Life Sciences 230 (2019) 132–140

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved

Eman Hassan[a,**], Mahmoud El-Neweshy[b,*], Marwa Hassan[c], Ahmed Noreldin[d]

[a] Department of Biochemistry, Faculty of Science, Mansoura University, Eldakahliya, Egypt
[b] Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt
[c] Department of Anatomy and Embryology, Faculty of Medicine, Helwan University, Cairo, Egypt
[d] Department of Histology and Cytology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

ARTICLE INFO

Keywords:
Apoptosis
Aromatase P450
Lead
Oxidative stress
Reproductive toxicity
Thymoquinone

ABSTRACT

Aims: The testis is one of the main target organs for lead (Pb) toxicity. The current study was investigated the mechanism (s) of the therapeutic potential of thymoquinone (TQ), the active principle of *Nigella sativa* seed, against testicular toxicity following subchronic Pb exposure in the light of cytopathic effects, apoptotic signaling pathways, oxidative stress, serum sex hormones levels and testicular aromatase gene expression.
Materials and methods: Thirty-two male albino rats were randomly allocated into control, PbAc (20 mg PbAc/kg bwt, orally), TQ (5 mg TQ/kg bwt dissolved in corn oil, orally), and PbAc + TQ groups for 56 successive days.
Key findings: PbAc-treated rats showed significant decrease of testes and epididymes weights, sperm count, motility and viability, spermatogenesis score and serum FSH, LH, testosterone and estradiol levels, as well as a significant decreased testicular antioxidant molecules (Superoxide dismutase enzyme and reduced glutathione), and a significant elevation of sperm abnormalities, oxidative biomarkers (Malondialdehyde and Nitric oxide) compared to a control group. In addition, Pb induced significant downregulation of aromatase gene expression, activation of Bax and Caspase-3 apoptotic pathways. Moreover, Pb caused complete seminiferous tubules hyalinization (38%), germinal epithelium sloughing (15%) and hypocellularity (8%). However, administration of TQ with PbAc improved sperm quality, testicular histology and oxidative/antioxidative status, and serum levels of LH, testosterone and E2 with respect to PbAc group. Additionally, TQ with PbAc significantly lessen the staining intensity and the area of Bax and Caspase-3 immunoexpression.
Significance: TQ might exert its acceptable therapeutic potential against Pb-induced testicular and spermotoxicity via anti-oxidative, endocrine and anti-apoptotic pathways.

1. Introduction

The impact of environmental toxicants on male fertility remains a matter of public health concern, primarily in industrial countries. Human and animal exposure to heavy metals (e.g., lead, cadmium, mercury, and arsenic) harmfully affects the male fertility. Many studies are available on male reproductive toxicity of lead (Pb) due to the common use of Pb in industries, medicine, and cosmetics [1]. The Pb toxicity causes dose-dependent testicular lesions with various degrees ranged from germinal epithelium degeneration to necrobiotic changes with a complete arrest of spermatogenesis [2,3]. Pb exposure induces DNA damage in spermatozoa and causes poor sperm parameters [1,4]. Many studies have attempted to explain the integrated mechanisms of Pb-induced testicular toxicity that are summarized into (i) lipid peroxidation (LPO) and overproduction of reactive oxygen species (ROS) in testicular tissues [5–7]. (ii) Direct toxic effect on spermatogenic and Leydig cells [6,8,9]. (ii) Suppression of anterior pituitary secretion of LH and FSH, thereby hormonal disruption [9,10]. (iii) Decrease the ability of sperm to fertilize ova [11,12]. (iv) Induction of spermatogenic cells apoptosis via activation of caspase-3 [13–15].

