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ORIGINAL ARTICLE

The Potential Protective Impact of Selenium on Cyclophosphamide Induced Hepatotoxicity and Nephrotoxicity in Adult Male Albino Rats

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ABSTRACT:

Background: Human exposure to cyclophosphamide (CP) is highly frequent because it is widely used chemotherapeutic and immunosuppressive. CP has potential toxic effects on liver and kidney that make it an issue of concern to public health. Selenium (Se) is an essential dietary element with a high nutritional value, immunomodulatory and antioxidant properties. This study aimed to evaluate the protective role of Se against toxic effects of CP in hepatic and renal tissues of adult male albino rats. **Methods:** The study was carried on 42 adult male albino rats were divided into 6 groups, 7 rats for each group. Group I (Negative control). group II (Positive control): divided into group IIA (Distilled water), group IIB (Normal saline), group III (Selenium), group IV (CP) and group V (CP + Se). The following markers were measured: serum ALT (Alanine aminotransferase), AST(Aspartate transaminase), LDH (Lactate dehydrogenase), Urea and Creatinine. Hepatic and renal tissue MDA (Malondialdehyde), PCC (Protein carbonyl content), catalase, GSH (Reduced glutathione) and IL-6 (Interleukin six). Histopathological and Immunohistochemical staining studies were demonstrated. **Results:** In CP administrated rats, serum ALT , AST, LDH , Urea and Creatinine levels increased. Also, CP induced elevation in hepatic and renal tissues MDA, PCC and IL-6 and a decrease in hepatic and renal tissues catalase and GSH. Histopathology and Immunohistochemical staining showed that: CP induced histological damages and strong immunoreaction decreased by co-treatment of Se. **Conclusion:** Administration of Se improved liver and kidney functions and histology beside improvement in oxidative stress and inflammation caused by CP.

Keywords: Selenium, cyclophosphamide, hepatotoxicity, nephrotoxicity, rats.

INTRODUCTION

The oral active version of the alkylating drug chiormethine, cyclic phosphoramide ester, or cyclophosphamide (CP), was created in 1958. CP is a cytotoxic immunosuppressive drug. It can decrease the generation of lymphokines and alter lymphocyte activity. The cornerstone for both of CP's therapeutic action and poisonous properties is its capacity to disrupt all rapidly replicating tissues **[1].**

Cyclophosphamide is frequently used to treat cancers such as small cell lung, ovary, breast, brain cancers and leukemia as well as autoimmune diseases like Goodpasture syndrome and rheumatoid arthritis. Patients with increasing interstitial lung disease (ILD) related to systemic scleroderma are managed with CP **[2].**

Cyclophosphamide is metabolized by hepatic microsomal cytochrome P450 to two active

metabolites: phosphoramide mustard and acrolein. CP possesses an anti-cancer action that is related to phosphoramide mustard. It is believed that the immunosuppressive and anticancer actions of CP are mediated by phosphoramide mustard, which also inhibits cell division by attaching to deoxyribonucleic Acid (DNA) **[3].**

Also, active metabolite acrolein interferes with the tissue's antioxidant defense system, causing oxidative damage and oxidative toxicity. The oxidative balance is the equilibrium between the generation and elimination of reactive oxygen species (ROS). When this equilibrium is upset, oxidative stress develops, which causes unwelcome difficulties in the biosystem **[3].**

Ijaz et al. [4] demonstrated that CP elevated the level of nuclear factor kappa beta (NF-KB) and some other inflammatory indicators such as tumor necrotizing factor alpha and interleukin six. Cyclophosphamide causes marked distortion of hepatic architecture, liver congestion, inflammation, hepatocyte degeneration and apoptosis **[5].**

Nephrotoxicity is one of the main harmful consequences of CP. It happens either because of CP building up inside the cell or because of the kidney's antioxidant defense system being depleted and ROS being produced. The primary outcome of oxidative stress induced by CP in renal tissue is the generation of inflammatory cytokines. Antioxidants can protect healthy tissues from CP damage **[1].**

A crucial dietary trace element with immune-modulating and antioxidant characteristics is selenium (Se). Many antioxidant enzymes, like glutathione peroxidase and thioredoxin reductase, benefit from the presence of se in their active sites. Immune-modulating impact of Se occurs by boosting T-cell proliferation, natural killer cell activity and inhibiting inflammatory mediator release. It has been proven that Se has a hepato-renal protection against cadmium, methimazole and Methomyl **[6].**

Aim of the work

The aim of this study is to evaluate the protective role of Selenium on toxic effects of Cyclophosphamide on biochemical parameters, histo-pathological and immune-histochemical changes in hepatic and renal tissues of adult male albino rats.

MATERIAL AND METHODS

The study was carried out at Forensic Medicine and Clinical toxicology& Histology and Cell Biology Departments, Faculty of Medicine, Zagazig University. The study extended for four weeks. The study was done on animals according Zagazig University - Institutional animal care and Use committee instruction. The Approval number is (ZU-IACUC/3/F/394/2022).The Animal House of The Faculty of Medicine at Zagazig University provided the rat species.

According to "The Guide for the Care and Use of Laboratory Animals," all animals were cared for in accordance with the Animal Care Guidelines and Ethical Regulations" **[7].** All of the animals appeared healthy before the test. They had a seven-day period of passive preparations to acclimate to their new surroundings, determine their physical well-being, and weed out any unhealthy animals. The animals were kept in regulated environments with an ambient temperature range of 22 ± 2ºC, relative humidity of 50 ± 5% and a 12-hour light-cycle in their own plastic cages, free from any sources of chemical pollution. To keep animals clean and prevent overcrowding and isolation, soft wood shavings were used as bedding and changed when the cages were washed on other days. The rats were fed a balanced diet that was rich in all the nutrients they needed to stay healthy before and during the administration of the medicines. It was made up of milk, bread, and barley. Water was offered in solitary, spotless containers.

