SELENIUM COULD ALLEVIATE POTASSIUM DICHROMATE-INDUCED EPIDIDYMAL, PROSTATIC AND SEMINAL VESICLE CHANGES IN ADULT RATS: POTENTIAL ROLE OF INHIBITING NF-KB PATHWAY

BY

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ABSTRACT

Background: Chromium is generated as a waste product from different industries. Its reproductive toxicity has been addressed in many studies that considered oxidative stress the main contributing factor for such toxicity. Selenium is essential for male reproductive health and crucially involved in regulation of cellular redox status. **Aim:** To elucidate the potential impacts of potassium dichromate (PDC) on the structure of the epididymis, prostate and seminal vesicles and to investigate the efficacy of selenium on counteracting these impacts. **Methods:** Thirty-six adult male rats were assigned into four groups: control, selenium, PDC, and PDC+selenium. The animals were subjected for a treatment period of four weeks. At the end of experimental period, blood and semen samples, epididymides, prostates and seminal vesicles were collected and processed for biochemical, morphological and immunohistochemical analyses. **Results:** PDC administration significantly deteriorated sperm parameters and triggered alteration of cellular redox homeostasis, evidenced by increased serum malonaldehyde levels with decreased enzymatic activity of the antioxidants: superoxide dismutase and catalase. Furthermore, PDC significantly unregulated the expression of the serum inflammatory markers; nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha and interleuken-1beta. Microscopically, all the examined tissues of PDC-treated rats displayed deteriorated microarchitecture as well as elevated 8-hydroxy-2-deoxyguanosine and microtubule-associated protein light chain3 immunoexpression, indicating increased oxidative DNA damage and autophagy activity. Selenium supplements to PDC-treated rats effectively alleviated all the tested parameters. **Conclusion:** Selenium supplements could effectively mitigate PDC-induced damage of the epididymis, prostate and seminal vesicles through counteracting oxidative stress, and reducing NF-κB activation and excessive autophagy evoked by PDC.

Key Words*: selenium, chromium, reprotoxicity, NF-κB , autophagy.*

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INTRODUCTION

The increasing prevalence of male infertility has aroused a serious global health challenge ***(Dobrakowski et al., 2018)***. In recent decades, environmental toxicants have gained interest as main factors for the escalating trend of male infertility ***(Kumar and Singh, 2022)***. Chromium is a common heavy metal naturally occurring in soil, plants, animals and gases ***(Pandey and Madhuri, 2014)***. It exists mainly in two valence states: trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) ***(Genchi et al., 2021)***.Although Cr(III) is essential for human and animal diet, with little or no toxicity, its hexavalent form Cr(VI) is 100 to 1000 times more toxic ***(Stoecker, 2004)***. Cr(VI) is commonly used in many industries including stainless steel and textile manufacturing, painting, welding and chrome plating ***(Sharma et al., 2022)***.

Occupational exposure to Cr(VI) is found among nearly half a million industrial workers worldwide ***(Pereira et al., 2021)***. Also, water contaminated with Cr(VI) represents a world-wide problem, as it is the major route of chromium exposure for the general population ***(Sharma et al., 2022)***.

Potassium dichromate (PDC) is a soluble Cr(VI) compound widely used in several industries **(Almukhtar et al., 2016)**. PDC was reported to induce reproductive toxicity in male rats ***(Bashandy et al., 2021)***. Furthermore, reduction of the index weights of the testes, epididymides, prostates and

seminal vesicles were detected in Cr(VI)-treated rats ***(Abd Elhafeez et al., 2019)***. Many mechanisms have been reported to explain chromium toxicity, including mainly overproduction of free radical species ***(Bashandy et al., 2021)***.

Furthermore, inflammation and autophagy induction have recently been proposed as other toxicity mechanisms of different heavy metals ***(Balali-Mood et al., 2021; Hu et al., 2022).*** Autophagy is an essential degradation and recycling process of damaged cellular organelles and proteins ***(Pickles et al., 2018)***. Although physiological autophagy levels are necessary for maintaining the stability of the intracellular environment, uncontrollable and excessive autophagy may induce autophagy-dependent cell death ***(Liu and Levine, 2015)***. During autophagy process, damaged organelles and proteins are enveloped into bilayered membranes forming autophagosomes, which then fuse with lysosomes for degradation ***(Niture et al., 2021)***. Autophagosome formation is regulated by many autophagy-related proteins, including LC3, which is considered a specific marker for autophagosomes and autophagy ***(Schläfli et al., 2015)***.

Selenium is an essential micronutrient involved in normal gonadal development, gametogenesis, and fertilization ***(Mirone et al., 2014)***. Selenium supplements at dosages of 200 µg daily were found to improve semen parameters in infertility cases ***(Safarinejad and Safarinejad, 2009)***. Selenium, in the form of selenoproteins, serves as an antioxidant that protects against reactive oxygen species (ROS) ***(Tinggi, 2008)***. Earlier evidence confirmed the positive impact of selenium on Cr(VI)-induced hepatotoxicity ***(Soudani et al., 2011a)***, neurotoxicity ***(Soudani et al., 2012)*** and nephroxicity ***(Wan et al., 2017)***.

AIM OF THE WORK

Based on the above literature, the current study was designed to elucidate the possible beneficial effect of selenium on structural damage induced by PDC on the epididymis, prostate and seminal vesicles, and to investigate the underlying mechanistic role of oxidative stress, NF-κB modulation and autophagic responses.

MATERIALS AND METHODS

1. Materials

1.1. Chemicals: PDC was procured from (El Gomhoria Company for Chemical and Medical Trading, Egypt) and selenium in form of sodium selenite was bought from (Sigma-Aldrich chemical com., USA).

1.2. Experimental animals:

Thirty-six adult male albino rats (weighing 150-200 g) were obtained from Animal house of Faculty of Medicine, Zagazig University. All the rats were subjected to 14 days of passive preliminaries to be adapted to their new environment and to ascertain their physical wellbeing. They were kept in separate well ventilated wide polypropylene cages with stainless steel tops and wood shavings for bedding, under standard conditions, with free access to the standard diet and water ad libitum. Temperature was maintained at 23±2°C. All rats received gentle care in compliance with Ethical Committee of Zagazig University and in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Zagazig University (ZU-IACUC) with no (ZU-IACUC/3/F/329/2022), Egypt.

Methods

1. Experimental design:

After acclimatization for two weeks, the rats were allocated equally into four groups. Each group contained nine rats as follow:

Control group: rats received only regular diet and tap water for 28 days, Se group: each rat was gavaged orally with sodium selenite (0.25mg/kg b.w.) dissolved in water once daily for 28 days, PDC group: each rat was given PDC (700 ppm equivalent to 67mg/kg b.w., orally) dissolved in water daily for 28 days, and PDC+Se group: received both PDC (67mg/kg) and sodium selenite (0.25mg/kg) once daily for 28 days.

Dose regimen of PDC in our research was selected to mimic levels of Cr(VI) encountered in industrial areas ***(Soudani et al., 2011b)***, taking into account that oral route is the major source of Cr(VI) exposure for the general *population* ***(WHO, 2020)***. Based on ***Zhou et al. (2017)*** study, the current selenium

dose represents adequate selenium nutrition for male rats. According to the OECD for testing of chemicals ***(OECD, 2008)***, 28 days of oral dosing of chemicals in rodents can provide information on reproductive organ toxicity.

At the end of experimental period, the animals were anaesthetized with intraperitoneal injection of 40 mg/kg sodium thiopental ***(Mohamed et al., 2020)***.Then, blood samples were collected in clean dry screw-capped tubes from the retro-orbital plexus for biochemical investigations. The animals were then sacrificed and midline abdominal incisions were done to get the epididymides, prostates and seminal vesicles. The left epididymides were immersed at 37° C in phosphate buffered saline (PBS) for the investigating sperm motility, count, and abnormality. The right epididymides, prostates and seminal vesicles were processed for light microscopic examination.

2. Characteristics of sperms:

The left epididymis was divided into small portions and placed in 3 ml of warm PBS to create a sperm suspension, which was then incubated for 10 minutes to allow the sperm to disperse into the buffered solution. Sperm motility evaluation was done by counting both motile and non-motile spermatozoa in the same field. The suspension was filtered, and the number of spermatozoa (million/1 ml) was calculated by passing a predetermined volume of the suspension through Neubauer's counting chamber hemocytometer ***(Narayana et al., 2005)***. Under a light microscope, a piece of the filtrate was stained with 1% eosin to determine the total quantity of aberrant sperms ***(Wyrobek and Bruce, 1975)***. The percentages of aberrant shaped sperms were calculated.

3. Biochemical analysis

Serum contents of CAT (catalase), MDA and SOD (superoxide dismutase) were assayed by colorimetric methods according to ***(Hugo and Lester, 1984; Nishikimi et al, 1972; Ohkawa et al., 1979)***, using available kits from (Biodiagnostic Company, Dokki, Giza, Egypt).