Abbreviations: ANOVA, One-way analysis of variance; cDNA, Complementary DNA; CYP19, Aromatase P450; E2, 17β-estradiol; ELISA, Enzyme linked immunosorbent assay; FSH, Follicular stimulating hormone; GSH, Reduced glutathione; LH, luteinizing hormone; LPO, Lipid peroxidation; MDA, Malondialdehyde; NO, Nitric oxide; Pb, Lead; PbAC, Lead acetate; ROS, Reactive oxygen species; RT-PCR, Reverse transcriptase-polymerase chain reaction; SOD, Superoxide dismutase; TQ, Thymoquinone

* Corresponding author at: Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Beheira, 22758, Egypt.
** Corresponding author.
E-mail addresses: dremanhassan77@gmail.com (E. Hassan), mahmoud.neweshy@alexu.edu.eg (M. El-Neweshy).

https://doi.org/10.1016/j.lfs.2019.05.067
Received 7 May 2019; Accepted 24 May 2019
Available online 25 May 2019
0024-3205/

Accordingly, much effort has been devoted to investigating various remedies to lessen the reproductive toxicity of Pb. Thymoquinone (TQ) is the active principle component extracted from *Nigella sativa* seed oil. TQ has been widely contemplated for its biological activities and remedial potential as antihypertensive, anticancer, antidiabetic, anti-inflammatory, antioxidant and pain relieving agent [16,17]. Previous investigations indicated that TQ has a protective effect against Pb-induced hepatic [18] and brain [19] damage. Moreover, it has been suggested that TQ play a role in retrieving the effect of various harmful conditions on male reproduction, such as heat stress [20], toluene-induced impairment of spermatogenesis in rats [21], methotrexate-stimulated testicular damage in mice [22], and ischemic testicular damage caused by torsion-detorsion in mice [23]. The high natural biological properties and low poisonous quality of TQ make it a promising target to the designed study. Two previous studies investigated the protective effect of TQ against Pb-induced low epididymal sperm count [24] and testicular lesions [3], whereas the mechanism (s) of this effect has not been completely elucidated. Thus, the current study was designed to investigate the mechanism (s) of the therapeutic potential of TQ on Pb-induced male reproductive toxicity in the light of cytopathic effects, apoptotic signaling pathways, oxidative stress, serum sex hormones levels and testicular CYP19 gene expression.

2. Materials and methods

2.1. Animals

Thirty-two male albino rats (130–150 g), aged 3–4 months were obtained from a closed bred colony at Mansoura University (Eldakahliya, Egypt). All rats were housed in cages under controlled conditions throughout the experiment. All rats fed on a standard diet in the form of dry chow pellets standard laboratory diet composed of 161 g/kg protein, 36.4 g/kg fat, 41.1 g/kg fiber and 12.1 MJ metabolizable energy, and received water ad libitum through the experiment. The experiment was approved by Local Ethics Committee of Medical Experimental Research Center of Mansoura University and followed the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

2.2. Experimental design

Two weeks later, acclimated rats were allocated into four groups ($n = 8$/each). Group I (Control) was given daily corn oil by gastric tube (0.5 ml/rat), the vehicle used for TQ. Group II (TQ) received daily oral TQ (purity ≥98.5, Sigma-Aldrich Chemical Co., St. Louis, USA) at a dose of 5 mg/kg body weight (bwt) dissolved in corn oil (0.5 ml/rat) by gastric tube; the dose of TQ was chosen according to Mabrouk [3]. Group III (PbAc) received oral lead acetate trihydrate (PbAc), purity ≥99.99% (Sigma-Aldrich Chemical Co., St. Louis, USA) via gastric tube at concentration 20 mg/kg bwt dissolved in distilled water [2]. Group IV (PbAc + TQ) received PbAc then, after 1 h, TQ at the same dose and route of the PbAc and TQ groups, respectively. The experiment was conducted for 56 consecutive days; the experiment period was chosen based on the time required to full spermatogenic cycle in rats [25].