42 adult male albino rats with body weight (B.W) 150-200 gm were used in the investigation. Thay were divided equally into 6 groups, 7 rats for each group. The groups were classified as follow:- group I (Negative control group): To assess fundamental parameters, each rat was given a standard diet and tap water group II (Positive control group): this group was divided into group IIA (Distilled water group): rats got 1 ml/kg of distilled water (solvent of Se) once daily by oral gavage. Group IIB (Normal saline group) each rat received 1 ml/kg of normal saline 0.9% NaCl (solvent of CP) once weekly intraperitoneal for 4 weeks (on 7 th &14 th & 21 st & 28 th days)**,** group III (Selenium treated group): Each rat received Se 1 mg/kg body weight B.W dissolved in distilled water once daily by oral gavage for 4 weeks **[8]**, group IV (Cyclophosphamide group): Each rat received 16.4 mg/kg B.W of CP (1/10 LD50)**[9]** dissolved in saline once weekly intraperitoneal for 4 weeks (on 7 th &14 th & 21 st & 28th days) CP dose was once weekly according to **[10]**and

group V (CP + Se group): For four weeks, each rat got the same doses and administration route of CP and Se.

METHODS:

The following was done 24 hours after the final dose at the end of the fourth week: Blood samples were taken from the retro-orbital plexuses of anesthetized rats to estimate various liver and kidney functions, such as: serum (ALT) and (AST) according to **[11]**, (LDH)) according to **[12],** creatinine according to **[13]**and urea according to **[14]**. As soon as possible, the liver and kidneys were painstakingly removed, thoroughly examined, and cleaned of any superfluous tissue. For histological and immunohistochemical analyses, one portion of the liver tissue and the left kidney were promptly fixed in 10% neutral buffered formalin. In order to acquire tissue homogenates for the investigation of oxidative stress indicators, the remaining liver tissue and right kidney were sent on dry ice and kept at -80 °C (MDA according to **[15]**, PCC according to **[16]**, Catalase according to **[17]** and GSH according to **[18]**) and inflammatory marker; Interleukin-6 according to **[19].** Haematoxylin (H) & Eosin (E) stain according to **[20]** and NF-KB immunohistostaining according to **[21].**

STATISTICAL ANALYSIS

The stastical analysis was done by Epi-info stastical package program version 6.04d, January 2001 according to **[22].** One way analysis of variance (ANOVA or F-test) and least significant difference (LSD) were used.

RESULTS

There was no significant difference regarding different laboratory markers among negative control "I", positive control "IIA & IIB" and Selenium " III" groups over the periods of the study (P>0.05) (**Table 1).**

The results of the present study showed a very highly significant increase in the mean values of serum ALT, AST, LDH in CP group in comparison with the control group. The results also showed a very highly significant decrease in the mean values of these enzymes in CP+ Se group when compared to CP group, but there was a very highly significant increase in in the mean values of serum ALT, AST, LDH in CP+ Se group when compared to the negative control group (P< 0.001). As regard serum kidney function tests (Urea and Creatinine), the results of the present study showed a very highly significant increase in the mean values of urea and creatinine in CP group when compared to the control group. The results also showed a very highly significant decrease in the mean values of urea and creatinine in CP+ Se group when compared to CP group, but there was a very highly significant increase in in the mean values of urea and creatinine in CP+ Se group when compared to the negative control group (P< 0.001). (**Table 2 &Table 3).**

As regard hepatic and renal tissue oxidative markers MDA and PCC, the results of the present study showed a very highly significant increase in their mean values in CP group when compared to the control group. The results of the present study showed a very highly significant decrease in the mean values of catalase and GSH in CP group when compared to the control group. The results also showed a very highly significant decrease in the mean values of MDA and PCC levels and showed a very highly significant increase in the mean value of catalase and GSH in CP+ Se group when compared to CP group The results also showed a highly significant increase in the mean value of MDA and PCC in CP+ Se group when compared to the negative control group (P< 0.01) and a highly significant decrease in the mean value of catalase and GSH in CP+ Se group when compared to control group (p< 0.01) in hepatic tissue. The results also showed a very highly significant increase in the mean value of MDA and PCC in CP+ Se group when compared to the negative control group and a very highly significant decrease in the mean value of catalase and GSH in CP+ Se group when compared to control group (p< 0.001) in renal tissue (**Table 2& Table 3).**

As regard hepatic and renal tissue IL-6, the results of the present study showed a very highly significant increase in the mean value of IL-6 in CP group when compared to the control group. The results also showed a very highly significant decrease in the mean value of IL-6 in CP+ Se group when compared to CP group, but there was a very highly significant increase in the mean values of urea and creatinine in CP+ Se group when compared to the negative control group (P< 0.001). (**Table 2 &Table 3).**

Histopathological results:

Light microscopic examination in both hepatic and renal tissues by hematoxylin & eosin stain (H&E.)

In the present study, histopathological findings of hepatic tissues in rats in control and Se groups showed normal structure **(Figure 1-1)**. While in CP group, the liver showed loss of the normal

organization of the hepatic lobules. Some hepatocytes showed dark stained pyknotic nuclei with pale cytoplasm while others showed vacuolated cytoplasm with dilated congested central and portal veins, dilated sinusoids and proliferation of bile duct (**Figure 1-2)**. The results of the present study revealed that rats which received Se+ CP showed apparent improvement of the histological organization of the hepatic lobules with apparent normal central vein. (**Figure 1-3).**

Histopathological findings of renal tissues in rats in control groups and Se treated groups showed normal structure **(Figure 2-1)**. While in CP group revealed hydropic degeneration with cytoplasmic vacuolation, necrosis of glomerulus and tubular epithelium with irregular bowman capsule, inflammatory cells infiltration and dilatation of tubules with acidophilic cast **(Figure 2-2)**. The results of the present study revealed that rats which received Se+ CP showed preservation of kidney structure with decreased interstitial infiltration and vacuolization **(Figure 2-3).**

Immunohistochemical staining using nuclear factor kappa beta (NF-KB):

Hepatic Immunohistochemical results: Immunohistochemical stained liver sections for detection of nuclear factor kappa beta (NF-KB) from negative "I" , positive control "IIA & IIB" and Se " III" groups showed negative immunoreaction **(figure 3A)**. Immunohistochemical stained liver sections for detection of nuclear factor kappa beta (NF-KB) from CP group "IV" showed strong positive immunoreaction **(figure 3B)**. Immunohistochemical stained liver sections for detection of nuclear factor kappa beta (NF-KB) from CP + Se group "V" showed weak positive immunoreaction **(figure 3C).**

Renal Immunohistochemical results: Immunohistochemical stained kidney sections for detection of nuclear factor kappa beta (NF-KB) from negative "I" , positive control "IIA & IIB" and Se " III" groups showed negative immunoreaction **(figure 4A)**. Immunohistochemical stained kidney sections for detection of nuclear factor kappa beta (NF-KB) from CP group "IV" showed strong positive immunoreaction **(figure 4B).** Immunohistochemical stained kidney sections for detection of nuclear factor kappa beta (NF-KB) from CP + Se group "V" showed weak positive immunoreaction **(figure 4C)**.

