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Research Article

ANTIOXIDANT EFFECT OF SELENIUM ON HEPATOTOXICITY INDUCED BY CHLORPYRIFOS IN MALE RATS

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ABSTRACT

The present study was undertaken to evaluate the protective effect of selenium against chlorpyrifos-induced hepatotoxicity in experimental rats. The way of application selected for the study was oral gavage for 28 consecutive days. Wister adult male rats were randomly divided into four groups. The first group was served as a control, whereas the remaining groups were respectively treated with sodium selenite (3 mg/kg b.wt.), chlorpyrifos (13.5 mg/kg b.wt., 1/10 LD_{50}) and a combination of chlorpyrifos and sodium selenite. The exposure of rats to chlorpyrifos promoted oxidative stress resulted in an increase and a decrease of liver malondialdehyde (MDA) and reduced glutathione levels compared to control, respectively. Also, decreases in liver glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) activities were observed. In addition, plasma transaminases (ALT&AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were increased. A significant decrease in body weight and an increase in absolute and relative liver weights were observed in chlorpyrifos-treated rats compared to the corresponding controls. The co-administration of Se attenuated the biochemical parameters cited above as well as the changes in body and liver weights. Liver histological studies have confirmed the changes observed in biochemical parameters and proved the beneficial role of Selenium. In Conclusion, the use of selenium appeared to be beneficial to rats, to a great extent in attenuating and restoring the oxidative damage sustained by insecticide exposure.

Keywords: Antioxidant enzymes, Chlorpyrifos, Histopathology, Lipid peroxidation, Oxidative stress, Selenium.

INTRODUCTION

For centuries, pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors[1]. The use of pesticides causes severe environmental and health hazards to organisms[2, 3, 4]. Due to their high insecticidal activity, low environmental persistence and moderate toxicity, the organophosphorus (OP) compounds are the most favored insecticides. They are widely used in agriculture and medicine. However, the unregulated use and its aerial application over large agricultural and urban areas have caused severe environmental pollution[5]. Exposure to OP is associated with toxic effects on humans and animals[2, 3, 6, 7].

One such organophosphate which has spurred interest is Chlorpyrifos (CPF). CPF is a broad-spectrum organophosphorus insecticide utilized extensively in agriculture[7]. CPF is thought to be primarily metabolized in the liver involving the intervention of multiple, specific cytochrome P450's through several reaction pathways[8]. CPF elicits a number of additional effects, including hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity, and neurobehavioral changes[4, 6, 9, 10].

Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn prompt oxidative stress in different tissues [3, 10, 11]. Many studies have implicated oxidative damage as the central mechanism of toxicity[12]. Oxidative damage primarily occurs through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues[13]. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers[14], and they may damage membranes by inducing lipid peroxidation[3, 15, 16].

Free radical generation is expected to induce hepatotoxicity. Therefore, supplementation of antioxidants can be considered as the alternative method for chelating therapy. In fact, several studies demonstrated that the cellular antioxidant activity is reinforced by the presence of dietary antioxidants[17, 18]. Accordingly, interest has recently grown in the role of natural antioxidants used as a strategy to prevent oxidative damage as a factor in the pathophysiology of various health disorders[3, 16, 18]. Among antioxidants, selenium (Se) used as nutritional supplements, is the essential elements in almost all biological systems. Se is an essential element for humans, which improves the activity of the seleno-enzyme. It is present in the active center of glutathione peroxidase (GPx), an antioxidant enzyme, which protects lipid membranes and macromolecules from oxidative damage produced by peroxides[19, 20]. Furthermore, other authors (Yuan and Tang; Akhtar et al.) reported that Se has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against oxidation injury [21, 22].

Since, the potential hazard of exposure to CPF on the liver is well documented, therefore the present study aimed to elucidate the hepatoprotective effect of Se when co-administered orally to adult male rat using biochemical alterations and histopathological findings as criteria.

MATERIALS AND METHODS

Animals

Healthy male Wister rats weighing 150 ± 5 g, were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (23 ± 2 °C). On standard pellet diet, tap water ad libitum, and daily dark/light cycle (12/12 hrs.), the rats were acclimatized for 1 week prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, and international guidelines for care and use of laboratory animals.