2.3. Sampling

After 24 h of the last treatment, blood samples were collected from the retro-orbital venous plexus under sodium pentobarbital anaesthesia. Serum was separated from blood clot by centrifugation. The separated serum samples were kept frozen at −20 °C for subsequent analysis of serum sex hormones levels (testosterone, estradiol (E2), LH and FSH). All rats were immediately sacrificed by decapitation. Testes and epididymides were removed, washed and weighted. Instantly after weighting, cauda epididymes were used to evaluate sperm concentration. In addition, the left testis of each rat was immediately fixed in 10% buffered formalin for at least 48 h to prepare paraffin-embedding blocks to evaluate the testicular histopathology, spermatogenesis scoring, and immunohistochemical detection of apoptosis. The right testis was crossly cut; one-split was thoroughly washed with ice-cold phosphate buffer saline and stored at −80 °C for consequent estimation of oxidative stress and antioxidant biomarkers. While the other split was immediately immersed in liquid nitrogen and stored at −80 °C for subsequent analysis of aromatase P450 gene (CYP19) expression by real-time quantitative polymerase chain reaction (qPCR).

2.4. Evaluation of sperm parameters

The epididymal sperm concentration was estimated as described previously [26]. Briefly, cauda epididymis was minced in 5 ml of warm physiological saline, and incubated at 37 °C for 30 min, then diluted 1:100 in a solution contain 25 mg eosin, 5 g NaHCO3 and 1 ml formalin 35% per 100 ml distilled water. 10 µl of diluted semen was examined under a light microscope at x200 magnification using Neubauer hemocytometer slide. Sperm progressive motility was evaluated microscopically and expressed as a rate of motile and non-motile sperms according to the described method [27]. In addition, the viability and abnormalities of epididymal sperm were evaluated as described previously [28]. Briefly, 2 µl of epididymal fluid was stained with warm eosin-nigrosin stain (20 µl) for 20 min at 37 °C. Smears from stained epididymal fluid were examined under 40× magnification with bright-field microscopy to calculate the percentage of unstained (live) and abnormal sperms.

2.5. Testicular histopathology and spermatogenesis scoring

Fixed testicular tissue specimens were processed using paraffin-embedding technique according to Culling [29]. Briefly, 5-µm thick sections were obtained from each testicular paraffin block and stained with hematoxylin and eosin (H&E). In each cross-section, twenty randomly selected seminiferous tubules (STs) were scored under a light microscope at X400; the mean score was determined for each group. The spermatogenesis was graded using Johnsen's Scoring System [30] with a score ranging from 1 to 10 according to the presence or absence of the main cell types and/or lesions.

2.6. Apoptosis detection by Bax and Caspase-3 immunohistochemistry

Deparaffinized paraffin sections were pretreated with 3% H2O2 in absolute methanol for 30 min at 4 °C, and then incubated with 10% (v/v) normal goat serum for an hour. After that, the sections were incubated with rabbit polyclonal anti-Bax (1:30, PU347-UP, San Ramon, CA, USA) or rabbit polyclonal anti-cleaved Caspase-3 (1:100, BioCare Medical, Cat. CP229C, Concord, CA, USA) overnight at 4 °C then incubated with biotin-conjugated goat anti-rabbit IgG antiserum (Histofine kit, Nichirei Corporation) for 1 h, followed by incubation with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation) for 30 min. After that, the sections were incubated for 3 min in diaminobenzidine tetra-hydrochloride (DAB)-H2O2 solution, and counterstained with Mayer's hematoxylin solution. Finally, the sections were examined at 100× and 10 photomicrographs were captured from different fields per section. In the captured photographs, the percent of testicular tissues that exhibited Bax or Caspase-3 immunoreactivity were measured using Image J software (Image J; v1.46r, NIH, Bethesda, MD, USA) [31].

2.7. Determination of testicular oxidant/antioxidant biomarkers

A 10% testicular tissue homogenate was prepared in 50 mM potassium phosphate buffer solution (pH 7.4) containing 1.15% KCl using a homogenizer with Teflon pestle, then the testicular homogenate was centrifuged at 8000 rpm/10 min, finally the supernatant was collected.

Malondialdehyde (MDA), the lipid peroxidation biomarker, and Nitric oxide (NO) concentration as total nitrite/nitrate were measured in aliquots of the testicular supernatant using the methods of Mabrouk and Ben [32] and Koltuksuz et al. [33], respectively. Additionally, superoxide dismutase enzyme (SOD) and reduced glutathione (GSH) content were estimated in aliquots of the supernatant according to the methods described by Nishikimi, Rao [34] and Sedlak and Lindsay [35], respectively.