Statistical analysis of the area percent of NF-KB immune-expression:

There was a very highly significant difference between the different groups in area percent of NF-KB immunoreaction ($P< 0.001$). (**Table 2)**

By using LSD to find relations between groups, there was very highly significant increase in the mean value of CP group when compared to the control group in liver ($P< 0.001$). The results also showed a significant decrease in the mean value of the area percent of NF-KB immune-expression in CP+ Se group when compared to CP group in liver ($P< 0.05$).The results also showed a very highly significant increase in the mean values of the area percent of NF-KB immune-expression in CP+ Se group when compared to the control group in liver ($P< 0.001$). **(Table 3).**

As regard the kidney, there was very highly significant increase in the mean value of CP group when compared to the control group in kidney ($P< 0.001$), while there was a very highly significant decrease in the mean value of the area percent of NF-KB immune-expression in CP+ Se group when compared to CP group in kidney ($P< 0.001$). Also, there was a highly significant increase in the mean values of the area percent of NF-KB immune-expression in CP+ Se group when compared to the control group in liver ($P< 0.01$) in kidney **(Table 3).**

Table (1): Statistical comparison among negative control, positive control "IIA & IIB" and Selenium " III" groups as regard mean values of different laboratory markers using One-Way ANOVA after 4 weeks**.**

Group(n=7) / Parameter	Group I (-ve control)	Group IIA (Distilled water group)	Group IIB (Normal saline group)	Group III (Se treated group)	F	P-value
Mean ±SD						
ALT (U/L)	31.52 ± 2.05	31.97 ±1.94	31.92 ± 1.8	32.57 ± 2.99	0.262	0.852
AST (U/L)	42.3 ± 1.81	42.41 ± 1.7	42.06 ± 2.05	41.2 ± 2.72	0.467	0.708
LDH (U/L)	170.74±2.22	171.44±1.33	171.47±2.61	172.8 ± 2.02	1.175	0.34
Urea (mg-dl)	15.37± 0.92	15.87±0.69	15.67±0.7	15.8±0.98	0.491	0.692
Creatinine (mg-dl)	0.62 ± 0.037	0.64±0.091	0.64 ± 0.031	0.64 ± 0.034	0.471	0.705
Hepatic tissue MDA (nmol/mg)	0.52 ± 0.04	0.51 ± 0.05	0.53 ± 0.07	0.53 ± 0.09	0.192	0.901
Hepatic tissue PCC (nmol/mg)	0.77 ± 0.11	0.76 ± 0.12	0.75 ± 0.1	0.75 ± 0.1	0.04	0.987
Hepatic tissue Catalase (ng/mg)	15 ± 1.3	15.1 ± 0.93	15.3 ± 1.2	15.8 ± 0.83	0.692	0.566
Hepatic tissue GSH (U/mg)	178.4 ± 1.5	177.4 ± 2.1	177.7 ± 1.95	178.8 ± 1.7	0.91	0.451
Renal tissue MDA (nmol/mg)	1.37 ± 0.1	1.36 ± 0.08	1.37 ± 0.05	1.34 ± 0.05	0.178	0.904
Renal tissue PCC (nmol/mg)	0.92 ± 0.08	0.91 ± 0.04	0.89 ± 0.08	0.91 ± 0.07	0.182	0.907
Renal tissue Catalase (ng/mg)	18.33 ± 0.91	18.49 ± 0.83	18.3 ± 0.74	18.73± 0.67	0.429	0.734
Renal tissue GSH (U/mg)	139.51±1.92	139.11 ± 1.6	139.23±1.39	140.1± 1.26	0.666	0.581
Hepatic tissue IL-6 (Pg/mg)	95.76 ± 2.52	95.81 ± 1.81	96.1 ± 0.63	95.1 ± 1.15	0.454	0.717
Renal tissue IL-6 (Pg/mg)	75.98 ± 2.02	75.86 ± 1.89	75.72 ± 1.81	75.23 ± 1.46	0.225	0.878

Data are presented as mean ± SD (standard deviation) and range

-ve: negative Se : Selenium ALT: Alanine transaminase

AST: Aspartate aminotransferase LDH: Lactate dehydrogenase (U/L): Unit / Liter

MDA: Malondialdehyde PCC: Protein carbonyl content IL-6 : Interleukin six

GSH: Reduced glutathione content nmol/mg : Nanomole/milligram U/mg : Unit/milligram

(mg/dl): Milligram/Deciliter ng/mg : Nanogram/milligram Pg/mg :Picogram/milligram

n: number of rats in each group F: ANOVA (analysis of variance) test.

P: level of significance (> 0.05): No significant difference

Table (2): Statistical comparison of the mean values of different laboratory markers and area percent of NF-KB immune-expression among the negative control, CP and CP+ Se groups using One-Way ANOVA test after 4 weeks.