Chemicals and Reagents

Chlorpyrifos 'CPF' (M. Wt. 350.6; 99% purity) was obtained from Dow AgroSciences (Indianapolis, Indiana, USA) and Sodium selenite (Na2SeO3) was purchased from Sigma (St. Louis, MO, USA). The assay kits used for biochemical measurements of malondialdehyde (MDA), ALT, AST, ALP, LDH, GSH, CAT, SOD and GPx were all purchased from Biodiagnostic Company, 29 Tahrir Str., Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

Experimental Design

The animals were randomly divided into 4 groups of six animals each. The route of administration selected for the study was oral

daily gavage for 28 consecutive days. Animals in Group 1 were served as control and given only standard pellet diet and corn oil (0.5 ml/rat). Animals in Group 2 were given daily Na_2SeO_3 at a dose of 3 mg/kg b.wt./day. Animals in Group 3 were given daily chlorpyrifos alone at a dose of 13.5 mg/kg b.wt. (1/10 LD_{50}, Tomlin, 2004) dissolved in corn oil (0.5 ml/rat) [23]. Animals in Group 4 were simultaneously given of CPF (13.5 mg/kg b.wt.) and Na_2SeO_3 (3 mg/kg b.wt./day).

This dose of CPF which corresponded to 1/10 of LD_{50} was selected on the basis of previous studies [6, 24], whereas, Na_2SeO_3 dose was selected based on the clinical application and on results from previous studies on human and experimental animals [25, 26].

During the experimental duration, body weights were weekly recorded and the doses were modulated accordingly. After completion of treatment period, blood samples were withdrawn from the animals under light ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary tube. Blood samples were collected, subjected to plasma separation and stored at -20 ^{0}C for biochemical analysis within one week. Rats were then killed by decapitation. Then, livers were dissected out, cleaned and weighed. Some samples were rinsed and homogenized (10%, w/v) in an appropriate buffer (pH, 7.4) and centrifuged. The resulting supernatants were used for biochemical assays. Other samples were immediately removed, cleaned and fixed in 10% formalin solution and embedded in paraffin for histological examination.

Biochemical Analysis

The plasma and liver supernatants obtained from different treatments were subjected to certain biochemical analyses spectrophotometrically by using Shimadzu UV- VIS Recording 2401 PC (Japan).

Protein content

Liver protein contents were measured according to the method of Lowry et al. using bovine serum albumin as standard [27].

Liver function tests

Plasma transaminases (ALT & AST) activities were determined by a colorimetric method according to Reitman and Frankel [28]. Plasma alkaline phosphatase (ALP) activity was determined by enzymatic colorimetric method according to Young et al. [29].

Indicators of liver cell necrosis

Liver and plasma lactate dehydrogenase (LDH) activity as indicator of necrotic cell death was determined using a kinetic method according to Vassault et al. [30].

Oxidative stress parameters

The liver malondialdehyde (MDA) concentrations, a lipid peroxidation index, were determined spectrophotometrically according to Draper and Hadley [31]. Briefly, an aliquot of liver extract supernatant was mixed with 1ml of 5% trichloroacetic acid and centrifuged at 3000g for 10 min. An amount of 1ml of thiobarbituric acid reagent (0.67%) was added to 500 ml of supernatant and heated at 90 ^{0}C for 15 min. The mixture was then cooled and measured for absorbance at 532 nm. The malondialdehyde values were calculated using 1, 1, 3, 3-tetraethoxypropane as standard and expressed as nano moles of malondialdehyde/g of liver.

Liver glutathione (GSH) levels in the liver was determined by the method of Ellman [32] modified by Jollow et al. [33]. The method is based on the development of a yellow color when 5, 5-dithiobtis-2 nitrobenzoic acid (DTNB) was added to compounds containing sulfhydryl groups. Five hundred microlitres of tissue homogenate in phosphate buffer were added to 3ml of 4% sulfosalicylic acid. The mixture was centrifuged at 1800g for 15min. Five hundred milliliters of supernatants were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10min. Total GSH content was expressed as mg/mg of protein in the liver.

Antioxidant enzymes

Catalase (CAT) activity was assayed by the method of Aebi [34]. Enzymatic reaction was initiated by adding an aliquot of 20 ml of the homogenized tissue and the substrate (H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of mmole H_2O_2 consumed/min/mg of protein.

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich [35]. The reaction mixture contained 50 mM of tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM l-methionine, 2 mM riboflavin and 75 mM nitro blue tetrazolium (NBT). The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units/mg of protein.

Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler [36]. The enzyme activity was expressed as nmole of GSH oxidized/ min/mg protein.

Histopathological Examination

For light microscopic investigations, specimens from liver were fixed in 10% phosphate buffer formalin, dehydrated in alcohols and embedded in paraffin. Five micron tissue sections were stained with hematoxylin and eosin stain (H&E) for general histopathological examination. Scoring of histopathological changes was done as follow: (-) absent; (+) mild; (++) moderate; (+++) severe, and (++++) extremely severe [37].

Statistical Analysis

The results were expressed as means ± SD. All data were done with the Statistical Package for Social Sciences (SPSS 11.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan's test for comparison between different treatment groups. Statistical significance was set at $p < 0.05$.

RESULTS

To address the hypothesis whether Se would prevent or attenuate CPF-induced oxidative stress and hepatotoxicity confirmed by biochemical perturbations and the histopathological findings, the principal and design of the experiment were conducted.

Table 1: Changes in food intake, body weight, and absolute and relative liver weights of control and treated rats.

Parameters	Treatment groups			
	Control	Se	CPF	CPF+Se
Initial body weight (g)	152.4±6.76 [a]	151.9±3.67 [a]	152.7±8.81[a]	150.6±4.28 [a]
Final body weight (g)	213.6±11.05 [ab]	220.1±18.27 [a]	184.0±11.77 [c]	201.3±13.37 [b]
Body weight gain (%)	28.5±4.52 [a]	30.7±4.92 [a]	16.9±4.97 [b]	24.9±5.53 [a]
Absolute liver weights (g)	7.24±0.77 [a]	7.63±0.35 [a]	8.99±0.66 [b]	7.66±0.57 [a]
Relative liver weights (g/100g bw)	3.41±0.52 [a]	3.48±0.23 [a]	4.90±0.50 [b]	3.82±0.35 [a]
Food intake (g/day/rat)	29.7±4.89 [a]	31.17±5.98 [a]	21.8±6.01 [b]	26.5±5.75 [ab]

Each value is a mean of 6 rats ± SD; [a, b, c] values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; % of body weight gain = [(final b .w t. – initial b .wt.)/ final b .wt.] X 100. CPF: Chlorpyrifos; Se: Selenium.

The Effects of CPF on General Rat Health

Generally, death was not observed in any of the experimental groups during the treatment period (28 days). However, in CPF-treated group, few clinical signs such as huddling, reduced activity, increasing weakness, slight diarrhea and hair loss were observed. The observed signs were related to the cholinergic crisis; is a consistent sign in organophosphate poisoning. However, except of huddling, no other significant clinical manifestation was observed following Se supplementation. Also, in CPF-treated rats, food intake was reduced by 25.6%. However, supplementation of Se attenuated the reduction in food intake to 10.8% (Table 1).

Evaluation of Body, Absolute and Relative Liver Weights

At the end of the experimental course, there was no significant difference in body, absolute and relative liver weights between Se and untreated rats. However a significant ($p< 0.05$) loss of body weight gain accompanied by a significant increase in the absolute and relative liver weights were recorded in rats treated with CPF compared to the control. The co-administration of selenium with CPF group restored these parameters to non significant difference compared to the control (Table 1).

Enzymatic Antioxidant Status in Liver

In the liver homogenates of CPF-treated rats, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities decreased significantly ($p < 0.05$) by 40.8%, 43.4% and 43.3% in male rats, respectively, when compared to the corresponding controls (Table 2). However, supplementation of Se regenerated SOD and GPx activities and partially ameliorated CAT activity when compared to CPF-group.

Table 2: Liver antioxidant enzyme activities of control and treated rats.

Parameters / Treatments	Control	Se	CPF	CPF+Se
Catalase (CAT) (µmoles H_2O_2 degraded/min/mg protein)	23.13±3.78 [a]	24.34±2.87 [a]	13.69±2.04 [b]	18.25±3.39 [c]
Superoxide dismutase (SOD (units/mg protein)	17.13±2.80 [a]	16.79±1.98 [a]	9.70±1.44 [b]	15.53±2.89 [a]
Glutathione peroxidase (GPx) (nmoles of GSH/min/mg protein)	90.3±15.00 [ab]	96.5±11.37 [a]	51.2±10.78 [c]	79.3±12.86 [b]

Each value is a mean of 6 rats ± SD; [a, b, c] values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; CPF: Chlorpyrifos; Se: Se: Selenium.