Serum levels of NF-κB, tumor necrosis factor-alpha (TNF-α) and interleuken-1beta (IL-1β) were estimated by the commercial ELISA kits (Ray Biotech, CUSABIO, United States) according to the manufacturer's instructions.

4. Histological Study:

For hematoxylin and eosin (H&E) staining, the specimens were fixed in formal saline for 12 hours to prepare paraffin blocks. 5 µm-thick sections were prepared, stained by H&E ***(Suvarna et al., 2013)*** and examined by light microscope LEICA DM500 at the Anatomy Department, Faculty of Medicine, Zagazig University, Egypt.

5. Immunohistochemical Study:

For immunohistochemical staining with 8-hydroxy-2-deoxyguanosine (8-OHdG, a marker for oxidative DNA damage) and microtubule-associated protein light chain3 (LC3, an autophagy marker), tissue sections were deparaffinized and rehydrated, then treated with 30% hydrogen peroxide for 30min to suppress endogenous peroxidase activity. Then, the slides were washed with PBS and treated with 5% normal goat serum at 37^{o}C for 20min to block non-specific binding sites. Next, the slides were incubated at 4^{o}C overnight with anti-8-OHdG polyclonal rabbit antibody (1:200 dilution, bs-1278R, Bioss, USA) and anti-LC3 polyclonal rabbit antibody (1:200 dilution, bs-8878R, Bioss, USA), according to the manufacturer's instructions, followed by incubation with a goat anti-rabbit secondary antibody. Finally, the slides were stained with diaminobenzidine and counterstained with hematoxylin ***(Ramos-Vara et al., 2008)***.

6. Morphometrical assessment

The image analysis was performed (Leica Q 500 MC program) at Anatomy and Embryology department, Faculty of Medicine, Zagazig university, to measure the following parameters, in ten non-overlapping different fields from each slide,at 400 magnification:

- The epithelial heights of the epididymal, prostatic and seminal vesicle tissues, and thickness of tunica mucosa of seminal vesicles (in H&E-stained sections).
- The optical density (OD) of 8-OHdG immuoexpression and the area percentage (%) of LC3 expression in immunostained

epididymal, prostatic and seminal vesicle sections.

STATISTICAL ANALYSIS

The biochemical and morphometrical measurements were statistically analyzed using SPSS 25 (statistical program for social science, version 25). Mean ± Standard deviation were used for descriptive data. The one-way analysis of variance (ANOVA) with post-hoc test was used to statistically evaluate the results obtained from control and treated groups. P value< 0.05 was considered significant statistically.

RESULTS

1. Characterization of Sperm

PDC group displayed significantly decreased sperm count and motility with increased total aberrant sperm shapes (tail and head) compared to other study groups. Co-administration of selenium with PDC caused a significant increase in sperm count and motility with significant decrease in deformed sperm shapes compared with PDC group **(Table1)**.

2. Biochemical results

PDC group revealed significantly higher serum MDA levels and lower serum CAT and SOD activity in relation to other groups. Co-administration of selenium with PDC caused a significant decrease in serum MDA levels associated with significant increase in serum CAT and SOD activity compared with PDC toxicity group **(Table2)**. Furthermore, PDC group exhibited significantly elevated serum inflammatory markers NF-κB, TNF-α and IL-1β in relation to other experimental groups. Co-administration of selenium with PDC caused a significant decrement in the previous markers compared with PDC group **(Table 3)**.

3. Histopathological results:

Light microscopic findings of both control and Se groups were comparable with no observable differences in all examined tissues. Thereby, they were represented as the control group in the figures.

3.1. Epididymis

Examination of the epididymal tissue of the control group revealed numerous circular epididymal tubules separated by thin interstitial connective tissue. The tubules had wide lumina filled with sperms, and were lined by pseudostratified epithelium formed of principal, basal and clear cells **(Fig. 1A, B)**. PDC group exhibited deformed epididymal tubules displaying hyperplasia and vacuolization of the epithelial lining with reduction of characteristic stereocilia. Furthermore, Halo cells appeared as small rounded cells with central rounded dark nuclei and pale cytoplasm. The interstitial spaces showed deposition of hyaline material, inflammatory infiltration, congested blood vessels and thickened smooth muscle layer **(Fig. 1C-F)**. PDC+Se group revealed obvious improvement of the epididymal histoarchitecture compared with PDC group **(Fig. 1G, H)**.

3.2. Prostate gland

Examination of H&E stained sections of the control group revealed normal architecture of the prostate gland. It was composed of variable shaped and sized adhered prostatic acini filled with homogenous acidophilic prostatic secretions. The glandular acinar epithelium was formed of simple cuboidal or columnar cells. Epithelial infoldings were observed in few prostatic acini **(Fig. 2A, B)**. In PDC group, the prostate gland exhibited apparent disturbance of the histological structure of both acini and stroma. Some acini revealed stratification and hyperplasia of the epithelial folds where the cells appeared with darkly stained nuclei and vacuolated cytoplasm. While other acini showed noticeable thinning of their epithelial lining. Furthermore, a number of prostatic acini demonstrated inflammatory infiltration inside their lumina. The prostatic secretions, in the majority of acini, were scanty and vacuolated, even some acini revealed complete absence of secretions. Regarding the fibromuscular stroma, a variety of lesions were observed; hyaline plaques, inflammatory infiltrates, congested dilated blood vessels and stromal hyperplasia were obvious in various sections **(Fig. 2C-H)**. On the other hand, relative preservation of the majority of the acinar and stromal structures was detected in PDC+Se group. Most of the acini retained their size and were lined with cuboidal epithelium with

little papillary folds; however, vacuolated epithelial cells and deeply stained nuclei were still noticed. The prostatic secretions were comparable to those of the control group. In addition, the fibromuscular stroma between the prostatic acini showed profound decrease in size and density relative to PDC group **(Fig. 2I, J)**.

3.3. Seminal vesicle

The seminal vesicles of the control group revealed folded tunica mucosa lined by pseudostratified columnar epithelium characterized by foamy eosinophilic cytoplasm and ovoid vesicular nuclei. Seminal secretions occupied the lumen of the gland **(Fig. 3A, B)**. The seminal glands of PDC group displayed remarkable thickening of the tunica mucosa and hyperplastic epithelial nodules within tunica muscularis. The hyperplastic epithelium revealed vacuolated cytoplasm and different shapes of nuclei; some nuclei appeared hyperchromatic with prominent nucleoli while others had darkly stained nuclei **(Fig. 3C, D)**. In PDC+Se group, the epithelium showed reduction of thickness and was nearly similar to normal. Most of the epithelial cells appeared similar to control except some still had vacuolated cytoplasm **(Fig. 3E, F)**.

4. Immunohistochemical Results:

4.1. Epididymis

Morphometric analysis of the OD of 8-OHdG and area % of LC3 epididymal expression revealed statistically significant increment in PDC group compared to both control and Se groups. Nonetheless, PDC+Se group showed significantly reduced 8-OHdG and LC3 expression in the epididymal tissue relative to PDC group **(Figs. 4, 5)**.

4.2. Prostate gland

PDC group exhibited significantly higher expression of the OD of 8-OHdG and area % of LC3 in the glandular prostatic epithelium compared to control and Se groups. On the other hand, PDC+Se group displayed significant improvement of the above mentioned parameters **(Figs. 6, 7)**.

4.3. Seminal vesicle

Significantly higher 8-OHdG OD and LC3 area % were recorded in the seminal vesicles of PDC group relative to both control and Se groups. Compared to PDC group, PDC+Se group revealed significant decrement of both parameters in the seminal vesicles **(Figs.8, 9)**.

5. Morphometric Results

PDC group displayed significantly increased epithelial heights in the epididymis, prostate and seminal vesicles compared to both control and Se groups, nevertheless PDC+Se group demonstrated a significant improvement of the aforementioned parameters relative to PDC group. Moreover, the thickness of tunica mucosa in the seminal vesicles was significantly increased in PDC group relative to control and Se groups. Compared to PDC group, there was a significant reduction in tunica mucosa thickness in PDC+Se group. The tested morphometric parameters in all examined tissues demonstrated insignificant differences between control and Se groups **(Table 4)**.

Table (1): Effect of selenium administration on sperm count, motility and aberrant sperm shapes induced by PDC toxicity in male rats. Analyzed Data are presented as the mean±standard deviation (n =9).

Parameter	Control group	Se group	PDC group	PDC+Se group
Sperm count (per epididymis × 10^6)	28.22±1.02	28.98±0.97	13.51±0.85[ab]	24.05±2.47[c]
Sperm motility %	87.75±3.79	88.75±3.87	34.94±3.28 [ab]	70.79±3.72[c]
Total aberrant sperm shapes %	5.25±1.05	4.827±0.98	18.57±2.97[ab]	8.32±2.50[c]

[a] *P <0.05 vs control group;* [b] *P <0.05 vs Se group;*
[c] *P < 0.05 vs PDC group (Se, selenium; PDC, potassium dichromate)*

Table (2): Effect of selenium administration on serum levels of CAT, MDA and SOD activity in PDC toxicity in male rats. Analyzed Data are presented as the mean ± standard deviation (n =9).