Group (n=7) / Parameter	Group I (-ve control)	Group IV (CP group)	Group V (CP+ Se group)	F	P-value
Mean ±SD					
ALT (U/L)	31.52 ± 2.05	81.34 ± 3.08	40.34 ± 1.85	865.78	<0.001**
AST (U/L)	42.3 ± 1.81	113.29 ± 2.93	50.52 ± 2	1995.54	<0.001**
LDH (U/L)	170.74 ± 2.22	363.71 ± 3.67	194.69 ± 1.6	11099.39	<0.001**
Urea (mg-dl)	15.37± 0.92	46.54 ± 1.55	19.59 ± 0.96	1444.67	<0.001**
Creatinine (mg-dl)	0.62 ± 0.037	1.4 ± 0.04	0.79 ± 0.07	465.206	<0.001**
Hepatic tissue MDA (nmol/mg)	0.52 ± 0.04	4.29 ± 0.65	1.36 ± 0.46	129.29	<0.001**
Hepatic tissue PCC (nmol/mg)	0.77 ± 0.11	4.03 ± 0.12	1 ± 0.08	2111.43	<0.001**
Hepatic tissue Catalase (ng/mg)	15 ± 1.3	8.43 ± 1.47	12.55 ± 1.49	38.15	<0.001**
Hepatic tissue GSH (U/mg)	178.4 ± 1.5	95.43 ± 0.73	176.23 ± 1.09	12231.76	<0.001**
Renal tissue MDA (nmol/mg)	1.37 ± 0.1	8.32 ± 0.64	3.64 ± 0.48	409.82	<0.001**
Renal tissue PCC (nmol/mg)	0.92 ± 0.08	3.82 ± 0.16	1.61 ± 0.09	1204.95	<0.001**
Renal tissue Catalase (ng/mg)	18.33 ± 0.91	10.23± 0.92	15.08 ± 0.87	143.91	<0.001**
Renal tissue GSH (U/mg)	139.5± 1.92	70.1± 1.27	131.5± 1.84	3489.194	<0.001**
Hepatic tissue IL-6 (Pg/mg)	95.76 ± 2.52	388.9± 2.94	142.2± 2.59	24029.20	<0.001**
Renal tissue IL-6 (Pg/mg)	75.98 ± 2.02	343.4± 3.59	121.7± 2.45	18719054	<0.001**
Area percent of Hepatic NF-KB Immunoreaction	2 ± 1	13 ± 1	7.6 ± 1.53	68.385	<0.001**
Area percent of Renal NF-KB Immunoreaction	2.67 ± 0.56	39.76 ± 1.53	8 ± 1	981.909	<0.001**

Data are presented as mean ± SD (standard deviation) and range
CP: Cyclophosphamide
-ve: negative
Se : Selenium
ALT: Alanine transaminase
AST: Aspartate aminotransferase
LDH: Lactate dehydrogenase
(U/L): Unit / Liter
MDA: Malondialdehyde
PCC: Protein carbonyl content
IL-6 : Interleukin six
GSH: Reduced glutathione content
nmol/mg : Nanomole/milligram
U/mg : Unit/milligram
(mg/dl): Milligram/Deciliter
ng/mg : Nanogram/milligram
Pg/mg :Picogram/milligram
NF-KB: Nuclear factor kappa beta
n: number of rats in each group
F: ANOVA (analysis of variance) test.
P: level of significance ** very highly significant (P<0.001)

Table (3): Least significance difference (LSD) comparison of the mean values of different laboratory markers and area percent of NF-KB immune-expression among the negative control, CP and CP+ Se groups using Post-Hoc test after 4 weeks.

Parameters	Groups	Group IV (CP)	Group V (CP+Se)
ALT	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
AST	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
LDH	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
Urea	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
Creatinine	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
(Hepatic) MDA	Group I (-ve) Control	<0.001**	0.003*
	Group IV (CP)		<0.001**
(Hepatic) PCC	Group I (-ve) Control	<0.001**	0.001*
	Group IV (CP)		<0.001**
(Hepatic) Catalase	Group I (-ve) Control	<0.001**	0.005*
	Group IV (CP)		<0.001**
(Hepatic) GSH	Group I (-ve) Control	<0.001**	0.002*
	Group IV (CP)		<0.001**
(Renal) MDA	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
(Renal) PCC	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
(Renal) Catalase	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
(Renal) GSH	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
Hepatic IL-6	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
Renal IL-6	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		<0.001**
Area percent of Hepatic NF-KB Immunoreaction	Group I (-ve) Control	<0.001**	<0.001**
	Group IV (CP)		0.035
Area percent of Renal NF-KB Immunoreaction	Group I (-ve) Control	<0.001**	0.001*
	Group IV (CP)		<0.001**

**: statistically very highly significant (P<0.001). *: statistically highly significant (P<0.01). statistically significant (P<0.05). -ve: negative

ALT: Alanine transaminase AST: Aspartate aminotransferase

LDH: Lactate dehydrogenase IL-6 : Interleukin six

MDA: Malondialdehyde PCC: Protein carbonyl content

GSH: Glutathione content NF-KB: Nuclear factor kappa beta

CP: cyclophosphamide Se: Selenium

Figure (1-1): A photomicrograph of a section in liver tissue of control adult albino rats (Groups I, II and III) showing:(A) Hepatocytes contain large pale nuclei, abundant cytoplasm (arrow) radiating from the central vein (CV). They are separated by blood sinusoids (S).(B)The portal area has normal appearance of bile duct (D), hepatic artery (A) and portal vein (V). **(H & E x 400)**