Oxidative Stress Biomarkers (LPO and GSH) In Liver

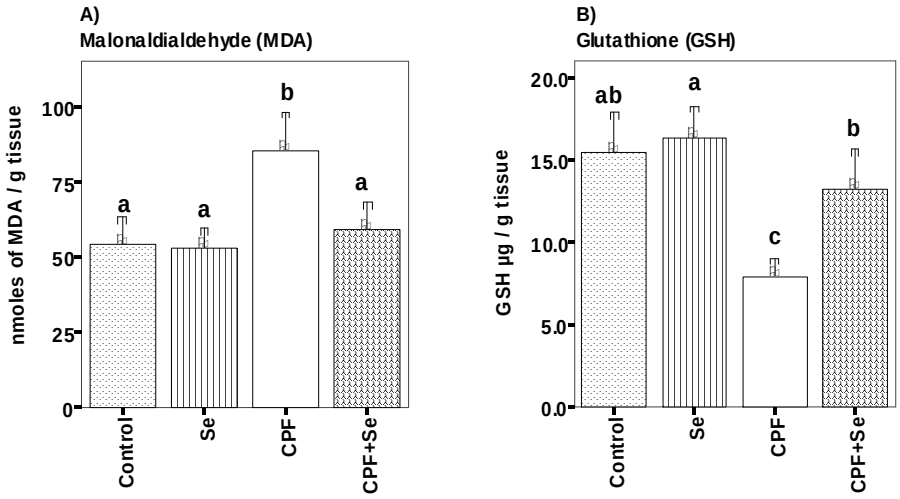

Fig. 1: Effects of selenium (Se) on liver MDA (Fig. 1A) and GSH (Fig. 1B) after CPF treatment.

Data represented as means of 6 rats ± SD; a, b, c columns are not sharing above letters (a, b, c) differ significantly at $p < 0.05$; CPF: Chlorpyrifos; Se: Selenium; MDA: Malonaldialdehyde; GSH: Glutathione reduced.

Administration of CPF led to a significant increment ($p<0.05$) in lipid peroxidation (LPO) as evidenced by the increase in liver MDA levels by +57.3% as compared to the control group. However, co-administration of Se to CPF-treated rats mitigated the augmentation in liver MDA levels (Fig. 1A). Reduced glutathione (GSH), natural antioxidant in liver tissue was significantly reduced in CPF treated group (–48.7%), when compared to the control group. Supplementation of selenium to CPF-treated group restored GSH level (Fig. 1B).

Table 3: Plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes of control and treated rats.

Parameters / Treatments	Control	Se	CPF	CPF+Se
ALT (IU/L)	32.3±6.19 [a]	33.2±4.83 [a]	57.7±8.21 [b]	36.5±5.39 [a]
AST (IU/L)	65.4±11.08 [a]	67.08±10.09 [a]	118.5±17.65 [b]	74.7±11.14 [a]
ALP (IU/L)	113.0±17.87 [a]	121.5±18.28 [a]	168.3±19.22 [b]	131.2±16.25 [a]

Each value is a mean of 6 rats ± SD; [a, b, c] values are not sharing superscripts letters (a, b, c) differ significantly at $p < 0.05$; CPF: Chlorpyrifos; Se: Se: Selenium.

Effects of CPF on Biochemical Markers In Plasma and Liver

The results of enzymes activities of male rats are shown in Table 3. Male rats exposed to CPF (13.5 mg/kg/day) showed a significant increase ($p<0.05$) in the plasma enzyme activities of ALT, AST and ALP levels comparable to control. Se-treated group did not show any significant changes as compared to the control. The treatment of (CPF +Se) decreased the enzymes activity as compared to CPF-treated group. The LDH activity measured as an indicator of necrotic cell death were increased by 89.3% in the plasma (Fig. 2A), but were decreased by –34.8% in the liver (Fig. 2B). Supplementation of Se to the CPF-treated group restored all the parameters cited above.

