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Original article

The Possible Protective Role of Crocin and Pumpkin Seed Oil Against Lead-Induced Testicular Cytotoxicity in Adult Male Albino Rat. Hormonal, Histological and Immunohistochemical study

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Abstract:

Background: Lead (Pb) disturbs of oxidant/antioxidant balance in testicular cells causing oxidative stress. Crocin (Cr) and pumpkin seed oil (PSO) have antioxidant effects and neutralize free radicals. **Aim**: This study was performed to assess the cytotoxicity of lead on testis and the possible protective effect of Cr and PSO. **Methods:** Forty eight healthy male rats were divided into six groups as follows: Control group, Cr group: each rat was received Crocin (200 mg/kg) given intraperitonealy (ip) once daily for 4 weeks, PSO group: each rat was orally received PSO (1 ml/kg) daily for 4 weeks , Pb group: each rat was received lead acetate, (8 mg/kg) ip daily for 4 weeks, (Cr+ Pb) group: each rat was received (200 mg/kg) crocin ip with 8 mg/kg Pb ip for 4 weeks and (PSO+ Pb) group: each rat was received PSO (1 ml/kg) orally in addition to ip lead acetate (8 mg/kg) daily for 4 weeks. The testicular tissues have been processed for a light microscopic study. Results**:** In Pb -treated rats, levels of FSH, LH, and testosterone significantly decreased. Decrease thickness of spermatogenic layers, mispresentation, Vacuolations, degeneration of spermatogenic cells, wide interstitial spaces, Weak PCNA and androgen receptor immunoexpression were observed. Both Cr and PSO treatment resulted in reversing Pb effects on testis presented in improvement in hormonal levels and histological architecture of most of seminiferous tubules with increase PCNA and androgen receptor immunoexpressions. **Conclusions:** Cr and PSO may have ameliorative effects after lead acetate toxicity on rat testis.

Keywords: Lead, Crocin, Pumpkin, Testicular cytotoxicity.

Introduction:

Lead (Pb) is one of the heavy metals and has non-biodegradable nature leading to its environmental accumulation and increasing its hazards (1). Pb is toxic metals to human, animals and plants (2). There are different routes of exposure to Pb, including ingestion of contaminated food and water, inhalation of Pb airborne, dermal contact, car exhaust gases, leaded gasoline varnish, pottery, leaded pipes and cosmetics (3, 4). Several factors such as age, sex, physical form, particle size, dose and hereditary factors result in individual

variations to lead toxicity (5). Lead is not metabolized in the body, but it is linked to glutathione (GSH) and excreted primarily in the urine (6).

Mechanisms implicated in lead oxidative stress inside the cells either indirect through its union to (GSH) or direct through inactivation of antioxidant enzyme like: Glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) (7). Pb generates reactive oxygen species (ROS) with subsequent stimulation of lipid peroxidation (8). ROS outbreak antioxidant defense system of cells, finally it disturbs of oxidant/antioxidant balance in cells causing oxidative stress (9, 10).

Crocin (Cr) is a water-soluble carotenoid obtained commercially from the stigma and petal of *Crocus sativus* (Saffron) and it is responsible for the red color of saffron (11). Crocin has attracted pharmaceutical scientific attention such as antidiabetic (12), neuroprotective properties (13), anti-inflammatory, antinociceptive (14), antioxidant (15), antidegenerative in the treatment of patients with neurological degeneration, anti-spasm (16), anti-depression (17) and anti-cancer (18). Recent studies have specified that Cr has protective effect on testicular toxicity caused by cisplatin and cyclophosphamide (19, 20). Some studies have shown that Cr attenuated kidney and liver oxidative toxicity in rats (21, 22).

Pumpkin is a herbaceous plant of the family Cucurbitacea, its seed filled with unsaturated fatty acids, especially omega, and trace elements (like magnesium, iron and zinc) (23). Pumpkin has several physiological and pharmacological properties such as antineoplastic, antioxidant, antibacterial, anti-diabetic, and anti-obesity (24). Pumpkin seed oil (PSO) is rich in β-carotene and vitamin E, which have powerful antioxidant and anti-inflammatory effects, they have role in neutralizing free radicals (25, 26).

The current study performed to investigate the changes of testicular structure in lead acetate treated rats, also further to demonstrate the possible protective effect of Cr and PSO against lead-induced testicular damage.

Materials and Methods:

Animals

This experimental study was done on forty-eight healthy adult male albino rats weighing 180-200 gram. The rats were obtained from the Laboratory Animals Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed in plastic cages at 23 ± 2°C, (12 h: 12 h light:dark) , humidity (55–60%), were allowed water and fed standard diet. The experiment was performed according in accordance with the ethical policies approval by committee recommendations of Faculty of Medicine, Benha University. The experiment was done from April 2022 to May 2022.

Preparation of materials:

Lead acetate (PbAc) [(CH3CO2)2], 99% pure) was obtained from (El-Gomhoria company, Egypt). Each 160 mg from lead acetate powder was dissolved in100ml distilled water, so each 1ml from the solution contained 1.6mg from Pb and each rat injected 1ml from the solution intraperitoneally in dose (8 mg/kg body weight) for 28 consecutive days [6]

Crocin (Cr) was obtained from (Sigma Chemical Co., St Louis, MO, Product No. 17,304 USA). Cr was dissolved in normal saline (0.09% NaCl) at 10 mg/ml concentration.