2.8. Assessment of CYP19 gene expression by qPCR

Total RNA was extracted from the frozen testis sample (four samples per group) using RNA Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration and quality were assessed using NanoDrop 2000 spectrophotometer (Thermo Scientific, California, USA). Synthesis of complementary DNA (cDNA) using Superscript II Reverse Transcriptase was done according to the manufacturer's guidelines (Thermo Scientific, California, USA). The PCR reaction for each sample was applied in triplicate using Step One Plus RT-PCR System (Applied Biosystem, USA) to evaluate CYP19 gene expression. CYP19 was amplified using 2× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA) following the manufacturer's protocol and primers (Forward primer:′5-TGGAACCTG CCCCCAGGACC-′3; Reverse primer: ′5-CCACGATGCGCCTTGAGCCA-′3) B-actin (Forward primer: ′5-AGGGAAATCGTGCGTGAC-′3; Reverse primer: ′5-CGCTCATTGCCGATAGTG-′3) was used as reference gene. The thermal condition of PCR reaction was performed as follows: pre-incubation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 15 s and extension at 72 °C for 15 s according to pervious method [36]. At the end of each extension, fluorescence intensities were assessed and relative quantitation of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [37].

2.9. Estimation of serum sex hormones levels

According to the procedure given along with the utilizing a commercial kit (Diagnostic System Laboratories Inc., Webster, USA): total serum concentrations of testosterone, LH and FSH were measured by enzyme-linked immunosorbent assay (ELISA); while serum concentration of E2 was measured by radioimmunoassay.

2.10. Statistical analysis

Statistical significance in each treated group in respect with the control group was determined using GraphPad Prism software 7.0 by one-way analysis of variance (ANOVA) followed with Dunnett's multiple comparison test. The effect of TQ administration on PbAc-induced changes was determined with respect to PbAc group (a positive control) using unpaired *t*-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Sex organs weights

As shown in Fig. 1, a significant decrease in the weights of paired testes ($p < 0.001$) and epididymes ($p < 0.05$) in rats treated with PbAc compared to the control group. Interestingly, TQ was able to prevent the toxic effects of PbAc on the weights of paired testes and epididymes. Additionally, rats only treated with TQ showed a significant increase ($p < 0.05$) in the weight of paired testes about 61.7% compared to the control.

3.2. Epididymal sperm quality

Epididymal sperm characteristics are presented in Fig. 2. Epididymal sperm concentration, motility and viability were significantly ($p < 0.001$) lowered in the PbAc group compared to the control group. On the other hand, TQ administration with PbAc significantly ($p < 0.0001$) improved the epididymal sperm concentration and motility by about 42% and 15%, respectively, and significantly ($p < 0.001$) alleviated the toxic effect of PbAc on sperm viability by about 45.48%. On the other hand, rats only treated with TQ exhibited significant ($p < 0.01$) increase in sperm concentration and motility compared to control rats. Sperm abnormality was significantly ($p < 0.001$) elevated in PbAc group compared to control group, while this effect was significantly ($p < 0.0001$) improved by coadministration of TQ with PbAc to the level of control.