Histopathological Examination

The histopathological examination of the liver tissue is shown in Fig. 3 and the semiquantitative histological scoring of liver damage is presented in Table 4. Liver sections from the control rats showed normal hepatic lobules formed of hepatocytes radiating from central vein to the periphery of the lobules (Fig. 3A1 and A2). However, only administration of Se showed normal appearance of hepatocytes (Fig. 3D). In contrast, the exposure of rats to CPF induced degenerative changes in the liver organ. CPF caused inflammatory cellular infiltration in between degenerated hepatocytes, kupffer cells proliferation, fatty infiltration and degeneration in hepatocytes (Fig. 3B1, B2, B3 and B4). Co-administration of Se improved the histopathological features (Fig. 3C). The liver of Se+CPF-treated rats showed marked improvement in their histological structure compared to CPF-treated group and represented by nil to mild degree in inflammatory cellular infiltration in between degenerated hepatocytes, kupffer cells proliferation, fatty infiltration and dilatation and congestion of portal and central vein (Table 4).

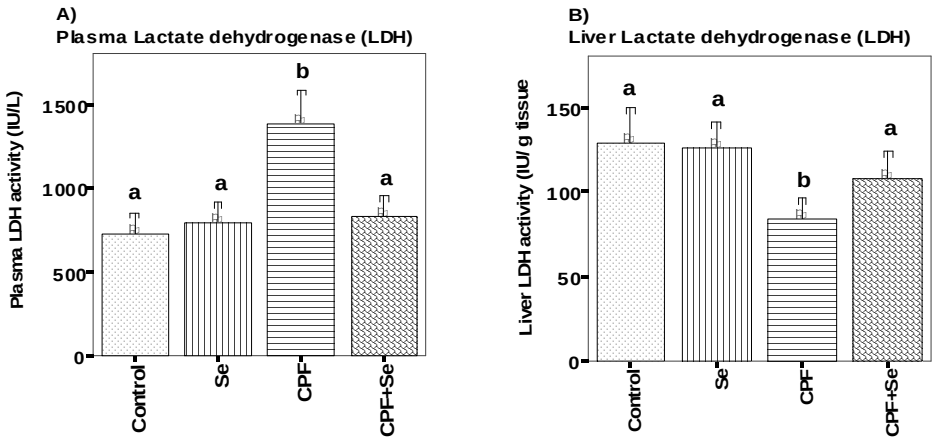

Fig. 2: Lactate dehydrogenase (LDH) levels in plasma (Fig. 2A) and in liver (Fig. 2B) of control and treated rats.

Data represented as means of 6 rats ± SD; a, b columns are not sharing above letters (a, b) differ significantly at $p < 0.05$; CPF: Chlorpyrifos; Se: Selenium; LDH: Lactate dehydrogenase.

Table 4: Semiquantitative scoring of architectural damage on histopathological examination of the rat livers in the different treatment groups.

Treatments	Control	Se	CPF	CPF+Se
Degeneration of hepatocytes	–	–	++++	+
Fatty change in hepatocytes	–	–	++	–
Inflammatory cells infiltration	–	–	+++	+
Diffuse kupffer cells proliferation in between hepatocytes	–	–	+++	+
Dilatation and congestion of central vein	–	–	++	+

CPF: Chlorpyrifos; Se: selenium.

(–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.

DISCUSSION

In toxicological studies, organ and relative organ weights are important criteria for evaluation of organ toxicity [3, 38]. In our study, rats exposed to CPF during 28 days showed a decrease in their body and organ weights. The reduction of daily food consumption in CPF-treated rats supported these findings. On the other hand, the reduction in body weight may be due to the overall increased degradation of lipids and proteins as a result of the direct effects of CPF as an organophosphate compound [6]. Moreover, the increase in liver weight could be attributed to the relationship between liver weight increase and various toxicological effects or to the reduction in body weight gain of experimental animals[3, 39, 40, 41, 42]. These results are consistent with many previous investigators with CPF and other OP compounds [2, 43, 44]. Co-administration of selenium to the CPF-treated group improved body and liver weights, which could be attributed to an increase in daily food consumption. Indeed, previous studies of Navarro-Alarcon and Cabrera-Vique showed an activated growth and development after intake of selenium [45].