Pumpkin seed oil (PSO) was purchased from (EL Captin Company, Al Obour City, and Cairo, Egypt).

3-Experimental design:

Rats were divided into six experimental groups (eight rats each) as follows: Control group: rats were provided an ordinary diet left with no medication. (Cr) group: each rat was received Corcin (200 mg/kg b.w./day) intraperitoneally for 28 consecutive days (27). (PSO) group: each rat was orally administered (PSO) (1 mL/kg b.w. /day) by gavage tube for 28 successive days (28). (Pb) group: each rat was received lead acetate (PbAc) (8 mg/kg b.w. /day) intraperitoneally for 28 successive days. (Cr+ Pb) group: each rat was injected (200 mg/kg b.w. /day) crocin intraperitoneally in association with intraperitoneal injection of (8 mg/kg b.w. /day) Pb for28 successive days. (PSO+ Pb) group: each rat was received oral administration of (PSO) (1 mL/kg b.w. /day) by gavage tube in addition to (8 mg/kg b.w. /day) Pb intraperitoneally for 28 successive days.

On the 28th day all animals were sacrificed under anesthesia with ketamine-xylazine (65:10 mg/kg i.p.), afterward blood was withdrawn from the retro-orbital vein, blood samples were collected into a clean test tube. Rats were decapitated immediately after blood collection, testes were dissected out, trimmed of excess fat tissues and fixed in10% neutral buffered formalin for histological and immunohistochemical studies.

Hormonal assay:

The blood samples were centrifuged at 1200×g for 20 min. Then, serum samples were stored at −20 °C till analysis. Level of testosterone, LH and FSH were analyzed.

Histological study:

Tissue of testis after formalin fixation for 48 hr. dehydrated in ascending concentrations of ethyl alcohol (70%–100%) and cleared in xylene before paraffin embedding. 4-5 µm sections were cut from paraffin blocks and mounted on slides, deparaffinized and processed for Hematoxylin and eosin (H & E) staining for light microscopic examination and imaging the section using a light microscope (Olympus CX 41, Japan).

Immunohistochemical Study:

The paraffin fixed testis were cut into 5µm sections and mounted on positively charged slides for both PCNA and androgen receptors immunohistochemistry. Sections were de-waxed, rehydrated Cell multiplying were determined after stimulation with HistoVT One (Nacalai Tesque, Kyoto, Japan) using polyclonal rabbit anti-proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Primary monoclonal and polyclonal antibodies for androgen receptors (Cat. No. MA1-150, Thermo Fisher Scientific Co., USA) and, respectively. After activation with HistoVT One (Nacalai Tesque, Kyoto, Japan). The immunocomplex was visualized by the avidin-biotin-peroxide method using the Vecstatin Elite ABC Rabbit IgG kit (Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's instructions. Sections were counter-stained with haematoxylin **(**29**)**.

Computer Assisted digital image analysis (Digital morphometric study)

Slides stained with AR and PCNA immunostain were photographed at ×400 magnifications using Olympus® digital camera installed on Olympus® microscope with 0.5 X photo adaptor and

saved as TIFF. The result images were analyzed on Intel® Core I7® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for stain quantification and distance measurement, Results were exported to Excel Sheet expressed as integrated density.

The mean thickness of the seminiferous tubule epithelium was measured at ×400 magnifications using image analyzer Leica (DMLB) and Leica Qwin software.

2 slides from each rat were prepared, 5 random non overlapped fields from each slide were analyzed.

Statistical methods:

Data was analyzed using Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Quantitative parametric data were presented in mean± SD. $p \leq 0.05$ was significant tested by using One-way analysis of variance (ANOVA) and tukey.

Results:

Hormonal Assay:

In (Cr) and (PSO) groups, there wasn't any significant difference in FSH, LH and testosterone levels from control group. In (Pb) group, there was significant decrease serum levels of the three hormones when compared with control at the end of experimental period. The serum levels of the three hormones significantly elevated (Cr + Pb) when compared with (Pb) group but still with significant difference when compared with control group. The effects of PSO on serum levels of testosterone and LH were noticed as significant increase when compared with Pb group but still with significant difference when compared with control and (Cr + Pb) groups, while serum level of FSH were significant decreased when compared with control group only and not in significant difference with (Cr + Pb) group ($p < .001$). These data were shown in table (1).

Table (1): Shows the level of sex hormones in serum in different studied groups.

	Serum testosterone (ng/ml)	**Serum FSH** (ng/ml)	**Serum LH** (ng/ml)
Control (C) group	5.37 ± 0.56	7.03 ±0.42	1.91±0.78
Crocin (Cr) group	5.82 ±0.93	6.93 ± 0.38	1.83 + 0.42
Pumpkin seed oil (PSO) group	5.54 ±0.53	6.75± 0.52	1.61±0.18
Lead Acetate (Pb) group	1.75 ±0.56 a, b, c	3.21±0.24 a, b. c	0.64±0.15 a, b, c
Crocin + Lead Acetate (Cr + Pb) group	3.91 ±0.74 a, b, c, d	4.96±0.77 a, b, c, d	1.49 ± 0.06 a, b, c, d
Pumpkin seed oil + Lead Acetate (PSO+ Pb) group	3.09 ±0.72 a, b, c, d, e	4.26±0.6 a, b, c, d	0.93 ± 0.03 a, b, c, d, e
p-value	<0.001	< 0.001	< 0.001

Data expressed as mean±SD , P:Probability *:significance <0.05

Test used: One way ANOVA followed by post-hoc tukey

a: Significance vs Control, b: Significance vs Cr, c: Significance vs PSO, d: Significance vs Pb, e: Significance vs Cr+ Pb.