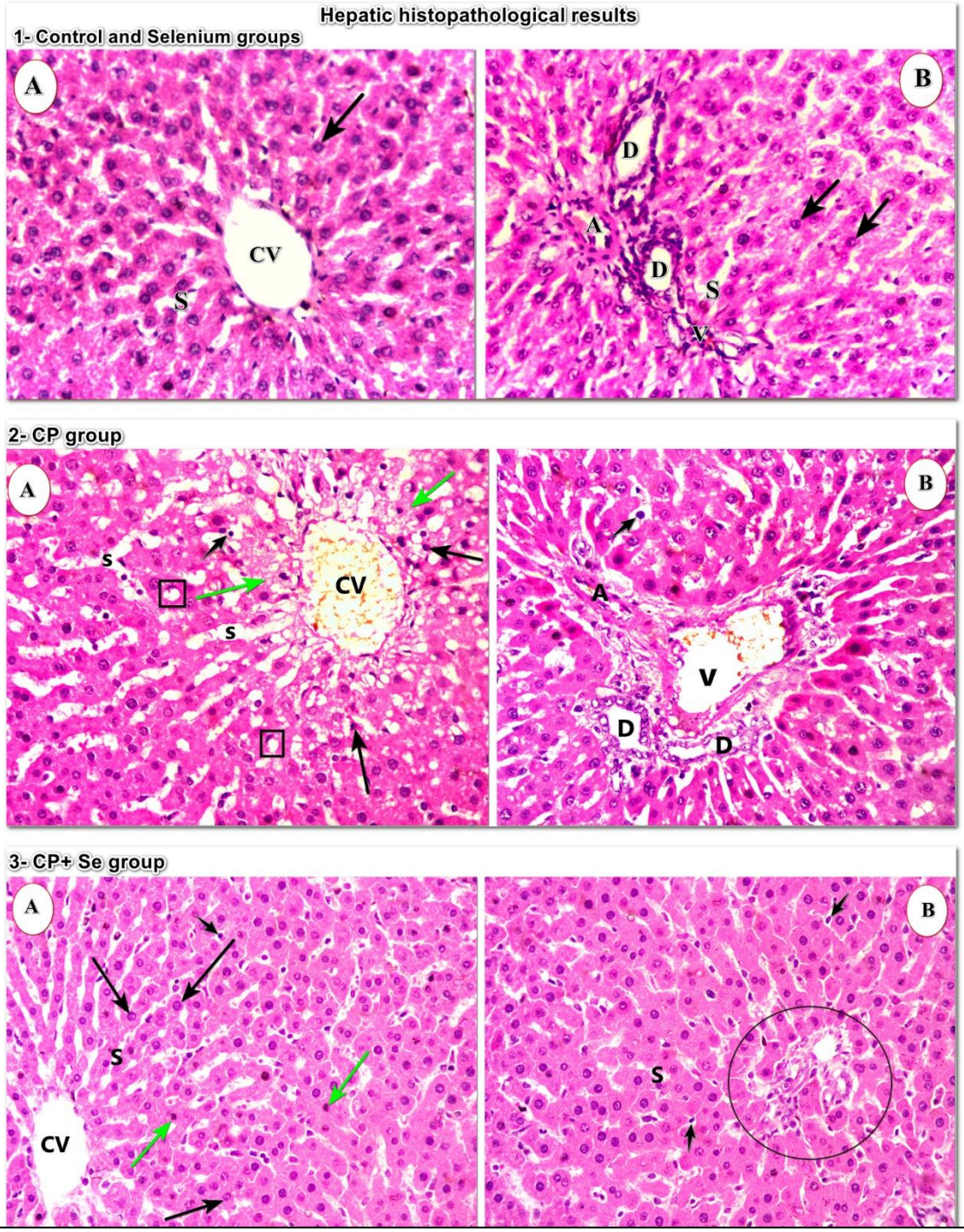

Figure (1-2): A photomicrograph of a section in liver tissue of CP administrated adult albino rat (Group IV) showing: **(A)** Congested central vein (cv), hepatocytes with dark stained pyknotic nuclei (arrow) and vacuolated cytoplasm (green arrow). Fatty infiltration (square) and dilated sinusoids in between (S) are detected. Notice Von Kupfer cells (arrowhead). **(B)** Proliferation of bile duct (D) and congested portal vein (V) are observed. **(H & E x**

Figure (1-3): A photomicrograph of a section in liver tissue of CP +Se administrated adult albino rat (Group V) showing: **(A)** Apparent improvement of the histological organization of the hepatic cords with apparent normal central vein (CV).Some hepatocytes have pale nuclei (arrow) while the others have pyknotic nuclei (green arrow). Some dilated blood sinusoids (S) are also seen. **(B)** Apparent normal portal area (Circle). Notice Kupfer cells infiltration (arrowhead). **(H & E x 400).**

Figure (2-1): A photomicrograph of a section in kidney tissue of control adult albino rats (Groups I, II and III) showing:(**A**) normal glomeruli with parietal layer (black arrow), visceral layer (green arrow) and normal urinary space (*). **(B)** normal tubules with normal epithelial lining (arrowhead). **(H & E x 400).**

