD and CAT function as mutually supportive antioxidative enzymes that provide ROS protection (Gałecka *et al*., 2008).

Natural products can be found in abundance in marine species. Many compounds derived from these organisms have sparked interest as both difficult structure elucidation and synthesis issues as well as cytotoxicity concerns. It is thought that marine organisms or their metabolites may provide a rich source of anticancer drug candidates. Chemical secretions formed and released from the ink sac of cephalopods are known as inks (Fahmy and Soliman, 2013).

When compared to the CP group, SIE significantly increased GSH, CAT, and SOD activity in the liver and kidney tissues while significantly reducing MDA and NO levels. This finding suggested that SIE had a protective effect against CP-induced tissue oxidative damage by increasing antioxidant enzyme activity and lowering lipid peroxidation levels. SIE has been shown to protect against oxidative damage caused by CP in previous studies (Ran *et al*., 2020).
According to the findings of this study, the SIE extract exhibited dose-dependent radical scavenging activity. Furthermore, the oxidation of polyunsaturated fatty acids produces low molecular weight end products, such as malondialdehyde, during lipid peroxidation.

Melanin, protein, carbohydrate, and lipid are all found in squid ink (Liu *et al*., 2011). Melanins are powerful antioxidants and free radical scavengers (Fahmy and Soliman, 2013). *Sepia* melanin, according to Katritzky *et al*. (2002), is a copolymer of eumelanin made up of approximately 20% units of 5, 6-dihydroxyindole (DHI) and 75% units of 5, 6-dihydroxyindole-2-acid carboxylic acid (DHICA). Zhang *et al*. (2003) reported that *Sepia* ink elevated superoxide dismutase SOD activity. Background researches showed that melanin of *Sepia* ink, like SOD, can catalyze O_2^- to H_2O_2, and thus avoid the free radical chain reaction triggered by O_2^- (Chen *et al*., 2007). Melanin of squid ink may act 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). Furthermore, in *vivo*, melanins absorb cationic metal ions such as iron and copper, which can dramatically alter the polymer's redox state by promoting the production of the highly reactive $HO^{\bullet}$ in a Fenton-type reaction (Fisher, 2003). In addition, two distinct metabolites in melanin, L-Dopa and dopamine effector molecules have been identified in concentrations sufficient to produce physiological effects. (Fahmy and Soliman, 2013).

Ellagic acid EA plays an important role in protein sulfhydryl repair mechanisms, in protection from the oxidative stress-induced cell damage via inhibition of single-strand break formation, reduction of intracellular calcium, and inhibition of lipid peroxidation (Gamal-Eldeen, 1997). Because EA is a polyphenol with four OH groups, it acts as a strong reduction agent, scavenging free radicals and reactive oxygen species (Bahri-Sahloul *et al*., 2009 and Michael, 2011).

When compared to the CP group, supplementing rats with EA caused less change in liver and renal antioxidant enzyme activities in the current study. EA supplementation improved the activity of liver superoxide dismutase and glutathione peroxidase enzymes.

EA scavenges superoxide radicals and hydrogen peroxide produced by isoproterenol-induced myocardial damage (Punithavathi *et al.*, 2010). Also, Lee *et al.* (2010) indicated that ellagic acid exerts its protective effects by inhibiting NADPH

oxidase-induced overproduction of superoxide, enhancing cellular antioxidant defenses. The antioxidants in the bloodstream were normalized after EA was administered. EA effectively scavenges $O_2^{\bullet-}$, hydroxyl radical, peroxy radicals and peroxynitrite (Murakami *et al.*, 1991 and Cozzi *et al.*, 1995); thus it could improve SOD activity.

Furthermore, Pari and Sivasankari (2008) found that EA administration reduced the activity of hepatic marker enzymes and MDA significantly. EA's antioxidant activity demonstrates that it scavenges free radicals (superoxide anion, hydroxy radical, and peroxy radical) and inhibits the formation of lipid peroxidation markers (Amador *et al.*, 1999 and Seeram *et al.*, 2005). Polyphenols have been shown to protect cells from oxidative stress; however, depending on the concentration and free radical source, polyphenol compounds may have both antioxidant and prooxidant properties; EA being a polyphenol can act as a chain-breaking antioxidant and may react directly with chain carrying peroxyl radicals thus terminating the propagation of these free radical-mediated reactions like NADPH- and ascorbate- dependent lipid peroxidation which is free radicals mediated (Majid *et al.*, 1991).

EA administration resulted in a highly significant improvement in liver GSH levels, according to the recent study Hassoun *et al.* (2006). Furthermore, (Majid *et al.*, 1991) also demonstrated that dietary administration of EA increased the levels of reduced glutathione in the liver. According to reports, EA not only acts as an antioxidant but also improves GSH-dependent protection (Khanduja *et al.*, 1999 and Özkaya *et al.*, 2010).

Furthermore, studies have shown that EA's two lactone groups (phenolic nucleus) can function as both hydrogen bond donors and acceptors. (Bala *et al.*, 2006), which might also involve free radical scavenging potential (Pari and Sivasankari, 2008).

CP caused severe necrobiotic changes in the liver and kidneys, as well as severe congestion and dilatation of the blood vessels, according to histopathology. CP may cause cellular injury by inducing oxidative stress through the production of free radicals and reactive oxygen species (ROS) (Ghosh *et al.*, 2002). Supplementation with ellagic acid or *Sepia* ink extract, on the other hand, reduced the pathological abnormalities caused by CP. Since EA has antioxidant properties, it has a hepatoprotective potential against tissue toxicity caused by CP. EA supplementation also reduced the toxicity of hepato-renal changes (Aslan *et al*., 2018). *Sepia* ink is a multifunctional bioactive mixture. It acts as an antioxidant (Wang *et al*., 2010), anti-inflammatory and anticancer properties (Fahmy and Soliman, 2013).

Conclusion

In conclusion, this study suggests that SIE and EA protect Liver and kidney tissues against CP toxicity. These protective actions of SIE and EA seem to be closely involved with the suppressing of plasma lipid peroxidation and increasing antioxidant enzyme activities. Therefore, SIE or EA may be used combined with CP in cancer patients, transplantation and autoimmune diseases to improve CP-induced injuries in oxidative stress parameters.

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