Histological results
H &E stain:

Sections of transverse sections of rat testes of the control group appeared with normal histological architecture with full tightened and well-organized seminiferous tubules, (ST) were encircled with normal basement membrane. Normal histological structure of seminiferous tubules with all stages of spermatogenic series from spermatogonia to mature spermatozoa was present. Sertoli cells appeared pyramid-shaped cells with oval nuclei, their bases lied against the basement membrane, and their tips point toward the cavity of tubule. The interstitial tissue showed groups of Leydig cells (Fig. 1a, 2a).

The histological results of sections of both (Cr) group and (PSO) groups did not demonstrate any difference from section of control group (Fig. 1b, 2b, 1c, 2c) respectively.
Sections of Pb treated group showed wide interstitial spaces. Irregular wavy basement membrane enveloped some seminiferous tubules and very thin basement membrane enveloped others. There was a decrease in thickness of germinal epithelium layer. There was disorganization of spermatogenic cells and empty spaces were seen between germ cells. Sloughing of the spermatogonia cells from the basement membrane with accumulation of dislodged spermatogenic cells and dense nuclei inside lumen were observed. Some spermatocyte showed vacuolations, pyknotic nuclei, fragmented nuclei and margination of their chromatin. Giant round spermatids were also observed. Some seminiferous tubules showed complete degeneration of spermatogenic epithelial series leaving remnants of apoptotic cells and pyknotic nuclei occupied most of the lumen of the seminiferous tubules. Vacuolations in interstitial cells were present and vascular congestion were observed (Fig.1d,2d,2e,2f).

Sections of (Cr+ Pb) group, Full tightened seminiferous tubules but congested blood vessel observed in interstitial tissue. There was improvement in histological architecture of some of seminiferous tubules which were lined spermatogenic cells in all different stages of development from Spermatogonia up to spermatozoa; others showed disorganization of spermatogenic epithelial series (Fig.1e, 2g).
Sections of (PSO+ Pb) group revealed the ameliorative effect of PSO on lead acetate toxicity represented in partial restoration in the testicular architecture, spermatogonia adjacent intimately to the basement membrane, presence of spermatozoa. Congestion of blood vessel persisted. Minimal widening of interstitial spaces was observed. Wavy basement membrane enveloped the seminiferous tubules (Fig.1f, 2h).