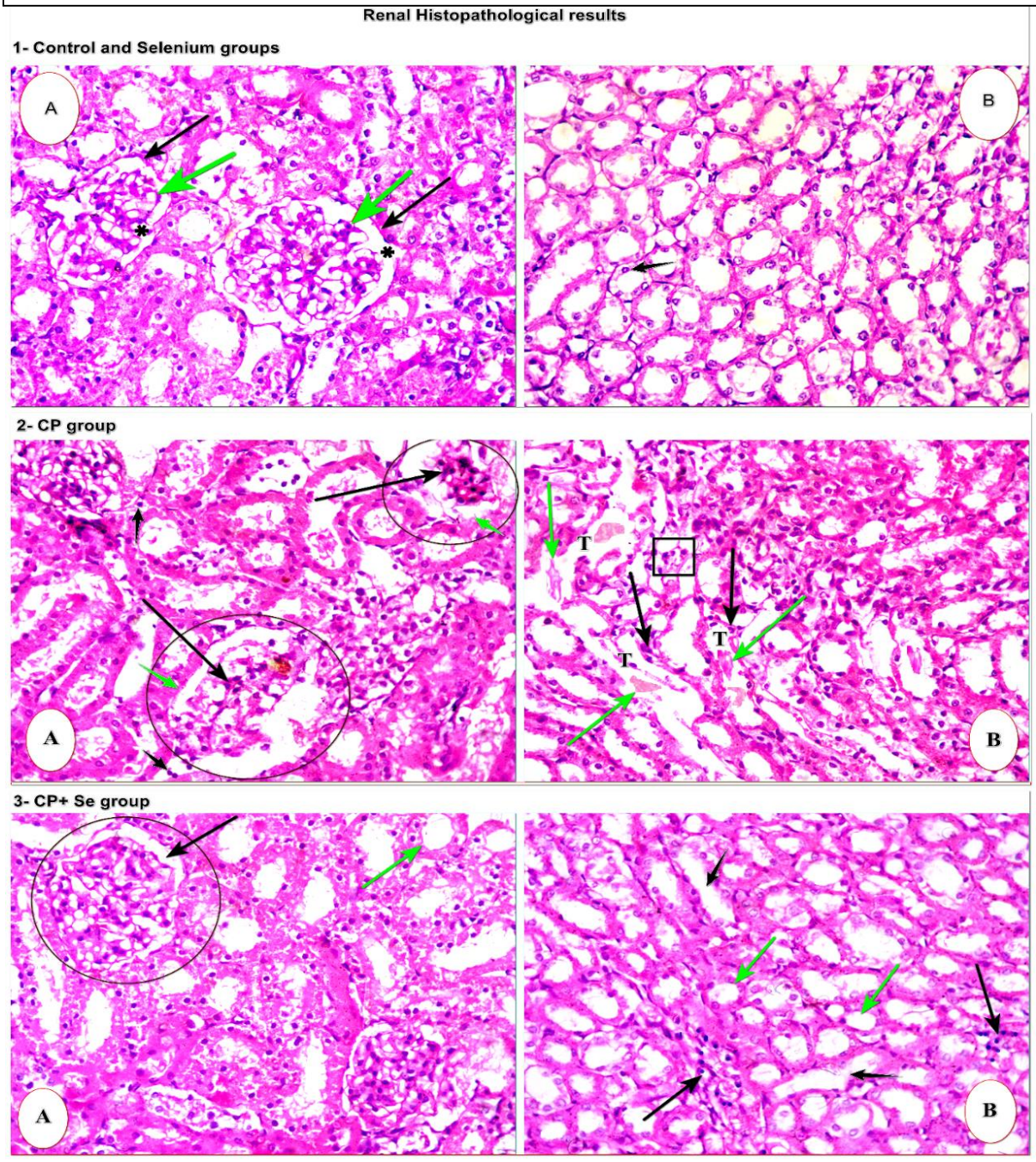

Figure (2-2): A photomicrograph of a section in kidney tissue of CP administrated adult albino rat (Group IV) showing: **(A)** Disorganized glomerulus (circle) with pyknotic nuclei (black arrow) and irregular bowman capsule (green head arrow). Notice cellular infiltration (black head arrow). **(B)** Tubular dilatation (T) with pyknotic tubular nuclei (arrow), and cytoplasmic vacuolation (square) are detected. Some dilated tubules have acidophilic cast (green arrow).
(H & E x 400).

Figure (2-3): A photomicrograph of a section in kidney tissue of CP +Se administrated adult albino rat (Group V) showing: **(A)** apparent normal glomerular architecture (circle) with apparent normal bowman space (arrow) and rearranging of normal tubular (green arrow). **(B)** Cellular infiltration appears among the tubules (Arrow). Some tubules are organized (green arrow) and others are still disorganized (arrowhead).
(H&E X 400).

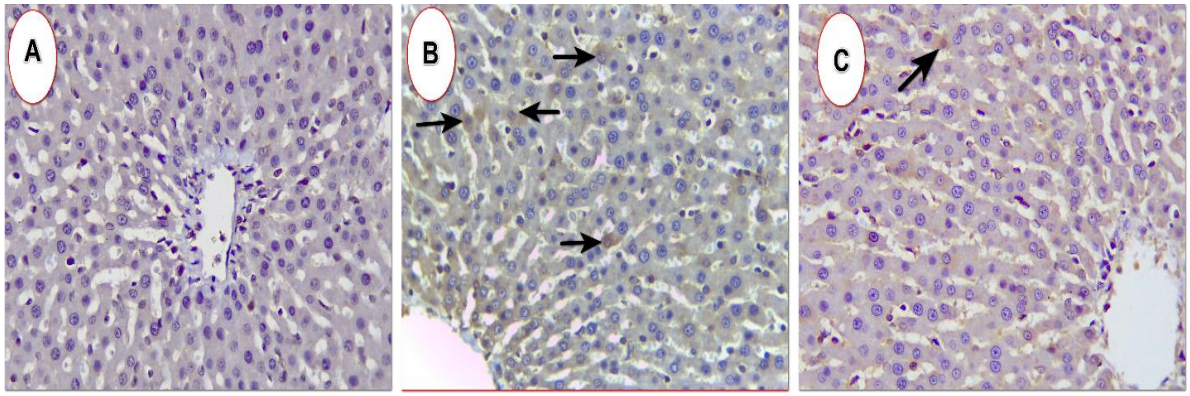

Figure 3: (A): A photomicrograph of a section in liver tissue of control adult albino rats (Groups I, II and III) showing negative immunoreaction for NF-KB. **(B):** A photomicrograph of a section in liver tissue of CP administered adult albino rat (Group IV) showing strong positive immunoreaction for NF-KB (Arrow). **(C) :** photomicrograph of a section in liver tissue of CP + Se administered adult albino rat (Group V) showing weak positive immunoreaction for NF-KB (Arrow). (**X 400)**