Parameter	Control group	Se group	PDC group	PDC+Se group
CAT (U/ml)	187.21±5.1	186.51±7.14	99.66±5.07[ab]	158.31±13.96[c]
MDA (mmol/ml)	23.41±3.12	22.78±2.96	51.07±2.90[ab]	36.16±5.88[c]
SOD (U/ml)	26.82±0.9	25.8±0.2	11.02±0.8 [ab]	20.91±0.4[c]

[a] P <0.05 vs control group; [b] P <0.05 vs Se group; [c] P < 0.05 vs PDC group. (Se, selenium; PDC, potassium dichromate; CAT, catalase, MDA; malondialdehyde; SOD, superoxide dismutase)

Table (3): Effect of selenium administration on serum levels of NF-κB, TNF-α and IL-1β in PDC toxicity in male rats. Analyzed Data are presented as the mean ±standard deviation (n =9).

Parameter	Control group	Se group	PDC group	PDC+Se group
NF-κB (ng/ml)	2.2±0.1	2.1±0.2	6.7±0.5 [ab]	3.0±0.4[c]
TNF-α (pg/ml)	6.2±0.3	5.9±0.1	8.2±0.7 [ab]	6.5±0.2[c]
IL-1β (pg/ml)	366±20.9	365.5±15.8	901.5±38.2 [ab]	410±20.9[c]

[a] P <0.05 vs control group; [b] P <0.05 vs Se group; [c] P < 0.05 vs PDC group (Se, selenium; PDC; potassium dichromate, nuclear factor-kappa B, NF-κB; tumor necrosis factor-alpha, TNF-α; interleukin-1 beta,IL-1β)

Table (4): Effects of selenium on PDC-induced morphometric insults of the epididymal, prostatic and seminal vesicle epithelial heights, and thickness of tunica mucosa of seminal vesicles. Data are represented as mean ±standard deviation (n=9)

Parameter	Control group	Se group	PDC group	PDC+Se group
Epididymal epithelial height (µm)	10.46±2.238	9.435±1.551	28.41±10.69[ab]	16.73±3.915[c]
Prostatic epithelial height (µm)	9.460±1.752	9.482±2.218	21.84±10.44 [ab]	13.68±2.681[c]
Epithelial height of seminal vesicle (µm)	17.00±4.087	18.12±3.183	22.63±3.354 [ab]	18.06±5.298
Tunica mucosa thickness (µm)	44.27± 16.59	42.88± 9.074	204.4± 13.77 [ab]	88.65±28.76 [c]

[a] P <0.05 vs control group; [b] P <0.05 vs Se group; [c] P < 0.05 vs PDC group (Se, selenium; PDC, potassium dichromate)

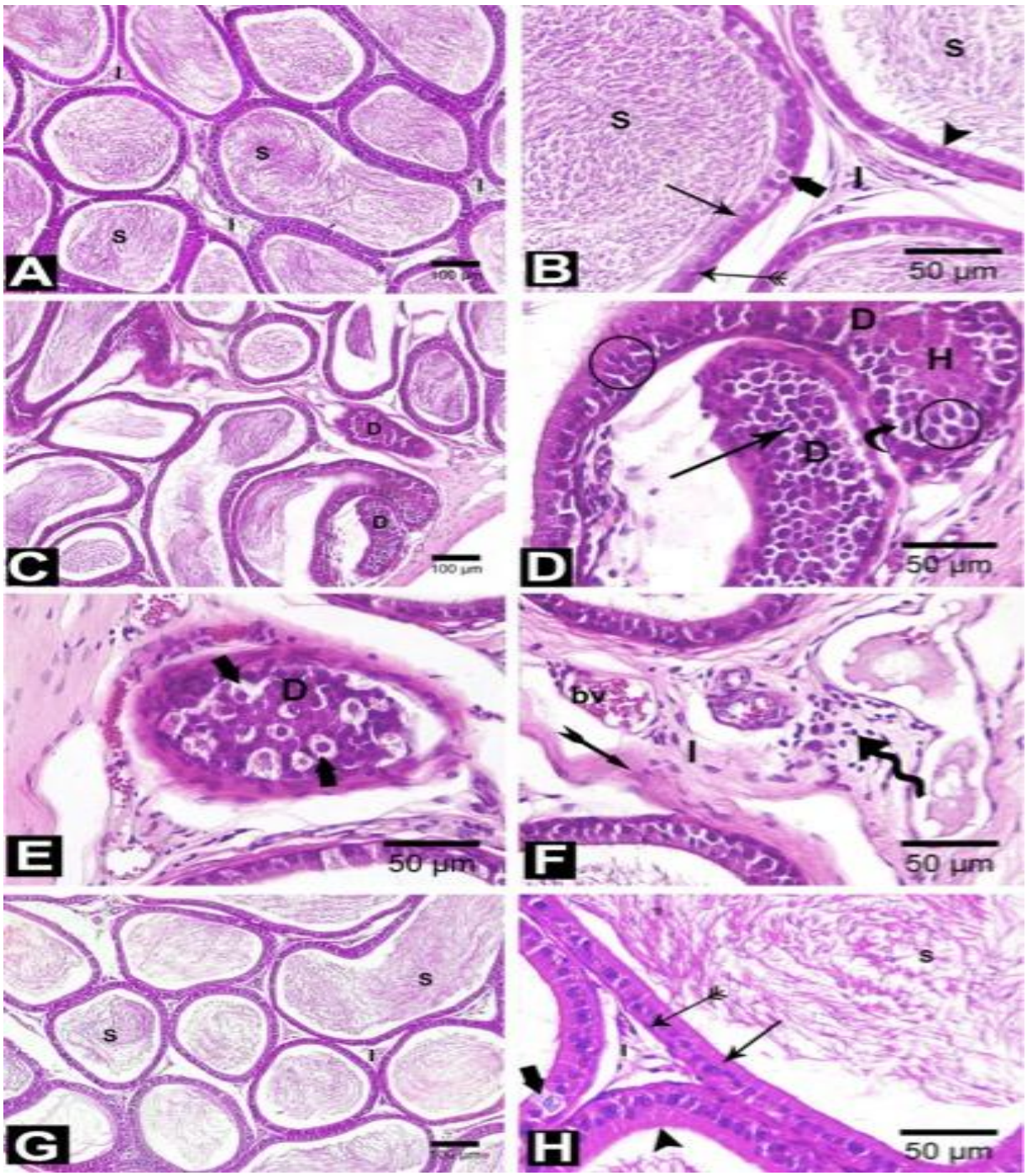

Fig. (1): *Photomicrographs of H&E-stained corpus epididymis sections of different experimental groups.* ***A, B:*** *Epididymal tubules of the control group containing numerous spermatozoa (S) into their lumina, and separated by narrow interstitial tissue (I). The tubules are lined by pseudostratified epithelial lining, composed of principal cells (arrow) with stereocilia (arrowhead), basal cells (double bifid tailed arrow) and clear cells (short thick arrow).****C-F:*** *PDC group demonstrating deformed epididymal tubules (D) with hyperplastic epithelial lining (circle), darkly stained nuclei (arrow), luminal hyaline material deposition (H), cellular vacuolization (curved arrow) and numerous Halo cells (short thick arrow). Wide interstitial spaces (I) revealing inflammatory infiltration (zigzag arrow), congested blood vessels (bv) and thickened smooth muscle layer (bifid tail arrow) are also observable.* ***G, H:*** *PDC+Se group displaying pseudostratified epithelial lining with stereocilia (arrowhead), principal cells (arrow), basal cells (double bifid tailed arrow) and clear cells (short thick arrow). Numerous luminal spermatozoa (S) and less wide interstitial spaces (I) are also evident.* **(H&E, A, C, G X100; Scale bar = 100µm; B, D, E, F, H X400; Scale bar = 50µm)**