Due to the role of liver in detoxification of environmental xenobiotics, it is at great risk of injury and induces hepatotoxicity. The results of the present study indicated that exacerbation of oxidative injury in liver of CPF-treated rats was more than control group as evidenced by elevated MDA levels and reduced GSH content. Indeed, the involvement of oxidative stress following exposure to OP has been reported [3, 43, 44]. Different mechanisms have been postulated to explain CPF-induced liver injury, such as lipid peroxidation and interaction with membrane components. In fact, lipid peroxidation represents one of the most frequent reactions resulting from free radicals' attack on biological structures [46]. GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST [47, 48]. Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation [49]. In this respect, several studies observed depletion of GSH in CPF-treated animals [6, 9]. It was found

that the co-administration of Se in CPF- induced toxicity has protected liver from lipid peroxidation and from any changes in GSH and antioxidant enzymes [50, 51]. This finding could be explained according to Ognjanovic et al.[52] by the important role of Se in preventing hydroxyl radicals' formation and in protecting the integrity and the functions of tissues [53].

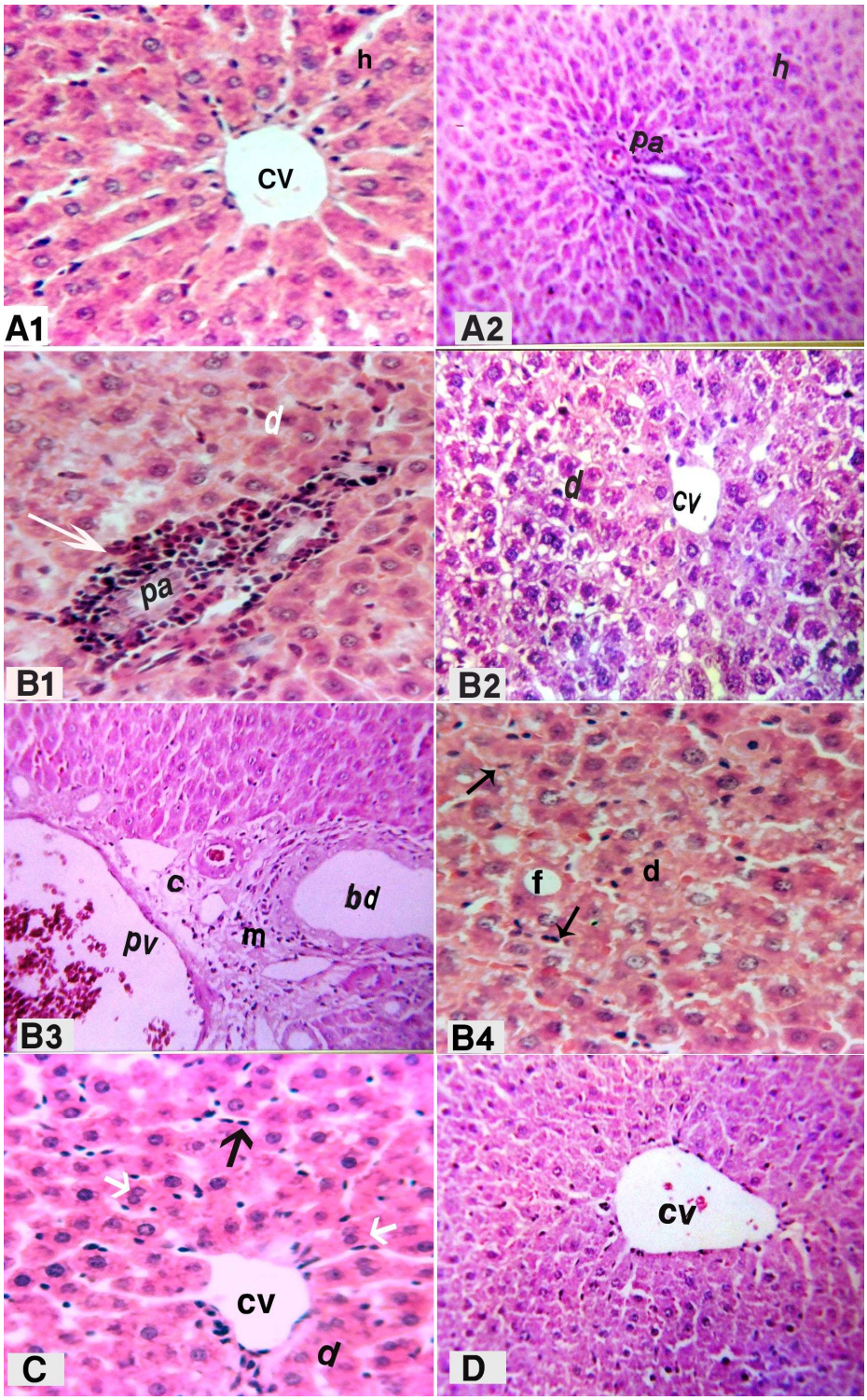

Fig. 3: Photomicrograph of H&E stained sections of liver from control rat showing normal histological structure of the central vein (CV), portal area (pa) surrounding hepatocytes (h) (Fig. 3A1-80x & A2-60x). Chlorpyrifos-treated rat liver showing inflammatory cells infiltration (white arrow) in the portal area (pa) with degenerative changes (d) (Fig. 3B1-80x), degeneration (d) in hepatocytes (Fig. 3B2-80x), , congestion was observed in the portal vein (pv) associated with dilatation in the bile duct (bd), inflammatory cells infiltration (m) and collagen proliferation (c) in the portal area (Fig. 3B3-80x) , fatty changes (f) with diffuse Kupffer cells proliferation (black arrow) in between the degenerated hepatocytes (d) (Fig. 3B4-80×). Se co-administrated with CPF treated rat liver showing binucleated hepatocytes (white arrow), diffuse Kupffer cells proliferation (black arrow) associated with degenerated hepatocytes (d) (Fig. 3C-80x). Sodium selenite (Se) treated rat liver showing normal appearance of hepatocytes as well as central vein (CV) (Fig. 3D-80x).

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. In the current study, CPF induced oxidative damage by producing reactive oxygen species and decreasing the biological activities of some liver antioxidant enzymes, like SOD, CAT and GPx. Our results were in line with previous studies which have shown that exposure to CPF generates lipid peroxidation and alters the antioxidant status of several tissues in rats [24, 41]. On the other hand, the effect of Se on the enhanced GPx activity may be attributed to the increase in the bioavailability of Se following co-treatment with sodium selenite [54]. Whereas, CAT became significantly higher than in control animals, reflecting, most probably, an adaptive response towards free radical damage in the liver, as reported by El Heni et al. [55].