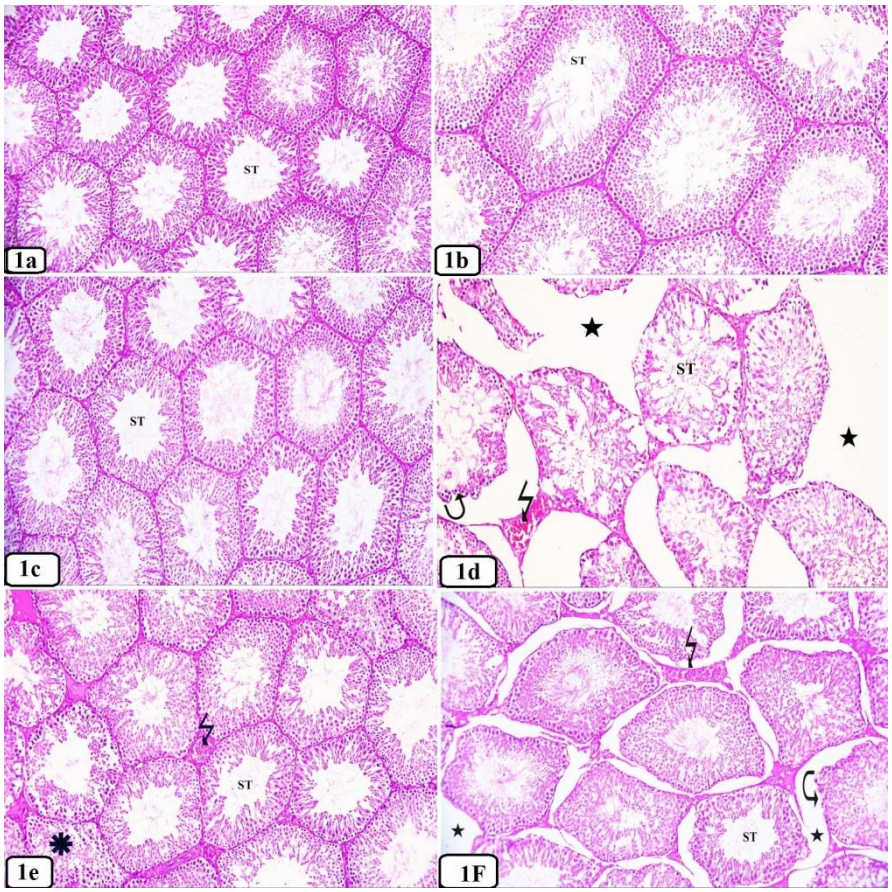

Fig. (1): A light photomicrograph of Transverse Sections (T.S) in testes for all experimental groups. (1a), (1b) &(1c) represent control, Cr and PSO groups respectively show normal histological architecture with full tightened seminiferous tubules (ST) which were encircled with normal basement membrane. (1d) represents (Pb) group and shows wide interstitial spaces (star) which contain congested blood vessel (zigzag arrow). Irregular basement membrane (curved arrow) enveloped the seminiferous tubules (ST). (1e) represents (Cr+ Pb) group and shows seminiferous tubules (ST) with full tightened, but interstitial tissue contains congested blood vessel (zigzag arrow). Some of seminiferous tubules (ST) show improvement in histological architecture, other show disorganization of spermatogenic epithelial series (astrike). (1f) represents ((PSO+ Pb) group shows minimal widening of interstitial spaces (star) which contain congested blood vessel (zigzag arrow). Irregular basement membrane (curved arrow) enveloped some seminiferous tubules (ST). H&E X100

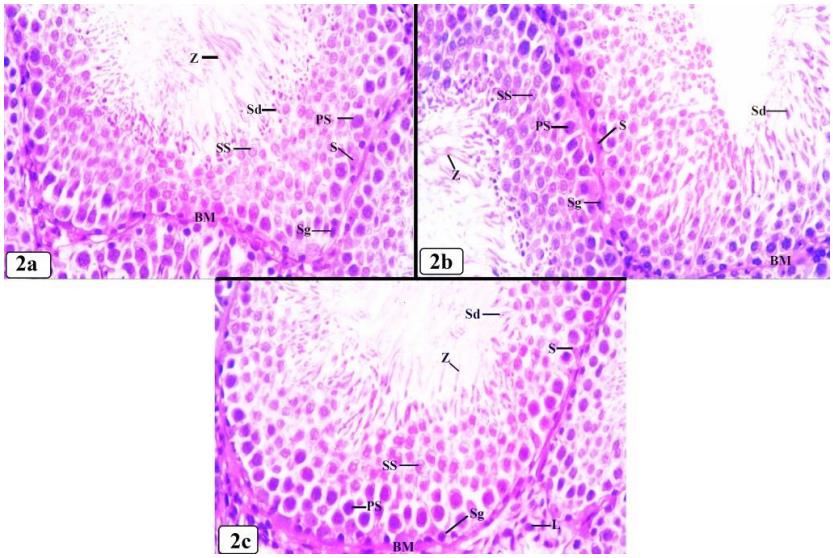

Fig. (2a,2b&2c): light photomicrographs of Transverse Sections (T.S) in seminiferous tubules of rat testes represent control, Cr and PSO groups respectively and show that the tubules are lined by multiple layers with regular arrangement of the spermatogenic cells. Spermatogonia (Sg) appear small and dark and close to basement membrane (BM). 1ry spermatocytes (PS) appear as the largest cells of all spermatogenic cell sequences having large vesicular nucleus. 2ry spermatocytes (SS) are of a small size and vesicular nucleus. Many elongated spermatids (Sd) are observed near the lumen of the tubules. Spermatozoa (Z) appear filling the lumen of the seminiferous tubules. H&E X400

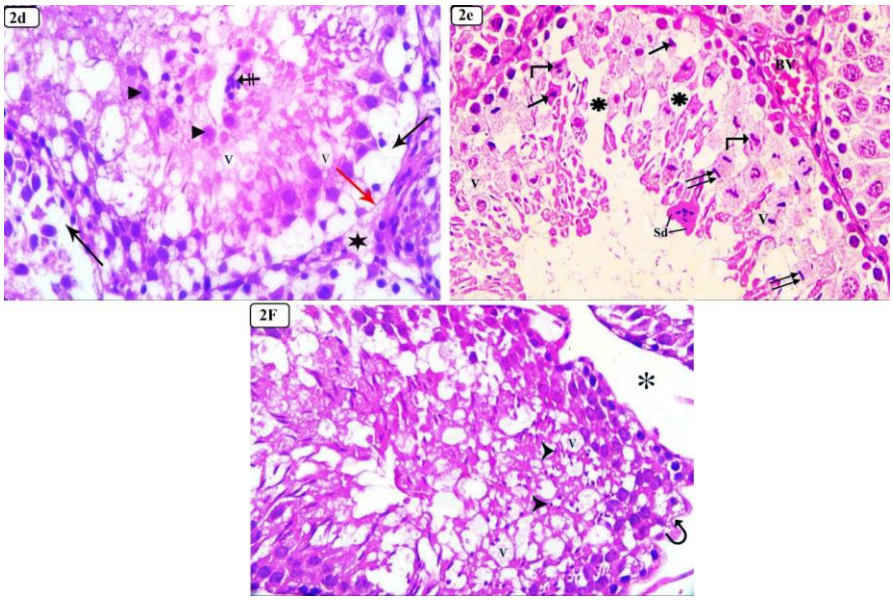

Fig. (2d,2e,2f): light Photomicrographs of seminiferous tubule of rat of (Pb) group;(2d) showing loss of the normal organization of the spermatogenic cells with vacuolation (V). The lumen of the tubule contains dense nuclei (crossed arrow) and dislodged spermatogenic cells inside lumen (arrow heads). The germ cells are sloughed from underlying basement membrane (black arrow). Basement membrane is thin (red arrow) N.B: vacuolation in the cells of interstitial space (star).