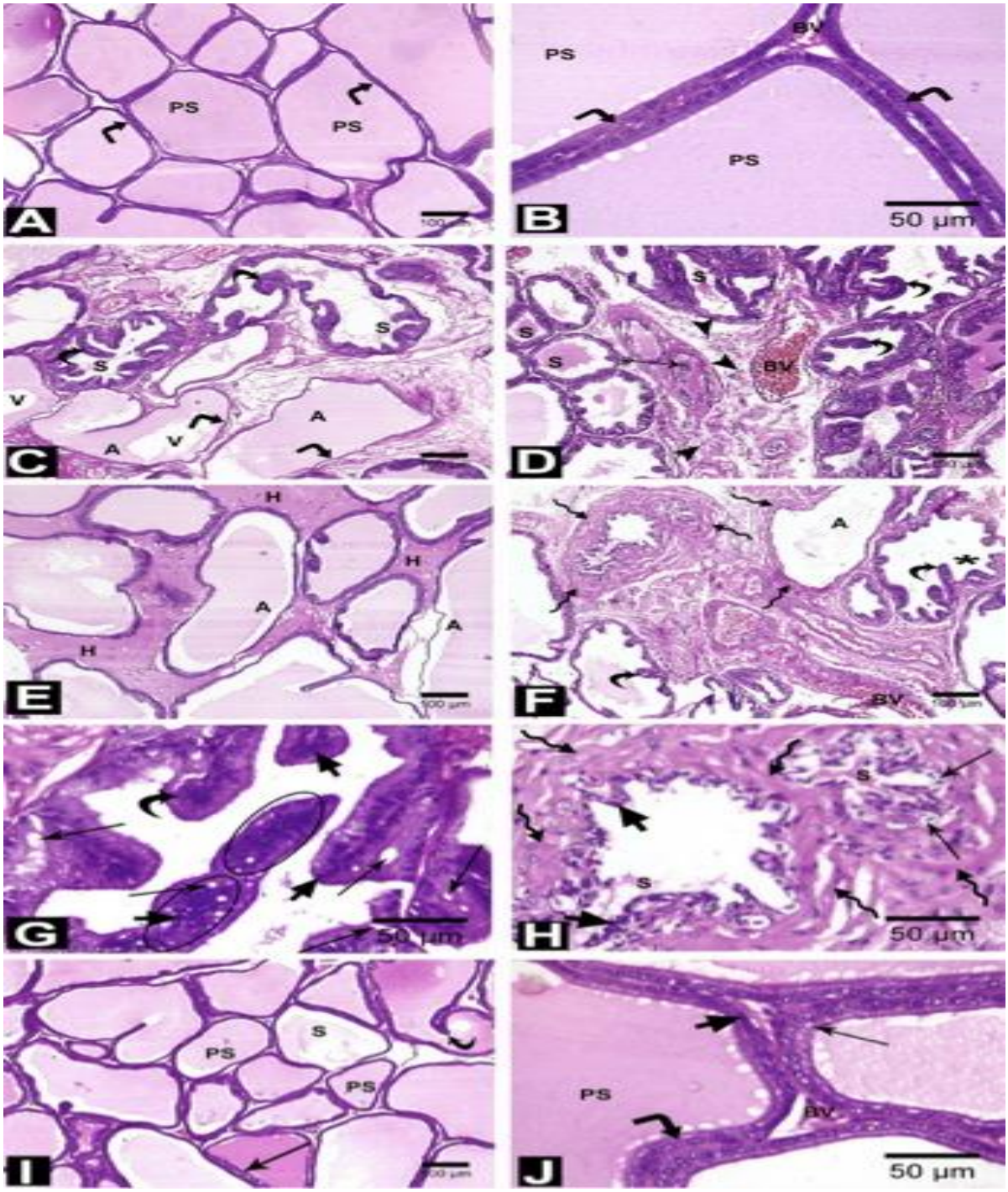

Fig. (2): *Photomicrographs of H&E-stained prostatic sections of different experimental groups.* ***A, B:*** *Prostatic acini of the control group filled with acidophilic prostatic secretions (PS) and lined by simple cuboidal or columnar epithelium (angled arrow). Minimal fibromuscular stroma containing blood vessels (BV) is also evident.* ***C-H:*** *PDC group revealing focal areas of atrophic acini (A) with marked thinning of their lining epithelium (angled arrow) in association with prostatic acini with epithelial hyperplasia (curved arrow) forming arborization folds (oval shape) characterized by dark stained nuclei (short arrow) and vacuolated cytoplasm (arrow). Scanty (S), vacuolated (V) or absent (*) prostatic secretions and inflammatory infiltrates (double bifid tailed arrow) are observed in some acini. The fibromuscular stroma shows hyaline plaques (H), inflammatory infiltrates (arrowhead), congested dilated blood vessels (BV) and thickened smooth muscle layer (zigzag arrow).* ***I, J:*** *PDC+Se group shows improvement of the glandular epithelial lining (angled arrow) except for some of epithelial hyperplasia (curved arrow) and vacuolated epithelial cells (arrow) with deeply stained nuclei (short arrow). The acini are filled with secretion (PS) and embedded in minimal stroma with blood vessels (BV).* **(H&E, A, C, D, E, F, I X100; Scale bar = 100µm; B, G, H, J X400; Scale bar = 50µm)**

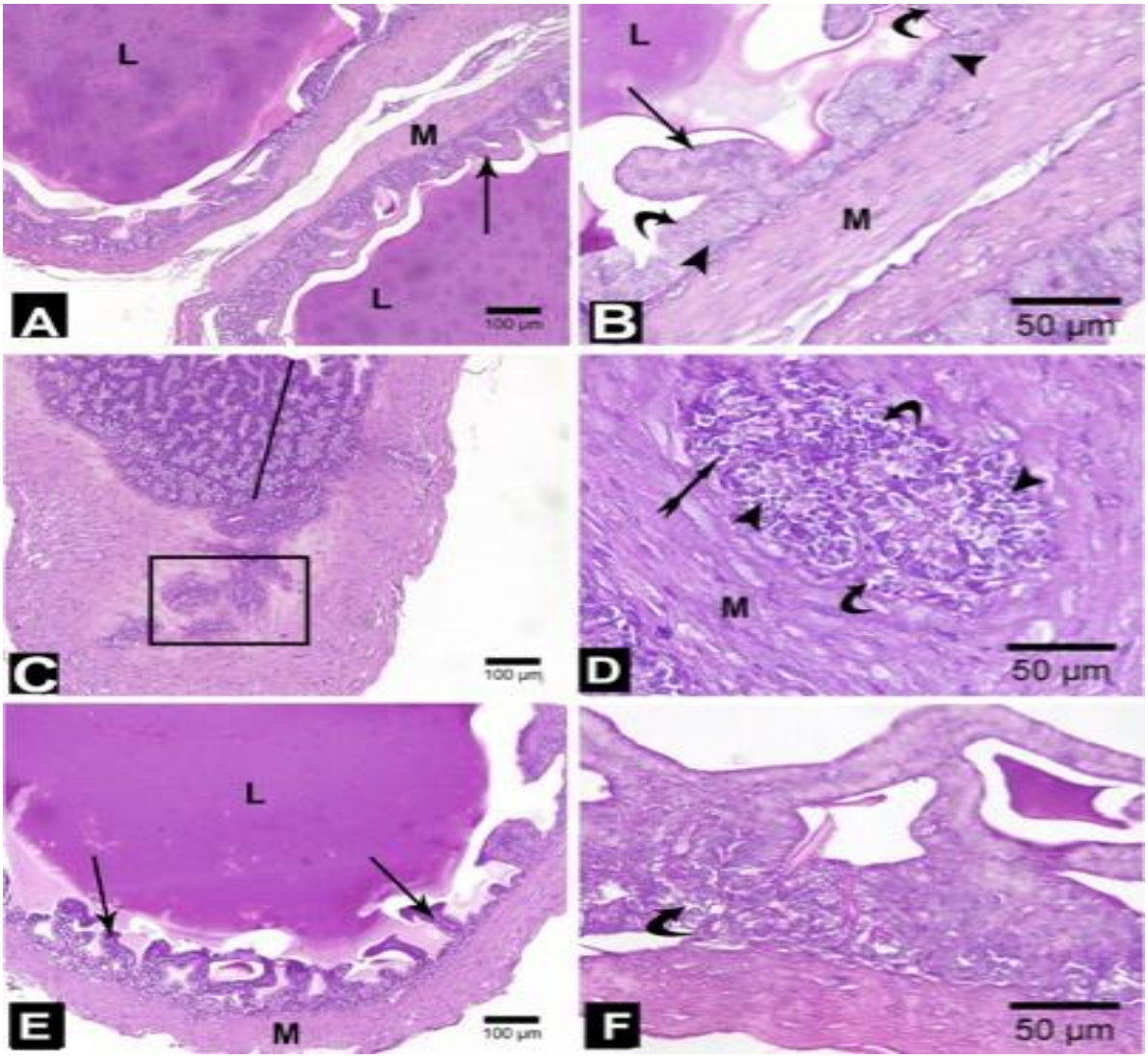

Fig.(3): *Photomicrographs of H&E-stained sections of the seminal vesicles of different experimental groups.* ***A, B:*** *Control group showing folded tunica mucosa (arrow) lined by pseudostratified epithelial lining with foamy eosinophilic cytoplasm (curved arrow) and ovoid vesicular nuclei (arrowhead), tunica muscularis (M) and seminal secretions occupying the lumen (L).* ***C, D:*** *PDC group revealing thickened tunica mucosa (line) and hyperplastic epithelial nodules (rectangle) within tunica musculosa (M). These nodules show highly vacuolated cells (curved arrow) and hyperchromatic nuclei with prominent nucleoli (arrowhead), in addition to some darkly stained nuclei (bifid tailed arrow).* ***E, F****: PDC+Se group displaying nearly normal epithelial lining (arrow), tunica musculosa (M) and seminal secretion into the lumen (L). Most of epithelial cells appeared similar to control except some still had vacuolated cytoplasm.* **(H&E, A, C, E X100; Scale bar = 100µm; B, D, F X400; Scale bar = 50µm)**