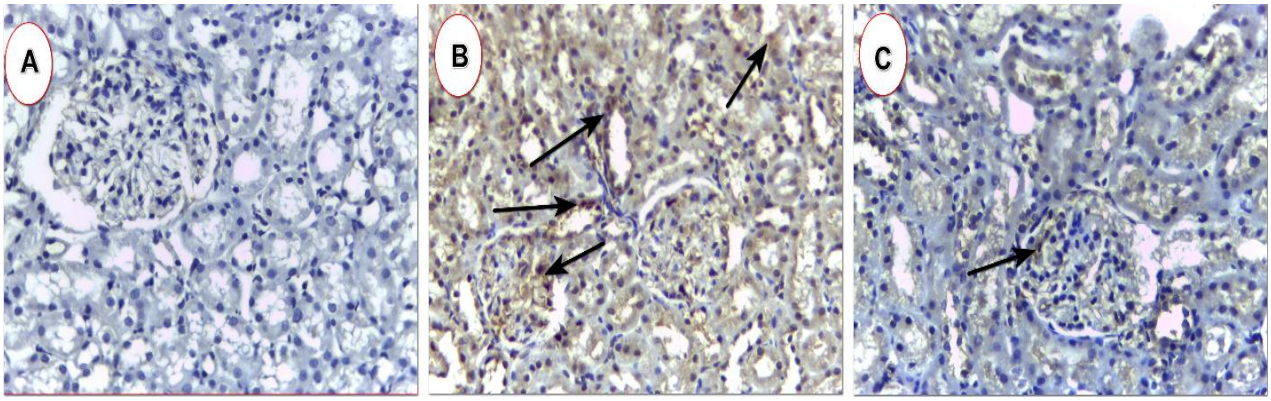

Figure 4: (A): A photomicrograph of a section in kidney tissue of control adult albino rats (Groups I, II and III) showing negative immunoreaction for NF-KB. **(B):** A photomicrograph of a section in kidney tissue of CP administered adult albino rat (Group IV) showing strong positive immunoreaction for NF-KB (Arrow). **(C):** photomicrograph of a section in kidney tissue of CP + Se administered adult albino rat (Group V) showing weak positive immunoreaction for NF-KB (Arrow).(**X 400)**

DISCUSSION:

The results of the present study showed a highly significant increase in the mean values of ALT, AST and LDH in CP group when compared to the control group. Additionally, the CP+ Se group's mean values for these enzymes decreased significantly when compared to the CP group, according to the data.

Similarly, **Althunibat et al. [23] & Cengiz et al. [24]** observed that serum ALT & AST and LDH levels were noticeably greater in the CP group compared to the control group, indicating liver injury in mice given CP 30 mg/kg for 10 days. In the same line **Li et al. [25]** found that Se could improve the elevated ALT, AST increased by CP administration.

While **Abraham et al. [26]** There was no noticeable difference in the levels of liver enzymes six and sixteen hours later CP administration with dose 150 mg/kg in adult femaleWistar rats weighing 200–250g, this can be explained by the short period of the study as 6 six hours not enough for adequate damage of liver cells and increasing the enzyme levels.

Elevated serum levels of these enzymes indicates liver damage that can be mediated by reactive oxygen species produced during drug metabolism, accumulation of lipid peroxidation and reactive oxygen species inhibits mitochondrial activity. Biological membrane disruption, creation of an immune response, and inflammation are among liver damage processes also.**[27].**

There was a highly significant rise in urea and creatinine in the CP group compared to the control group. Additionally, when comparing the CP+ Se group to the CP group, the results revealed a highly significant decline in the mean value of urea and creatinine.

The findings of the present investigation are consistent with **Ijaz et al. [4] & Uyumlu et al. [28],** who discovered a substantial rise in urea and creatinine levels in rats exposed to CP.

Similarly, **Alaqeel et al. [29],** determined that the expected considerable loss in renal function was brought on by CP treatment. This result is a classic indicator of nephrotoxicity brought on by CP.

In addition, **Alshahrani et al. [30],** discovered that, compared to the healthy control group, the blood urea nitrogen (BUN) and creatinine content considerably increased in the CP rats given a single 150 mg/kg injection of the drug.

However, **Subramaniam et al. [31]** found that single dose of intravenous CP 100 mg/ day in forty-eight years old male patient can cause acute hepatitis and increase in serum aminotransferase level but without marked increase in urea and creatinine serum level. Serum urea was 6.6 mmol/L (normal range 2.5–6.4) Serum creatinine was 116 umol/L (normal range 62–106). This difference may be due to shorter period of administration and the small dose as it equals 1.43 mg/kg in adult male with average weight 70kg which is considered a low dose in comparison with 16.4 mg/kg dose we used in rats.

Elevated Impaired renal function is indicated by elevated levels of urea and creatinine. The CP derivative acrolein activates the formation of nitric oxide and peroxy-nitrite, which are both responsible for the cell death brought on by intracellular ROS in the renal tissue. **[32].**

As regard hepatic and renal tissue oxidative markers MDA and PCC According to the study's findings, the mean value increased significantly in the CP group compared to the control group. In addition, the catalase and GSH levels in the CP group were significantly lower than those in the control group.

Additionally, the results demonstrated a highly significant decline in the mean value of MDA and PCC levels and a highly significant rise in the mean value of catalase and GSH in the CP+ Se group compared to the CP group.

Similarly, **Rezaei et al. [33] & Abraham et al. [34]** detected a comparable rise in MDA levels and a fall in GSH levels in the liver tissues after CP exposure. **Hamzeh et al. [35]** found a highly significant increase in MDA and PCC and a highly significant decrease in GSH level in rat hepatic tissue when injected by CP intraperitoneal.

In the line of the present experiment, **Qian et al. [36]** reported a comparable increase in MDA and decrease in catalase levels in hepatic tissues on CP exposure.

Hu et al. [37] showed a considerable increase in MDA level and a fall in catalase and GSH levels in renal tissue of rats intraperitoneally injected with CP once weekly. **Goudarzi et al. [38]** showed the same change in MDA, catalase and GSH levels with highly significant increase in PCC level in renal tissue in mice injected with CP.

Bokhary et al. [39] & Hasan et al. [40] investigations support the findings of the current study, which revealed a considerable rise in oxidative stress markers (MDA and PCC) and a significant decrease in the antioxidant enzymes (CAT and GSH) levels in both hepatic and renal tissue. In the same way, **Li et al. [25]** observed Se treatment could significantly inhibit CP induced changes in the MDA level, glutathione peroxidase and catalase.

Dwivedi and Jena [5] demonstrated that hepatocytes require more adenosine triphosphate (ATP) to carry out a few activities than other cells. Hepatocytes have comparatively more mitochondria which makes them vulnerable to oxidative stress-mediated toxic damage.

In contrast with our study **Merwid-Ląd et al. [41]** showed that injection of CP for consecutive 10 days in mice led to non-significant decrease in both GSH and catalase levels in hepatic tissue and significant drop in MDA level in hepatic and renal tissue. , they explained that by daily dosing of CP in sequence may exert protective effect against tissue peroxidation through decreasing neutrophil accumulation.