(2e): Showing, some spermatogenic cells shows vacuolations (v), pyknotic nuclei (arrows), fragmented nuclei (karyorrhexis) (angled arrows) or margination of their chromatin (double arrow). The cells are separated by wide gaps (astrikes). The lumen shows giant rounded spermatids (Sd) with lacking of spermatozoa. Notice, large congested blood vessel (Bv) in the interstitial space.

(2f): Showing, degeneration of spermatogenic epithelial series leaving remnants of apoptotic cells and pyknotic nuclei (arrow head) occupied most of the seminiferous tubule. Vacuolations (V) are seen in the seminiferous tubule. Basement membrane appears markedly wavy (curved arrows). Wide interstitial-space-separates-the-tubule-(astrike). H&E X400

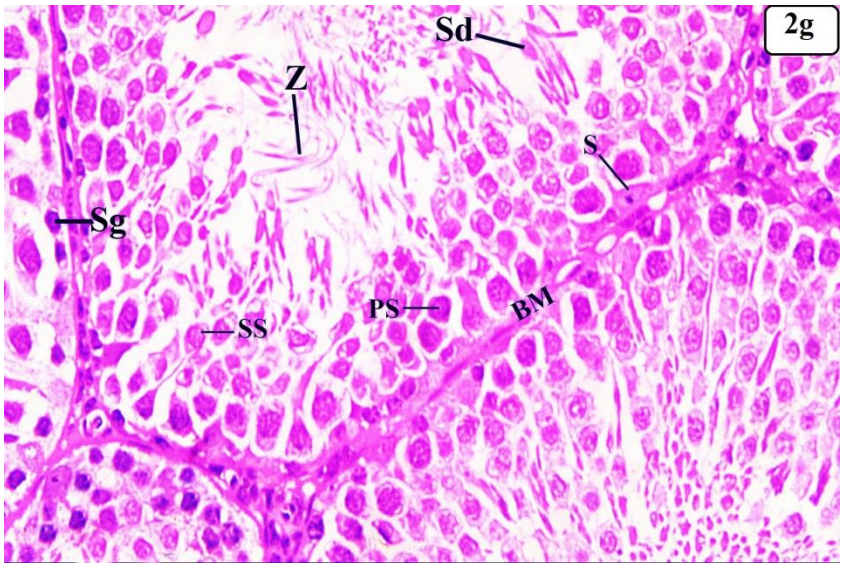

Fig. (2g): A light Photomicrograph of seminiferous tubule of rat of (Cr+ Pb) group showing seminiferous tubules are lined by germinal epithelium which represents the spermatogenic cells in different stages of development from Spermatogonia (Sg), 1ry spermatocytes (PS), 2ry spermatocytes (SS), Spermatids (Sd), up to spermatozoa (Z). Sertoli (S) lies on basement membrane (BM). H&E X400

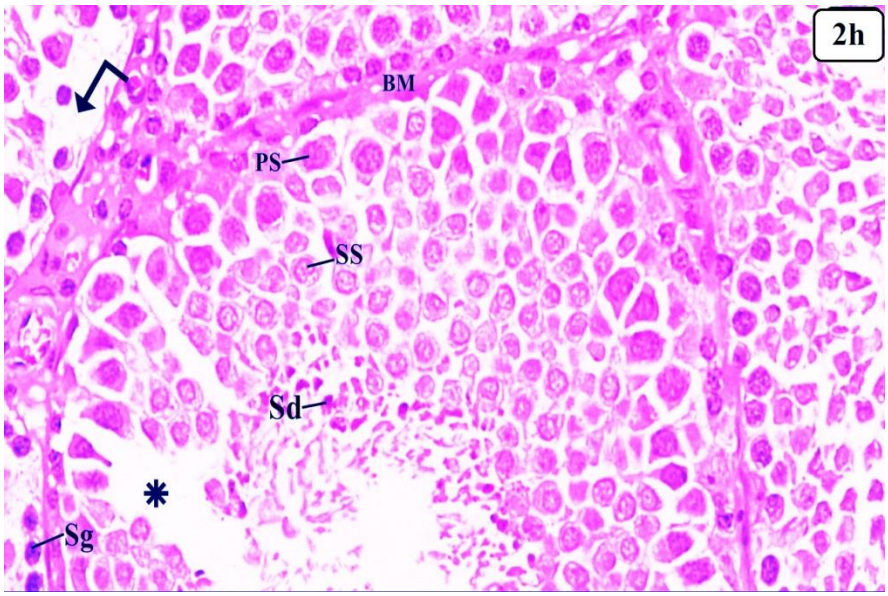

Fig. (2h): A light Photomicrograph of seminiferous tubule of rat of (PSO+ Pb) group showing regular arrangement of the spermatogenic cells sequences as Spermatogonia (Sg), 1ry spermatocytes (PS), 2ry spermatocytes (SS) and Spermatids (Sd). There is wide gap (astrikes) between cells and some germ cells are sloughed from underlying basement membrane (angled arrow). N.B: basement membrane (BM). H&E X400