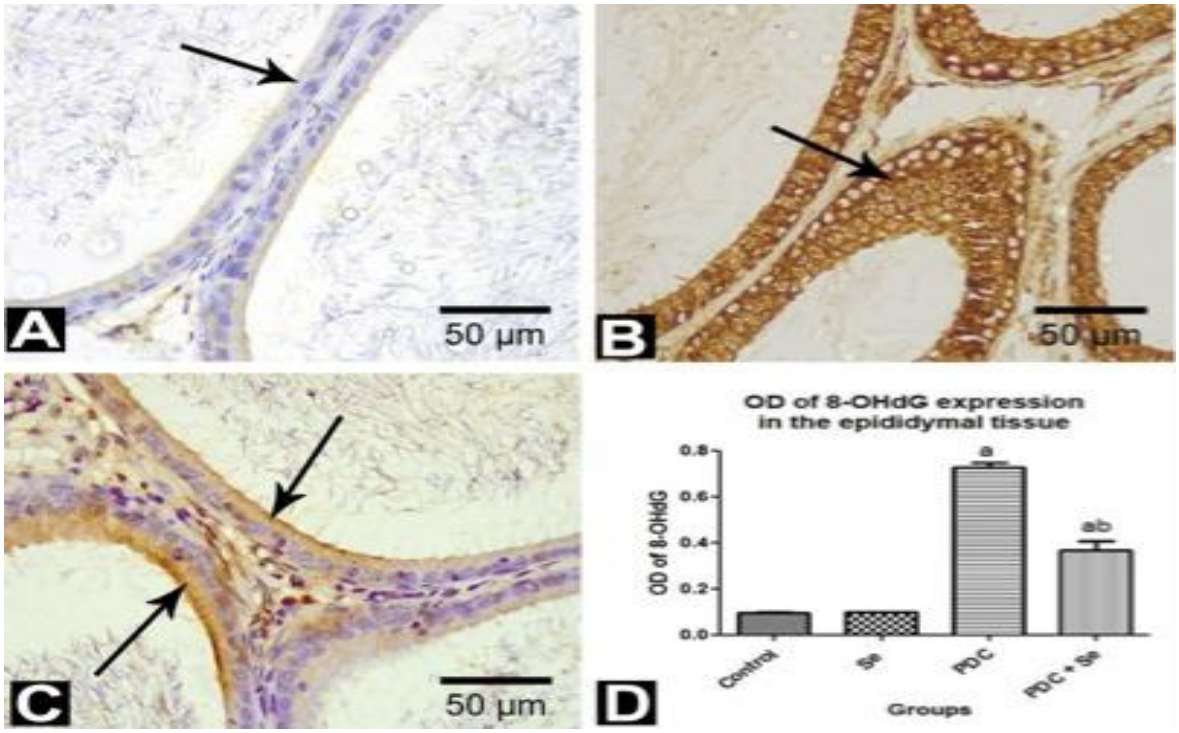

Fig.(4): *Photomicrographs of 8-OHdG-immunostained epididymal sections from the control (A), PDC (B) and PDC+Se (C) groups.* ***A:*** *Negative immune reaction in the epididymal cells (arrow).* ***B:*** *Intense immune reaction in nearly all the lining epithelial cells (arrow).* ***C:*** *Moderate immune reaction in some epithelial cells (arrow). (Scale bar=50µm, 8-OHdG immunostaining X400).* ***D:*** *A histogram showing the OD of 8-OHdG immunoreactivity in different experimental groups. a:P< 0.05 vs control or Se groups, b: P<0.05 vs PDC group.*

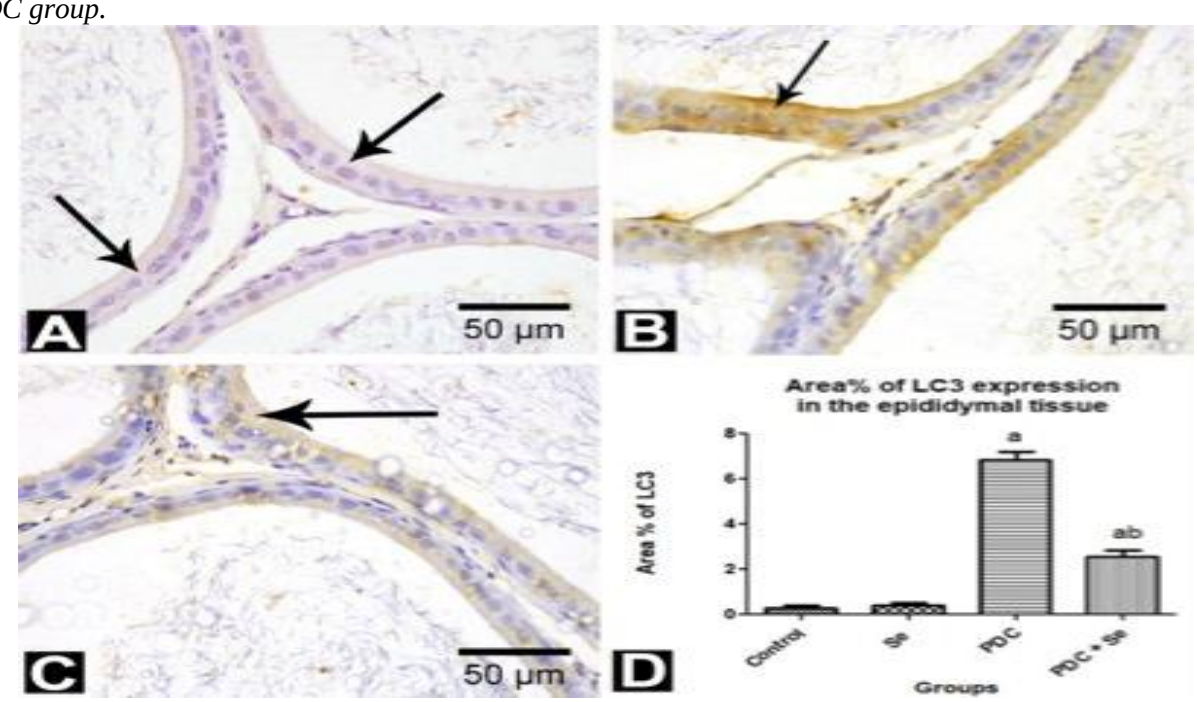

Fig.(5): *Photomicrographs of LC3-immunostained epididymal sections from the control (A), PDC (B) and PDC+Se (C) groups .****A:*** *Negative immune reaction (arrow).* ***B:*** *Strong positive cytoplasmic reaction (arrow).* ***C:*** *Faint reaction (arrow) in the cytoplasm of the lining epithelial cells. (Scale bar =50µm, LC3 immunostaining X 400).* ***D:*** *A histogram showing the area % of LC3 expression in different experimental groups. a: P< 0.05 vs control or Se groups, b: P<0.05 vs PDC group.*