Another biochemical marker used to evaluate liver function was lactate dehydrogenase (LDH) activity. Its activity decreased in liver tissue and increased in plasma in the CPF-treated group. This may be attributed to a generalized increase in membrane permeability, as reported by [56]. Moreover, elevation of LDH activity indicates cell lysis and death as well as the switching over of anaerobic glycolysis to aerobic respiration. LDH can be used as an indicator for cellular damage and cytotoxicity of toxic agents [57]. Besides, the decreased levels of liver LDH activity result from superoxide anions and hydroxyl radicals which cause oxidative damage to the cell membrane [58]. The co-administration of selenium with CPF-treated rats improved LDH activity due probably to its antiradical/antioxidant efficacy.

In addition, ALT, AST and ALP are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity [59, 60]. In our finding, we demonstrated that CPF administrated to rats provoked a marked elevation in plasma AST, ALT and ALP activities which indicating hepatocellular damage as previously reported by El-Demerdash and Kalender et al.[60, 61]. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation [62], indicating a necrosis and inflammatory reactions [63]. Selenium tended to alleviate plasma transaminases and ALP as demonstrated by us and by other studies [60, 64].

The hepatic function tests corroborated the histopathological lesions observed in the present study. Degeneration and necrosis in the hepatocytes, inflammatory cells infiltration, and Kupffer cells proliferation were frequently observed in CPF-treated group. These observations indicated marker changes in the overall histoarchitecture of liver in response to CPF, which could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membrane components of the cell. Our results are supported by other studies conducted on CPF and other OP insecticides [3, 4, 41, 42]. The co-treatment of Se improved the histological alterations induced by CPF, which could be attributed to the antiradical/antioxidant and metal-chelating efficacy of this metal. Moreover, these results are in good accordance with those obtained by other studies which have postulated the beneficial role of Se on histopathological and enzymatic changes of rats [50, 51, 65].

In conclusion, the results of present study showed that CPF induced generation of free radicals that caused oxidative damage to macromolecules and cell membrane accompanied by histopathological alterations. Also, the results of the present study implicated the capability of CPF to induce hepatotoxicity. In contrast Se reduces oxidative stress by virtue of its antioxidant properties thus improving the structural integrity of cell membrane and eventually alleviates the histopathological changes as well as the biochemical perturbations. Based on our present observations, we propose that Se may provide a cushion for prolonged therapeutic option against toxins-induced hepatotoxicity without harmful side effects.

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