As regard hepatic and renal tissue IL-6, according to the study's findings, the mean level of IL-6 in the CP group increased significantly more than it did in the control group. Additionally, the CP+ Se group's mean value of IL-6 decreased significantly when compared to the CP group, according to the results.

In addition, **Alruhaimi [42]** found that CP in mice caused the amount of IL-6 in hepatic tissue to rise noticeably. **Hu et al. [24]** showed a dramatic increase in IL-6 level in rats intraperitoneally injected with CP once weekly in renal tissue.

Mahmoud et al. [43] and El-Kholy et al. [44] found that CP administration in rats caused NF-KB activation Pro-inflammatory cytokines such tumor necrosis factor-alpha (TNF-alpha) and

IL-6 were produced in response to oxidative stress, injuring the hepatic tissue.

In the present study, histopathological findings of hepatic tissues in rats in control and Se Groups displayed typical organization. The liver in the CP group displayed a lack of the hepatic lobules' usual structure. Several hepatocytes had dark-stained pyknotic lesions nuclei with pale cytoplasm while others showed vacuolated cytoplasm with dilated congested central and portal veins, dilated sinusoids and proliferation of bile duct.

These results are in line with **Yadav et al. [45]** who discovered that rats given CP showed severe damage to their livers, including numerous apoptotic cells and steatosis. Transparent void vacuoles in the cytoplasm of hepatocytes were used to describe them. Despite the modest central vein congestion, the central vein's enlargement was closely scrutinized. Intense sinusoidal dilatation, bile duct hyperplasia, and inflammatory cell infiltration were all subsequent effects of CP therapy.

Mostafa et al. [46] found that injection of CP to rats led to observable vacuolation of hepatocyte cytoplasm, hepatocyte enlargement around the central vein, and occasional cell necrosis.

In contrast to present study results, **Bhat et al. [47]** found that 100 mg/kg single dose CP administration for one week in Swiss albino mice only caused mild peri-venular infiltration. The hepatocyte and sinusoids appeared normal.

As regard CP+ Se group, the present study revealed improvement of the histological organization of the hepatic lobules with apparent normal central vein. Hepatocytes were radiating from the central vein, they had vesicular nuclei, some of them were binucleated and others had pyknotic nuclei with vacuolated cytoplasm with less dilated congested blood sinusoids and the portal area showed apparent normal structure.

To explain this improvement **Chen et al. [48]** reported that selenoproteins reduced liver damage by decreasing protein expressions in the NF-kB/IkB pathway and increased the antioxidant capacity and inflammatory response of the liver in rats suffering from heatstroke.

In the present study, histopathological findings of renal tissues in rats in control groups and Se treated groups showed normal structure while in CP group revealed hydropic degeneration with cytoplasmic vacuolation, necrosis of glomerulus and tubular epithelium with irregular bowman capsule, inflammatory cells infiltration and dilatation of tubules with acidophilic cast.

These changes run in parallel with the results of **Hu et al. [37] & Obaid et al. [49] and Mohamed et al. [50],** who reported marked histopathological changes in the kidneys indicated by atrophy of most glomeruli, with widening in bowman's space. The tubules showed degenerative change including cellular swelling and vacuolated cytoplasm. There was prominent dilatation in tubules with eosinophilic luminal hyaline cast material and interstitial bleeding with inflammatory cell infiltration.

In addition, **Uyumlu et al. [28]** detected CP-induced nephrotoxic changes including glomerular degeneration, tubular cell shedding, swelling of tubule cells, mononuclear cell infiltration, edema and hemorrhage. **Małyszko et al. [51]** highlighted that one of the primary causes of significant morbidity in cancer survivors is dose-related CP-induced nephrotoxicity.

The findings of the current investigation demonstrated that rats given Se displayed preservation of kidney structure with decreased interstitial infiltration and vacuolization.

The nephro-protective effects of Se has been previously described in animal models against mycotoxin-induced nephrotoxicity in rats and cisplatin carbon tetrachloride **Liu et al. [52]; Boutellaa et al. [53] and He et al. [54].** Also, **Bjørklund et al. [55]** detected that Se has a protective effect against heavy metal induced kidney aging process. Mercury, Selenite and selenomethionine are the major Se compounds that can bind to and detoxify lead and cadmium.

Boutellaa et al. [53] revealed that Se's role in cell membrane biosynthesis may be the cause of the nephro-protective actions of Se in rats against Carbon Tetrachloride nephrotoxicity.

Immunohistochemical examination of liver and kidney samples from the group of CP rats used in this investigation, showed strong positive reaction to NF-KB. These findings of the present study coincides with **Rezaei et al. [33],** who declared that CP exposure induced positive immunoreactivity of NF-KB in liver cells. Administration of Se was successful in lowering the positive cells in CP-treated animals. Aside from that **Althunibat et al. [23]** detected that CP administration increased NF-KB expression and gives positive immunoreaction in mice.

Ahmad et al. [56] & Mohamed et al. [50] revealed a positive immunoreaction for NF-KB renal tissue in rats injected with CP with significant elevation in its expression.

Considerable improvement in the immunoreactions was gained using Se with CP. These findings run in parallel with **Eddie-Amadi et al. [57]** who proved that Important trace

elements (zinc, Se, and zinc+Se combination) decreased hepatorenal injury by demonstrating anti-inflammatory and anti-apoptotic actions in female albino rats exposed to a heavy metal mixture that caused hepatorenal toxicity by blocking the NF-KB pathway.

CONCLUSION

Cyclophosphamide administration resulted in toxic effects significantly elevated liver and renal function tests indicate an effect on the liver and kidney. Hepatic and renal tissues both underwent histological and immunohistochemical alterations as a result of CP induced toxicity by inducing oxidative stress that was evident through significant increase in oxidants, significant decrease in antioxidants and induction of inflammation. Administration of Se produced improvement of liver and kidney functions and histology beside improvement in oxidative stress and inflammation caused by CP in liver and kidney tissue. Se also improved the histological alternation caused by CP and decreased NF-KB expression in liver and kidney.

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