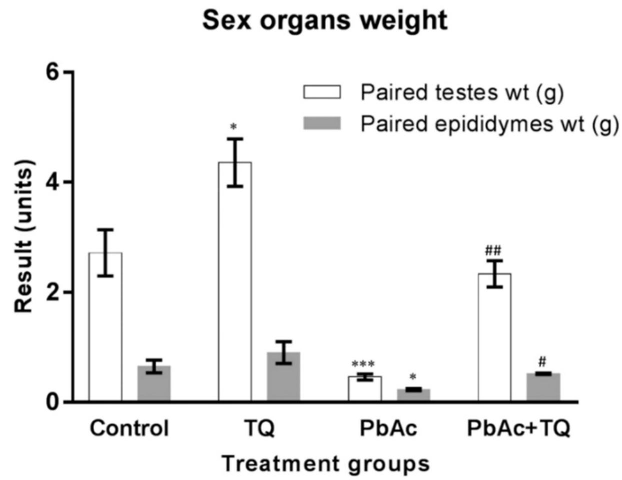

Fig. 1. Alleviating effect of oral thymoquinone administration on the changes in the weights of paired testes and epididymes of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. * Significance at $p < 0.05$; *** Significance at $p < 0.001$ with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test), # Significant change at $p < 0.05$; ## Significant change at $p < 0.01$ with respect to PbAc group as a positive control (Unpaired *t*-test).

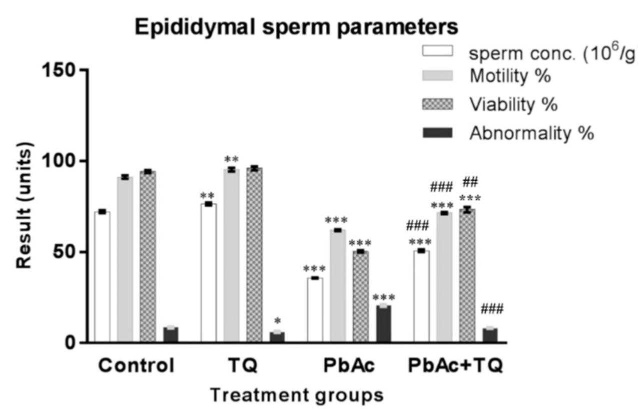

Fig. 2. Alleviating effect of oral thymoquinone administration on the epididymal sperm characteristics of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. * Significance at $p < 0.05$; ** Significance at $p < 0.01$;*** Significance at $p < 0.001$ with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test), ## Significant change at $p < 0.001$; ### Significant change at $p < 0.0001$ with respect to PbAc group as a positive control (Unpaired *t*-test).

3.3. Testicular histopathology

PbAc caused marked testicular lesions (Fig. 3A) compared to control and TQ groups (Fig. 3A and B, respectively) where 62% of the examined