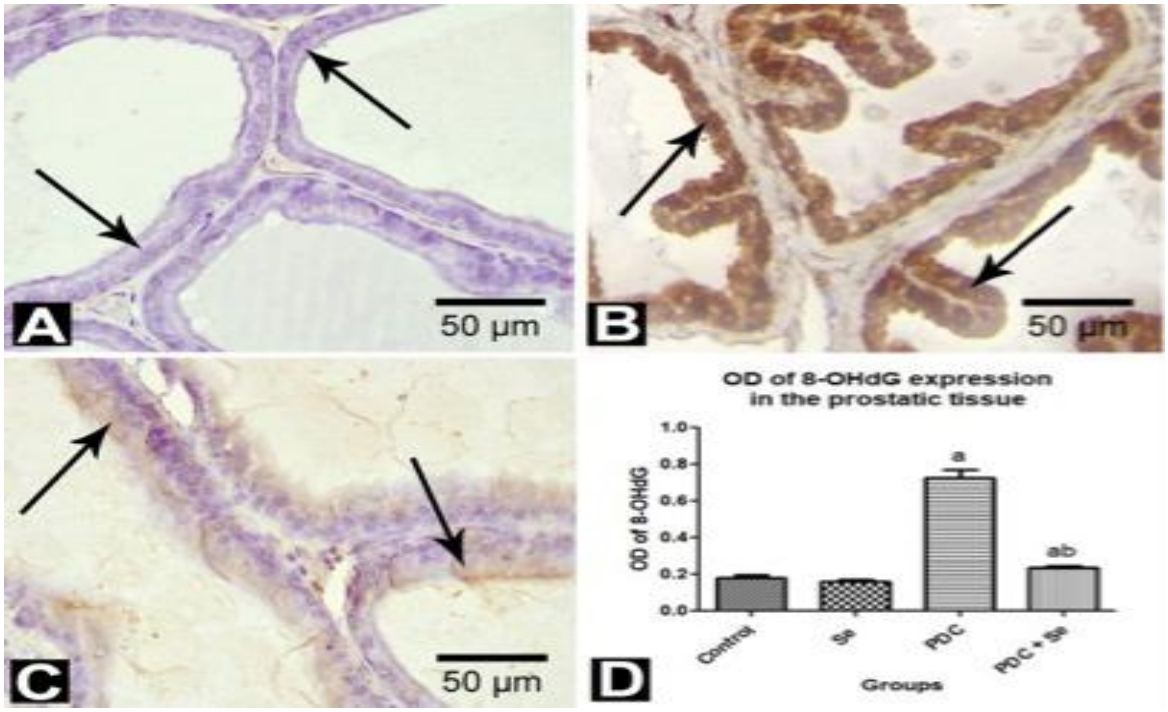

Fig. (6): *photomicrographs of 8-OHdG-immunostained prostatic sections from the control group (A), PDC (B) and PDC+Se (C) groups.* ***A:*** *Negative 8-OHdG expression in the epithelial lining cells of prostatic acini (arrow).* ***B:*** *Extensive positive cytoplasmic reaction for 8-OHdG in the acinar cells (arrow).* ***C:*** *Mild cytoplasmic reaction in some acinar cells (arrow) (Scale bar=50µm, 8-OHdG immunostaining X 400).* ***D:*** *A histogram showing the OD of 8-OHdG immunoreactivity in different experimental groups. a: P< 0.05 vs control or Se groups, b: P<0.05 vs PDC treated group.*

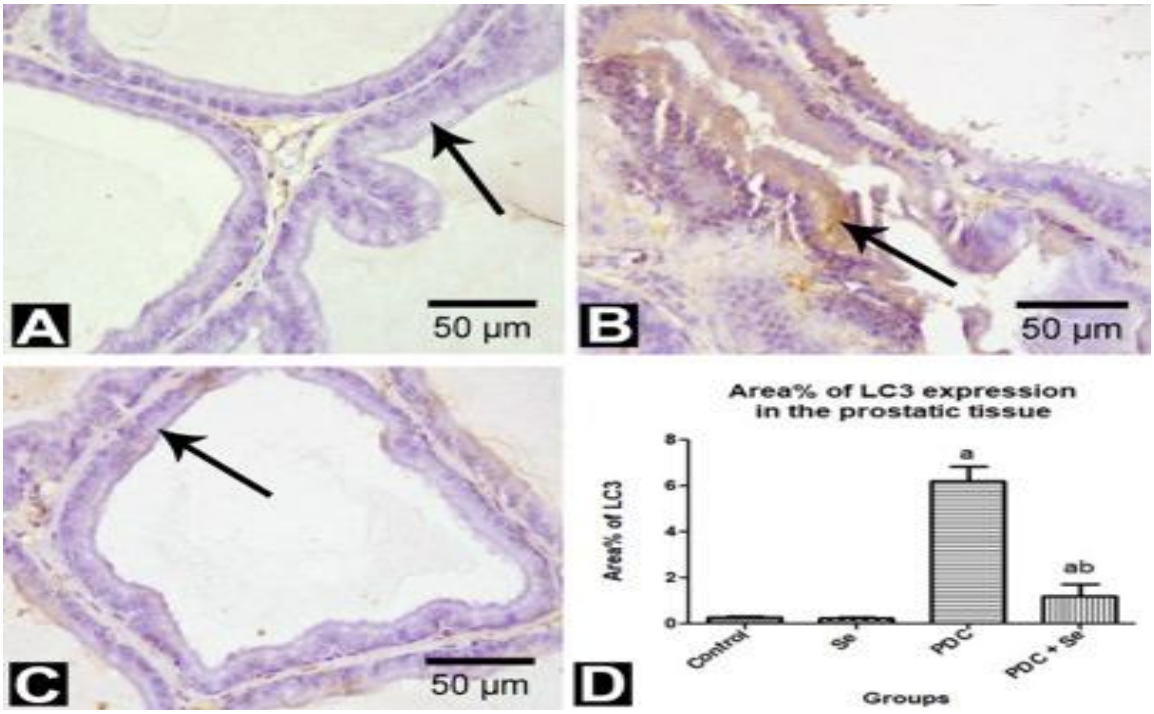

Fig. (7): *Photomicrographs of LC3 immunoexpression in prostatic sections from the control group (A), PDC (B), and PDC+Se (C) groups.* ***A:*** *Negative LC3 expression in the cytoplasm of the acinar cells (arrow).* ***B:*** *Intense positive cytoplasmic immunoreaction for LC3 in the acinar cells (arrow).* ***C:*** *Faint or even negative cytoplasmic reaction for LC3 in the acinar cells (arrow) (Scale bar =50µm, LC3 immunostaining X 400).* ***D:*** *A histogram showing the area % of LC3 expression in different experimental groups. a: P< 0.05 vs control or Se groups, b: P<0.05 vs PDC treated group.*

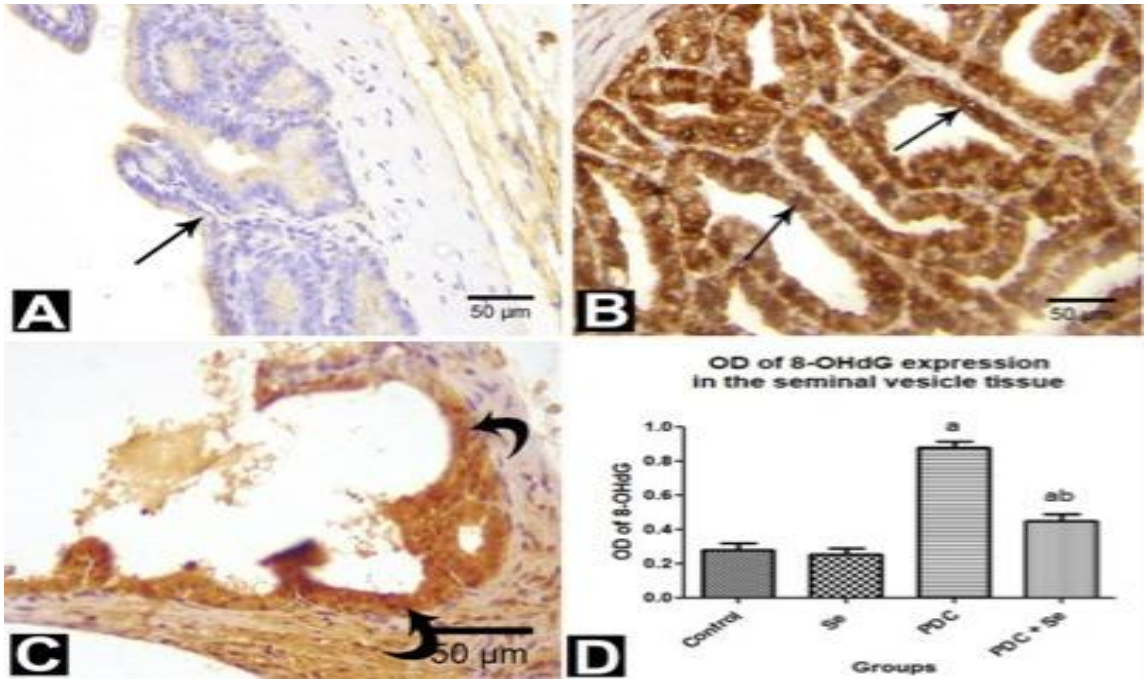

Fig. (8): *Photomicrographs of 8-OHdG immune-expression in seminal vesicle sections from the control group (A), PDC (B) and PDC+Se (C) groups.* ***A:*** *Negative 8-OHdG immune-expression in the cytoplasm of the epithelial lining of the seminal vesicle (arrow).* ***B:*** *Massive positive cytoplasmic immunoreaction for 8-OHdG in nearly all the cells (arrow).* ***C:*** *Positive cytoplasmic reaction for 8-OHdG in epithelial cells (curved arrow) (Scale bar=50µm, 8-OHdG immunostaining X 400).* ***D:*** *A histogram showing the OD of 8-OHdG immunoreactivity in different experimental groups. a: P< 0.05 vs control or Se groups, b: P<0.05 vs PDC treated group.*