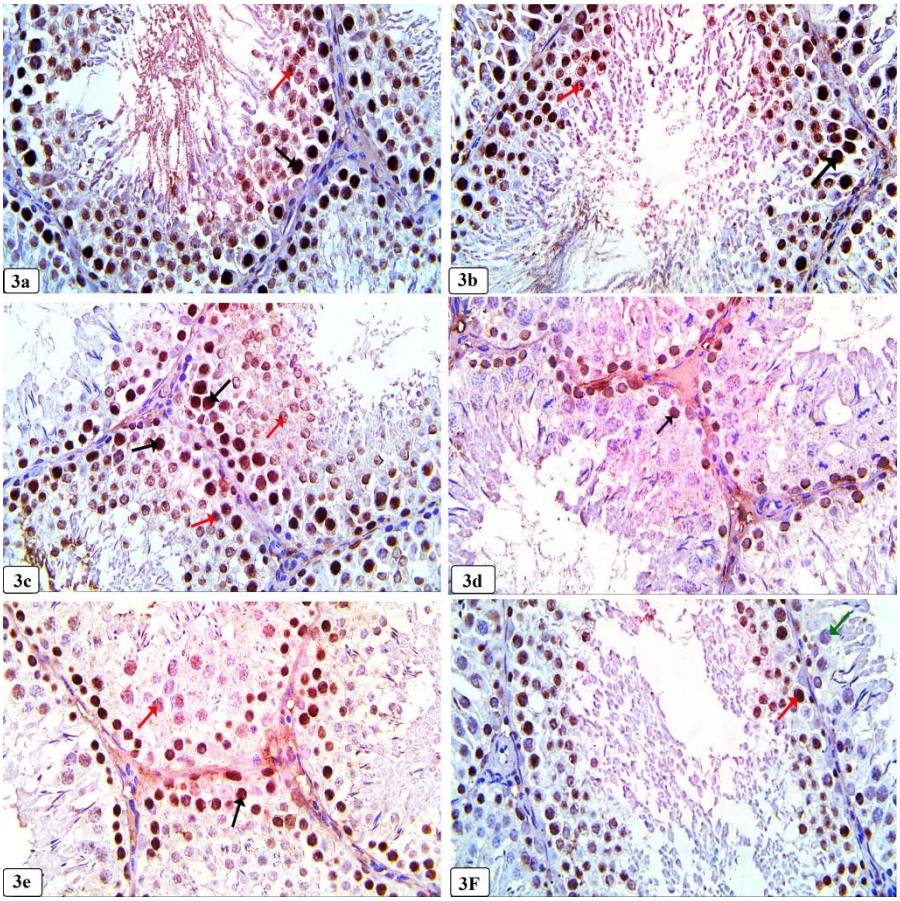

Fig. (3): A light photomicrograph of Transverse Sections (T.S) in testes for all experimental groups. (3a), (3b) &(3c) represent control, Cr and PSO groups respectively, showing strong PCNA immunoexpression in primary spermatocytes (black arrow) and moderate PCNA immunoexpression in secondary spermatocytes (red arrow). (3d) Testes of lead treated rats, showing weak immunoexpression of PCNA in the basal layer only. (3e) Testes of lead and crocin treated rats showed strong PCNA immunoexpression in primary spermatocytes (black arrow) and moderate PCNA immunoexpression in secondary spermatocytes (red arrow). (3f) Testes of lead and PSO treated rats showing moderate immunoexpression of PCNA in primary spermatocytes (red arrow) in some seminiferous tubules and week PCNA immunoexpression (green arrow) in other seminiferous tubules. PCNA x 400