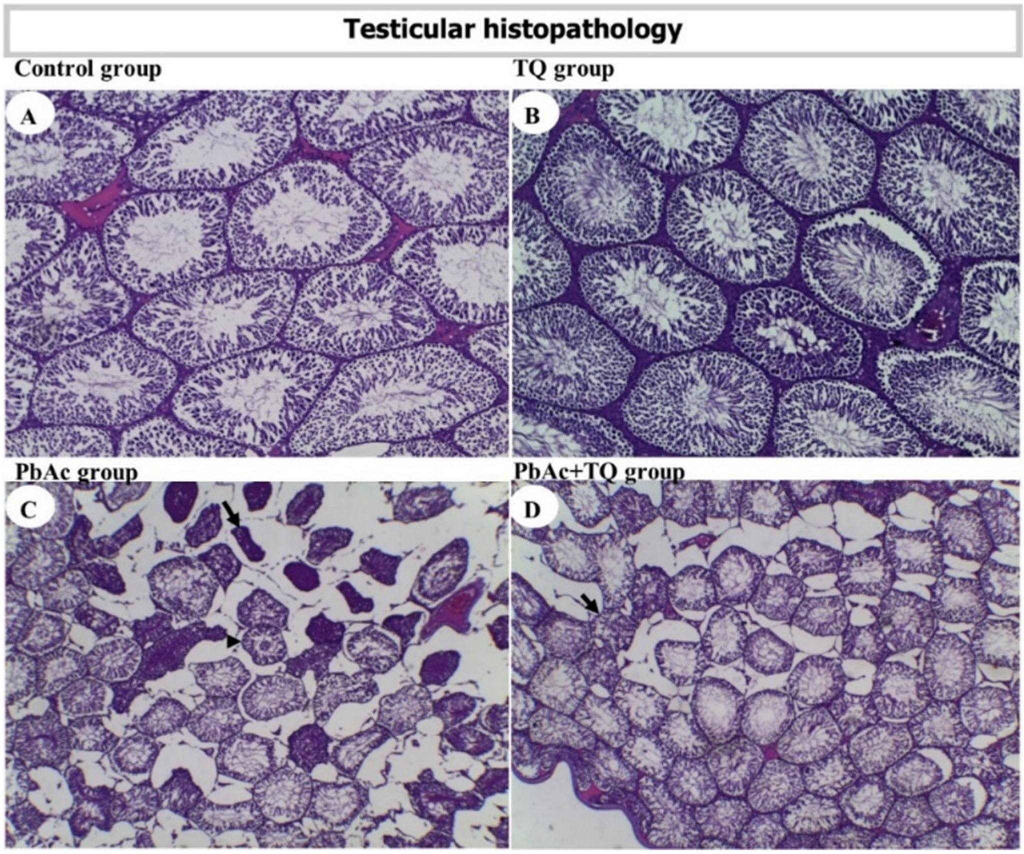

Fig. 3. Oral thymoquinone administration improves lead acetate-induced testicular damage: (A) control group appeared with normal seminiferous tubules (STs) lined with multilayer of spermatogenic cells and their lumina contain free spermatids (X200), (B) TQ group showed normal STs and interstitium architecture (X200). (C) Rats treated with lead acetate (20 mg/kg bwt) for 56 days exhibited marked histopathological changes include necrosis and hyalinization (long arrow), impacted STs with sloughed germinal epithelium (arrowhead) and many STs with hypocellularity (X100). (D) Rats treated with lead acetate and thymoquinone showed few hyalinized and impacted STs (arrow) with few STs with hypocellularity while the most STs histologically normal (x100). Sections were stained with H&E.

seminiferous tubules (STs) showed various histopathological lesions consisted of disorganization and complete hyalinization (38%), tubular blockage with sloughed germinal epithelium (15%) and germinal epithelium hypocellularity (8%), while the remained 38% of STs showed active spermatogenesis. While, TQ Treatment largely lessen PbAc-induced structural damage (Fig. 3D) by decreased the percent of abnormal STs to 29% consisted of disorganization (14%), tubular blockage with sloughed germinal epithelium (8%) and germinal epithelium hypocellularity (7%).

3.4. Spermatogenesis score

As shown in Fig. 4 the spermatogenesis score was significantly ($p < 0.001$) declined in PbAc treated rats compared to the control. Moreover, oral TQ coadministration shortly after PbAC significantly ($p < 0.0001$) enhanced the spermatogenesis score by about 79.7%.

3.5. Apoptosis detection

Bax antigen was detected in different germinal epithelium, Sertoli cells and Leydig cells. Staining intensity was more pronounced in testes of PbAc-treated group, this staining intensity was reduced in testis of rats treated with TQ and PbAc as presented in Fig. 5. The testicular area exhibited Bax immunoreactivity in PbAc-treated rats was 65.63 ± 2.29%, which significantly ($p < 0.0001$) reduced by TQ administration to 24.38 ± 1.61 (Fig. 7).

Un approximate 55.88 ± 1.6% of testicular tissues in PbAc-treated rats showed strong staining intensity of Caspase-3 antigen in Sertoli cells and the resident germ cells and to a lesser extent Leydig cells. Again, Caspase-3 staining intensity caused by PbAc was reduced upon administration of TQ. Additionally, the testicular area that displayed Caspase-3 immunoreactivity was significantly ($p < 0.0001$) decreased as shown in Figs. 6 and 7.