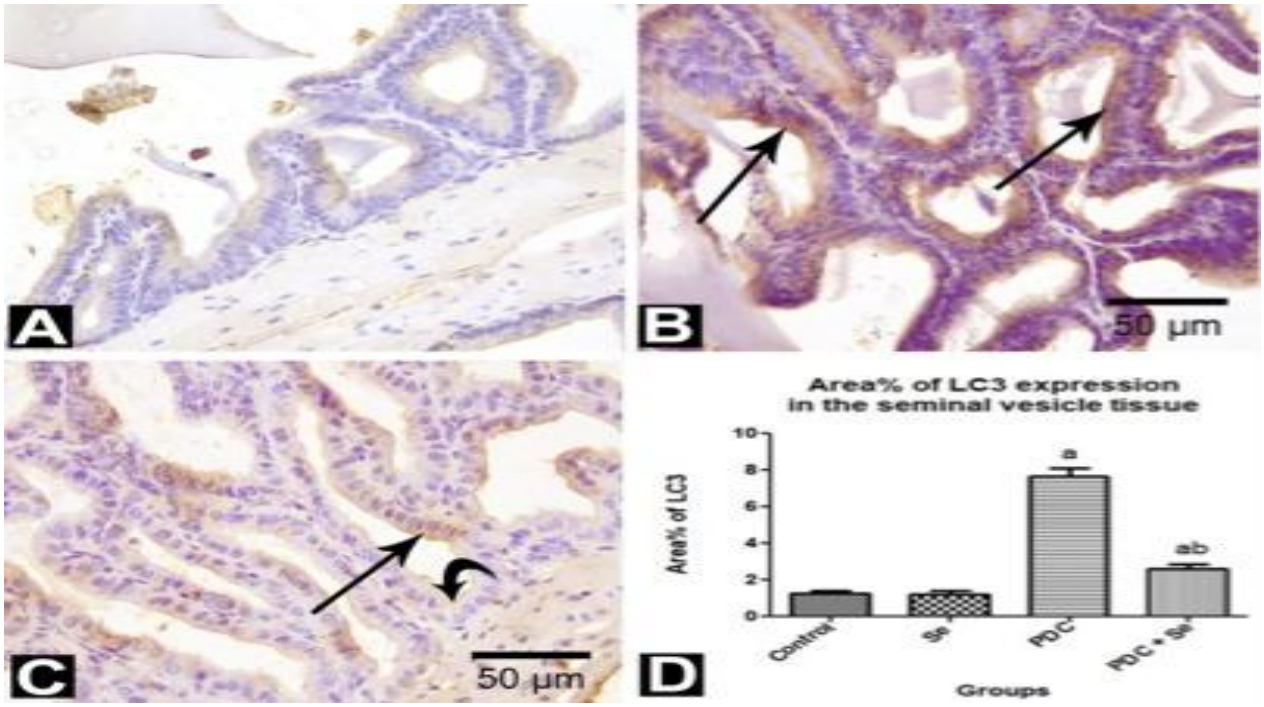

Fig. (9): *Photomicrographs of LC3 immunoexpression in seminal vesicle sections from the control group (A), PDC (B), and PDC+Se (C) groups.* ***A:*** *Negative LC3 expression in the cytoplasm of the epithelial cells* ***B:*** *Positive cytoplasmic immunoreaction for LC3 in the epithelial cells (arrow).* ***C:*** *Negative cytoplasmic reaction for LC3 in most of the epithelial cells (curved arrow) while some still have faint positive reaction (arrow) (Scale bar =50µm, LC3 immunostaining X 400).* ***D:*** *A histogram showing the area % of LC3 expression in different experimental groups. a: P< 0.05 vs control or Se groups, b: P<0.05 vs PDC treated group.*

DISCUSSION

Cr(VI) pollution represents a significant environmental threat, raising concerns over its impacts on human health. PDC administration, in the present investigation evoked significant alterations of sperm parameters and cellular redox balance, upregulation of inflammatory and autophagy markers as well as deterioration of the epididymal, prostatic and seminal vesicle histoarchitecture. These deleterious impacts were effectively alleviated by selenium treatment.

Consistent with our present findings, elevated oxidative stress status was recorded in dental technicians and tannery workers occupationally exposed to chromium ***(Berniyanti et al., 2020; Dubey et al., 2022)***. Additionally, ***Pokhrel et al. (2020)*** observed inverse correlation between urinary chromium levels and sperm motility in men attending infertility clinics. As Cr(VI) enters the cell, it is metabolically reduced to Cr(III), resulting in ROS production ***(Patlolla et al., 2009)***, which eventually interferes with spermatozoa motility via reducing phosphorylation of axonemal proteins, essential for spermatozoa movement ***(de Lamirande and Gagnon, 1992)***. Furthermore, Cr(VI) was proven to inactivate antioxidant enzymes, either due to its direct binding to enzyme active sites or to the displacement of metal co-factors from these active sites **(*Kalayarasan et al., 2008; Pedraza-Chaverrí et al., 2005)***.

Moreover, the current study revealed that PDC treatment significantly unregulated the serum inflammatory markers; NF-κB, TNF-α and IL-1β. Consistently, Cr(VI)-induced oxidative stress was shown to aggravate immune inflammation and NF-κB pathway inflammatory responses in recent human and animal studies ***(Hu et al., 2022; Zhao et al., 2022)***. Metal-induced oxidative stress could trigger activation of the transcription factor, NF-κB owing to its redox sensitivity, which in turn provokes expression of different inflammatory genes and propagates pro-inflammatory cascade ***(Das and Al-Naemi, 2019; Ptaschinski and Lukacs, 2018)***.

Interestingly, selenium treatment to PDC-intoxicated rats, in our research, effectively corrected abnormal sperm parameters and alleviated oxidative stress and inflammatory status induced by PDC. In the same line, ***Murbat et al. (2018)*** stated that selenium treatment could increase sperm count, motility, viability and normal morphology in infertile men. In addition, efficacy of selenium in counteracting Cr(VI)-induced oxidative stress and pro-inflammatory cytokine upregulation was recently reported in different animal models ***(Choudhuri et al., 2020; Zhao et al., 2022)***. This positive effect of selenium could be credited to its own antioxidative activity and structural role in different enzymatic antioxidants ***(Qazi et al., 2019)***. In addition, it was believed that selenium could regulate NF-κB activity by directly oxidizing critical sulfhydryl groups of this transcription factor ***(Mal'tseva et al., 2022)***.

In the current investigation, PDC treatment elicited profound structural epididymal changes, represented by vacuolization and hyperplasia of the epithelial lining with appearance of Halo cells. Hyperplasia of the epididymal lining is regarded as a morphological sign of direct epididymal toxicity **(*Kempinas and Klinefelter, 2014)***, indicating the direct toxicant impact of PDC on the epididymis. In addition, epididymal vacuolization was previously described in Cr(VI)-intoxicated Bonney monkeys ***(Aruldhas et al., 2006)***; the investigators identified disintegrated spermatozoa inside the vacuoles of several principal cells, suggesting increased phagocytic activity of these cells. Moreover, the obvious increase of the infiltrating immune cells, Halo cells, in the epididymal tubules of PDC-treated rats, in our research, reflects imbalance of the epididymal immune status ***(Noblanc et al., 2020)***.

The current research also illustrated PDC-induced oxidative DNA damage of the epididymal epithelium, evidenced by significantly elevated 8-OHdG expression. Indeed, excessive ROS levels are well documented to oxidize guanosine, due to its lower redox potential, to 8-OHdG, promoting DNA damage ***(Noblanc et al., 2020; O'Flaherty, 2019***). In this regard, oxidative stress was previously reported in the epididymal tissue of Cr(VI)-intoxicated rats

(Kim et al., 2012). Oxidative stress is considered a main modulator of chronic low grade inflammation, myofibroblast differentiation and extracellular matrix production ***(Richter and Kietzmann, 2016; Thuan et al., 2018)***, explaining the observed interstitial inflammatory cell infiltration, congested capillaries, abundant smooth muscles, and hyaline material deposition in PDC-treated rats in our study.

Given the crucial epididymal role in spermatozoa maturation and acquisition of motility and fertilizing ability ***(Tarique et al., 2020)***, PDC-triggered epididymal injury could be linked, at least in part, to poor sperm quality detected in the present study. Contrary to our findings, ***Oliveira et al. (2010)*** did not identify any histopathological alterations in the epididymal tissue of mice injected subcutaneously with PDC (10 mg/ kg) for four days. This contradiction could be ascribed to differences in exposure route and dosage.

The present research further declared the beneficial role of selenium on PDC-induced epididymal injury and oxidative DNA damage. In the same line, selenium was reported to improve diabetes- and diclofenac-induced epididymal toxicities in rats ***(Owumi et al.,2020; Sahu et al., 2020)***. Efficacy of selenium treatment in counteracting heavy metal-induced DNA damage was confirmed by ***Battin et al. (2011)*** who ascribed the antioxidative activity of selenium mainly to metal-binding mechanism rather than radical scavenging.