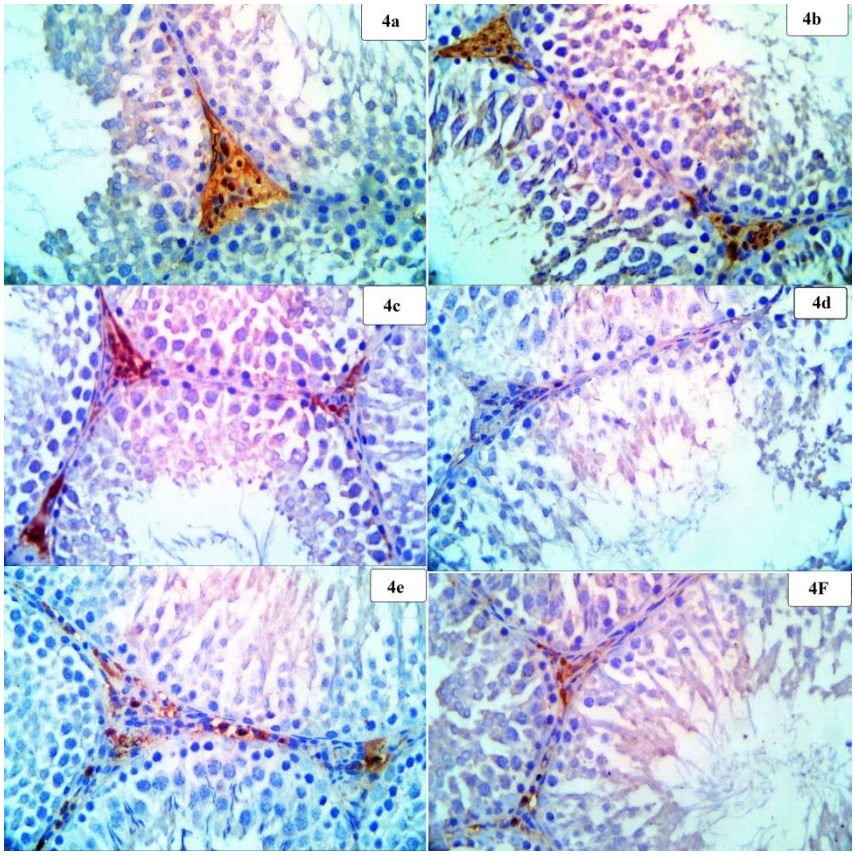

Fig. (4): A light photomicrograph of Transverse Sections (T.S) in testes for all experimental groups. (4a), (4b) &(4c) represent control, Cr and PSO groups respectively, showing intense immunoexpression of androgen receptor in the interstitial cells and lack of immunoreaction in seminiferous tubules. (4d)Testes of lead treated rats, showing minimal AR immunoexpression. (4e) Testis of lead and crocin treated rats showing moderate AR immunoexpression in the interstitial cells. (4f) Testes of lead and PSO treated rats showing moderate AR immunoexpression in the interstitial cells. AR X400

Immunohistochemical results:

Control, Cr and PSO groups, showed strong PCNA immunostained primary spermatocytes and moderate PCNA immunostained secondary spermatocytes. The (Pb) group showed weak PCNA immunostained cells. Sections of (Cr+ Pb) group showed strong PCNA immunostained primary spermatocytes and moderate PCNA immunostained secondary spermatocytes. Sections of (PSO+ Pb) group showed moderate PCNA immunoexpression in some seminiferous tubules and week PCNA immunoexpression in other seminiferous tubules. This was seen in (Fig. 3).

Control, Cr and PSO groups, showed intense immunoexpression in the interstitial cells. Sections of (Pb) group showed minimal AR immunoexpression. Sections of (Cr+ Pb) group showed moderate AR immunoexpression in the interstitial cells. Sections of (PSO+ Pb) group showed moderate AR immunoexpression in the interstitial cells. This was seen in (Fig.4).

Morphometric results:

AR and PCNA immunoreactivity was identical with no statistical significance difference in Cr and PSO groups in comparing with the control group.

In (Pb) group there was a significant decrease in AR immunoreactivity in comparison with control group. Both (Cr+ Pb) and (PSO+ Pb) groups showed

significant elevation in AR immunoreactivity comparison with (Pb) group but still in significant decrease with control group. (PSO+ Pb) group showed significant decrease from (Cr+ Pb), P <0.001. These data were shown in table (2).

In (Pb) group there was significant decrease in PCNA immunoreactivity in comparison with control group. Both (Cr+ Pb) and (PSO+ Pb) groups showed significant elevation in PCNA immunoreactivity in comparison with (Pb) group but still in significant decrease with control group. (PSO+ Pb) group showed significant decrease from (Cr+ Pb), P <0.001. These data were shown in table (2)

No significant difference was observed between control, Cr and PSO in the mean thickness of the spermatogenic cell layers in the seminiferous tubules, but it significantly decreased in (Pb) group in comparison with control group . Both (Cr+ Pb) and (PSO+ Pb) groups showed significant increase in the mean thickness of the spermatogenic cell layers in comparison with (Pb) group but still in significant decrease with control group , P <0.001. These data were shown in table (2)

Table (2): Shows the mean area% of AR, PCNA immunoreactivity and mean thickness of the spermatogenic cell layers in different studied groups.

	Control	Cr	PSO	Pb	Cr+ Pb	PSO+ Pb
AR($IDx10^5$)	768.6±90.87	775.6±81.12	783.6±82.01	192.6±26.59^{abc}	483.2± 49.53 ^{abcd}	335.6±34.76 ^{abcde}
PCNA ($IDx10^5$)	860.5±99.04	887.4±91.83	883.6±102.9	354.1±41.91 ^{abc}	556.1±52.78 ^{abcd}	479.3±53.09 ^{abcde}
Mean±SD thickness (nm)	169.6±18.04	166.1±18.15	164.1±20.57	70.56±7.350 ^{abc}	119.0±16.50 ^{abcd}	96.92±7.498 ^{abcde}

Data expressed as mean±SD , P:Probability *:significance <0.05

Test used: One way ANOVA followed by post-hoc tukey

a: Significance vs Control, b: Significance vs Cr, c: Significance vs PSO, d:Significance vs Pb , e: Significance vs Cr+ Pb.

Discussion:

The lead induces testicular toxicity through oxidative stress that makes an imbalance between generation and removal of reactive oxygen species (30).

The present study was planned to evaluate harmful effect of lead acetate on the histological patterns and hormonal functions of testis and the possible protective effect of crocin and Pumpkin seed oil.

In the current study, lead treated group showed significant reduction in serum level of testosterone, LH and FSH as compared to control group, this was in agree with (31,32,33,34). Decrease serum testosterone may be due to direct toxic effect of Pb on leydig cells causing its apoptosis (35). Pb inhibits steroidogenic enzyme production in Leydig cells resulting in reduced testosterone secretion (36). Pb causes degeneration of pituitary gland gonadotrophic cells leading to decrease LH level and subsequent loss of stimulation of

testosterone secretion (37). Researches revealed that when level of lead in blood reached above 40 μg/dL led to decrease FSH and LH levels, supporting idea of secondary effect of lead on hypophysis in the long-term exposure of Pb, while its level was less than 40 μg/dL, FSH and LH levels increased (38). Another study reported a rise in LH levels after lead toxicity (39). This difference in hormones levels in different studies may be due to the difference in duration of lead exposure, technique of measuring or route of administration.

In this study administration of crocin significantly increased LH, FSH and testosterone hormones compared to lead treated group, this was in the same line with results reported by (40, 41). In this study PSO significantly upgraded sex hormonal levels after lead testicular injury and this was in agree with (42).

The histological examination in this study revealed that lead toxicity could cause histological changes indicating impairment of spermatogenesis process, included focal degeneration of spermatogenic series in most of seminiferous tubules, disorganization, vacuolations of spermatogenic cells, dislodged spermatogenic cells inside lumen and congestion in testis blood vessels. These pathological findings were the same as (33,38,43). Lead crosses the blood-testis barrier, accumulate in the testis, and damage germinal cells (44). Many studies have shown that lead acetate decrease level of testicular antioxidant enzymes such as (SOD), (GPx) and (CAT), shifting the oxidant/antioxidant profile towards oxidant side (36, 1, 45). Previous study demonstrated that exposure to lead leads to increasing level of malondialdehyde (MDA) which causes lipid peroxidation (46). The pathogenesis of lead toxicity occurs by oxidative stress due to excessive generation of (ROS) that induces apoptosis (47,48). There were gaps between cells in seminiferous tubules and we suggested that the cause may be due to reduction of number of Sertoli cells.

In the current study, there was degeneration of spermatogenic epithelial series leaving remnants of apoptotic cells and pyknotic nuclei occupied most lumen of the seminiferous tubule, these findings were observed by (49). Other signs of apoptosis were found in this study as pyknotic nuclei, karyorrhexis and margination of their chromatin. Other investigators documented an increase in Bax and caspase 3 expressions, and reduced Bcl2 expression in the testicular cells after lead toxicity (36,1).

In this study, sections of Pb treated rats showed wide interstitial spaces between seminiferous tubules indicating interstitial edema that may be the cause of thin wavy basement membrane. This was in contrast with other authors who found thick basement membrane and attributed this to stimulation of myeloid cell to produce of collagen fibers and extracellular matrix (24). We suggested that this difference may be due to different duration of lead exposure.

The immunohistochemical results revealed marked reduction in PCNA immunoexpression in Pb treated rats. This was in the same line with study that recorded a significant reduction in the PCNA index in rats after testicular cytotoxicity (50). PCNA is not only involved in DNA replication but also in DNA repair so presence of positive PCNA expression in testicular cytotoxicity a response for spermatogenesis dysfunction (51).

In the present study there was marked reduction in androgen receptor immunoexpression in the interstitial cells of testis of lead treated rats, this was the same result as (8,25, 52). We suggested that, this may due to apoptosis of interstitial cells of Leydig and edema in the interstitium.

The hormonal results of rats treated with crocin with lead were confirmed by the histological findings that revealed great improvement in the architecture of testis, marked increase in PCNA and AR immunoexpressions. Our results are reliable with the results of previous experiments that studied the protecting effects of crocin against cisplatin, cyclophosphamide, paraquat, nicotin and cadmium -induced testicular destructive changes (19, 20, 27, 40, 53) respectively. Another study demonstrated that crocin decreased apoptosis in the germinal epithelium of seminiferous tubules in testicular tissue after exposure to electromagnetic fields (54). Cr inhibits the intrinsic apoptosis pathway by increasing bcl-2 expression and decreasing the caspase-3 expression, and also diminishes DNA damage through reduction of expression of p53 (27). The antioxidant property and lipid peroxidation inhibition capacity of Cr are due to reaction of Cr with free radicals (ROS) to convert them into more stable products (55). Many studies have proved that Cr could effectively restore oxidant-antioxidant balance and prevents lipid peroxidation by decreasing testicular MDA levels and enhancement of SOD activity (19, 27, 56).

In this study ,PSO had ameliorative role in lead induced testicular toxicity, this was indicated by improvement of testicular function by significant increase of hormonal levels and improvement of testicular architecture with significant increase in PCNA and AR immunoexpressions; this was in the agreement with those reported by (57) who found that PSO normalized the testicular tissue architecture and sex hormones levels. Phenolic compounds, vitamins A, E and zinc in pumpkin interpret its antioxidant action by neutralizing free radical generation and can diminish DNA damage in the rat testis (58). A Study concluded that PSO have antioxidant effect by down –regulation of gene expression Nrf2 that was increased after lead acetate exposure (57). PSO supplementation could inhibit lipid peroxidation through lowering level of MDA (59). Other studies proved that PSO is strong antioxidant as it elevated (GSH), (CAT) and decreased DNA fragmentation in testis (60 ,61).

Conclusion

It can be concluded that Cr and PSO may have ameliorative effects after lead acetate toxicity on rat testis at endocrine, histological and immunohistochemical levels.

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