The present histopathological investigation of the prostatic tissue of PDC-exposed rats displayed epithelial hyperplasia in association with focal atrophic lesions and interstitial inflammation. In fact, exposure to environmental agents is considered a causative factor in the development of prostatic injury and subsequent inflammation ***(De Marzo et al., 2007)***. Focal atrophic lesions develop as an adaptive response to inflammation, which leads to decreased membrane integrity, intracellular calcium influx and eventually oxidative damage ***(Billis, 2010)***.

Cellular injury could further trigger proliferative regeneration of the prostatic glandular epithelium, referred to as proliferative inflammatory atrophy ***(Vral et al., 2012)***. Proliferative inflammatory atrophy is regarded as a precancerous lesion closely related to the development of high grade prostatic intraepithelial neoplasia and carcinoma ***(Wang et al., 2009)***.

Indeed, the prostatic tissue is particularly vulnerable to oxidative stress due to rapid cellular turnover and lack of DNA repair enzymes ***(Hamid et al., 2011)***. In accordance, the present research revealed oxidative DNA damage in the prostatic tissue of PDC-treated rats. In the same context, Cr(VI) exposure has recently been identified as a risk factor for cancer prostate ***(Deng et al., 2019)***; oxidative DNA damage with subsequent defects in DNA repair has been considered the main causative factor for Cr(VI) carcinogenicity ***(Balali-Mood et al., 2021)***.

Selenium treatment, in the current study, dramatically mitigated PDC-mediated prostatic injury and oxidative DNA damage. Consistently, dietary selenium intake was proposed to reduce the risk of developing prostatic hyperplasia and neoplasm transformation via reducing the deleterious impacts of oxidative stress ***(Eichholzer et al., 2012; Udensi and Tchounwou, 2016)***. Furthermore, men supplemented with selenium for six months exhibited decreased episodes of prostatitis, prostate glandular epithelium microarchitecture stability and preserved DNA integrity, confirming the intimate association between serum selenium levels and prostate health ***(Karunasinghe et al., 2019).***

Concerning the current microscopic findings of the seminal vesicles, PDC treatment induced remarkable thickening of tunica mucosa and hyperplastic epithelial nodules, in addition to oxidative DNA damage. Structural lesions of the seminal vesicles were also reported in rats exposed to Cr(VI) during the suckling period ***(Savici et al., 2020)***. Epithelial hyperplasia represents a form of cellular adaptation to stress, however unfortunately it takes place at the expense of normal function ***(Kumar et al., 2017)***. The prostate gland and seminal vesicles are responsible for secretion of seminal plasma

which provides a favorable medium for spermatozoa metabolism, motility, and function ***(Wang et al., 2020a)***. Furthermore, the seminal plasma is enriched with immunological modulators that protect against fertilized egg rejection ***(Politch et al., 2007)***. Accordingly, chromium-induced alterations of prostatic and seminal glands should be considered as causative factors for sperm dysfunction and impaired sperm-oocyte fusion triggered by chromium toxicity ***(Pokhrel et al., 2020)***.

In fact, selenium in seminal plasma is secreted by the glandular epithelium of the accessory sex glands; prostate and seminal glands, and it is intimately related to dietary selenium intake ***(Qazi et al., 2019).*** According to ***Hosny et al. (2020)***, selenium supplement to rabbit bucks could improve seminal plasma composition, reflecting the positive impact of selenium on the functions of these accessory sex glands. In agreement with these data, selenium treatment to PDC-intoxicated rats, in the present investigation, considerably mitigated the reprotoxic effects triggered by PDC, as noted by obvious improvement of the histoarchitecture and morphometric indices of prostatic and seminal glands.

In the current research, PDC treatment triggered excessive autophagy by enhancing the immune expression of LC3 in the epididymal ducts, prostatic glands, and seminal vesicles. In this regard, Cr(VI) was reported to promote excessive testicular autophagy via upregulation of autophagy-associated proteins ***(Zhuge et al., 2022)***. Furthermore, Cr(VI) treatment triggered autophagosome formation and LC3 upregulation in chicken liver ***(Wang et al., 2020b)***. Recent research on Cr(VI)-exposed fish illustrated that Cr(VI) could induce hepatic and renal autophagy via inducing mitochondrial dysfunction and oxidative stress ***(Kumar et al., 2022)***. Cr(VI)-induced oxidative stress and autophagy were also demonstrated in broiler cardiomyocytes ***(Li et al., 2022)***. NF-κB inflammatory pathway is considered an important mediator of autophagy induction; It has been reported to enhance autophagic process via direct upregulation of proteins and genes related to autophagosome formation, including LC3 ***(Verzella et al., 2020)***.

The present work further clarified that selenium treatment effectively counteracted PDC-induced autophagy via downregulation of LC3 expression. In accordance, selenium was documented to decrease the number of autophagosomes and downregulate LC3 levels and autophagy activity in the hepatic tissue of PDC-intoxicated broilers **(Wang et al., 2023)**. In the same context, anti-autophagic effect of selenium was reported in many heavy metal toxicities; selenium was proven to inhibit cadmium-induced autophagy in primary hepatocytes via promoting antioxidant enzyme expression **(Zhang et al., 2017)**. Moreover, selenium provoked antioxidant and anti-autophagic impacts on arsenic-treated rat pheochromocytoma cells **(Rahman et al., 2018)**as well as lead-treated chicken testes **(Huang et al., 2019)**. Overall, these findings confirm the beneficial role of selenium against excessive autophagy induced by Cr(VI).

CONCLUSION

Selenium supplements (0.25mg/kg daily for four weeks) could effectively protect against PDC-induced damage of the epididymis, prostate and seminal vesicles through counteracting oxidative stress and reducing NF-κB activation and excessive autophagy evoked by subchronic PDC exposure. Therefore, selenium should be considered as a promising reproprotective agent for chromium-exposed workers and populations with potentially high exposure. Further investigations are warranted to ascertain the exact mechanisms of chromium-induced reproductive toxicity.

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السيلينيوم يمكن أن يخفف من تغيرات البربخ والبروستاتا والحويصلات المنوية التي يسببها ثاني كرومات البوتاسيوم في الجرذان البالغة: الدور المحتمل لتثبيط مسار العامل النووي كابا ب

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الملخص العربي

المقدمة: يعد الكروم منتج نفايات لصناعات مختلفة وقد تم تناول سميته على الجهاز التناسلي في العديد من الدراسات التي اعتبرت أن الإجهاد التأكسدي هو العامل الرئيسي المساهم في هذه السمية. السيلينيوم ضروري للصحة الإنجابية للذكور ويشارك بشكل فعال في تنظيم حالة الأكسدة الخلوية.

الهدف: توضيح التأثيرات المحتملة لثاني كرومات البوتاسيوم على بنية البربخ والبروستاتا والحويصلات المنوية والتحقق من فعالية السيلينيوم في مواجهة هذه التأثيرات.

المواد والطرق المستخدمة: تم تقسيم ستة وثلاثين من ذكور الجرذان البالغة إلى أربع مجموعات: المجموعة الضابطة، مجموعة السيلينيوم، مجموعة ثاني كرومات البوتاسيوم، ومجموعة ثاني كرومات البوتاسيوم والسيلينيوم. تم إخضاع الحيوانات للعلاج لمدة أربعة أسابيع. في نهاية التجربة، تم جمع عينات الدم والسائل المنوي والبربخ والبروستاتا والحويصلات المنوية ومعالجتها لإجراء التحاليل الكيميائية الحيوية والمورفولوجية والكيميائية المناعية.

النتائج: أدى تناول ثاني كرومات البوتاسيوم إلى تدهور كبير في خواص الحيوانات المنوية، كما تسبب في خلل توازن الأكسدة الخلوي وارتفاع ملحوظ في دلالات الالتهاب (العامل النووي كابا ب وعامل نخر الورم ألفا و إنترلوكين -١ بيتا) في مصل الدم. مجهريًا، أظهرت جميع الأنسجة التي تم فحصها للجرذان المعالجة بـثاني كرومات البوتاسيوم بنية متدهورة بالإضافة إلى زيادة تلف الحمض النووي المؤكسد ونشاط الالتهام الذاتي المفرط. وقد خفف تناول مكملات السيلينيوم للجرذان المعالجة بثاني كرومات البوتاسيوم بشكل فعال من جميع التغيرات المذكورة سابقا

الخلاصة: مكملات السيلينيوم يمكن أن تخفف بشكل فعال الضرر الناجم عن ثاني كرومات البوتاسيوم في البربخ والبروستاتا والحويصلات المنوية من خلال مواجهة الإجهاد التأكسدي، وتقليل تنشيط العامل النووي كابا ب والالتهام الذاتي المفرط الناجم عن ثاني كرومات البوتاسيوم.