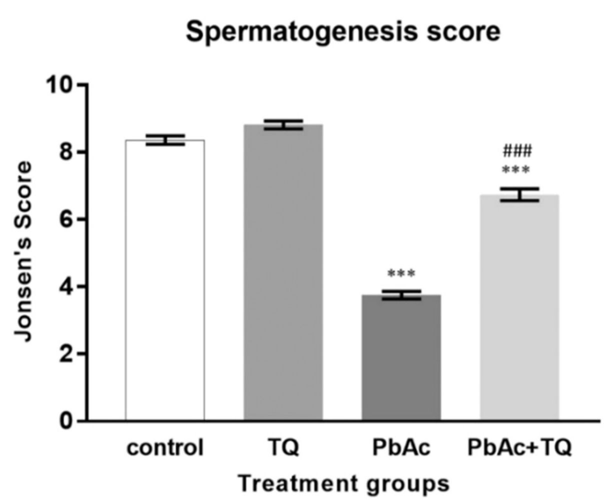

Fig. 4. Alleviating effect of oral thymoquinone administration on spermatogenesis score of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. *** Significance at $p < 0.001$ with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test). ### Significant change at $p < 0.0001$ with respect to PbAc group as a positive control (Unpaired *t*-test).

3.6. Testicular oxidative stress

PbAc induced significant ($p < 0.001$) increase in testicular MDA and NO compared to the control. Testes of rats only received TQ exhibited significant ($p < 0.05$) decrease of MDA content but not showed a significant change in NO. Furthermore, TQ treatment shortly after PbAc significantly alleviated the toxic effects of PbAc on testicular MDA and No by 82.5% and 15.8%, respectively. The latter results are

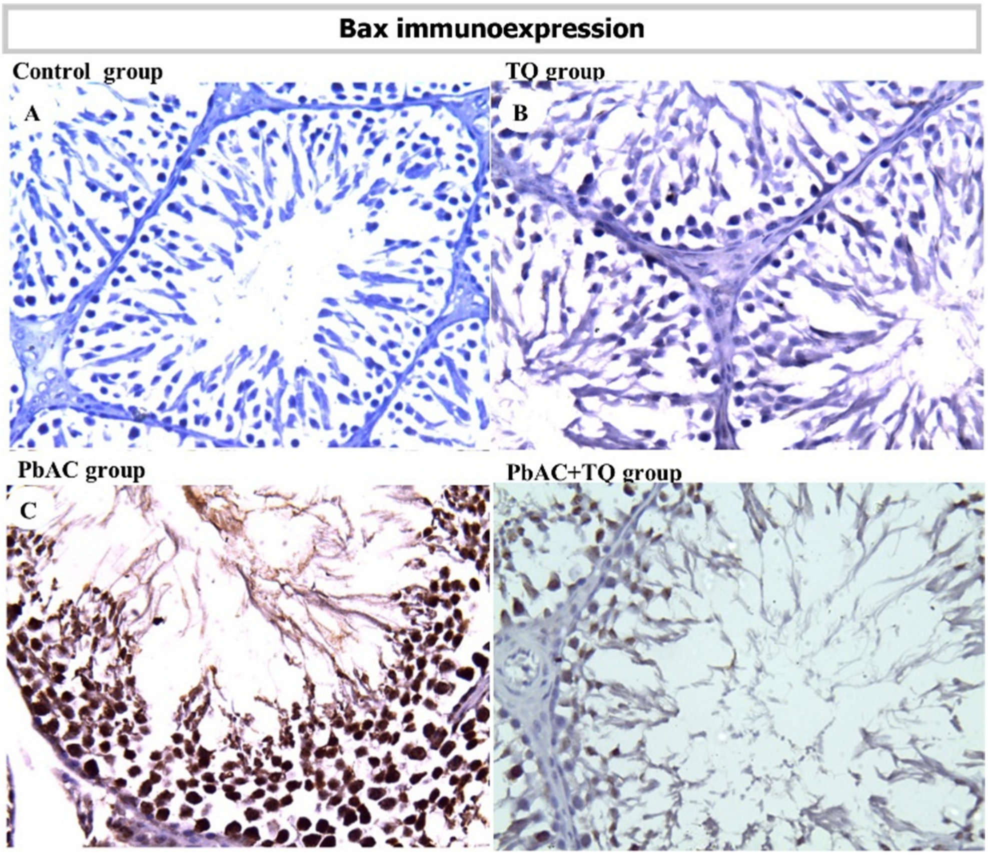

Fig. 5. Immunohistochemical localization of Bax antigen in testes of the control and treated groups. (A) Control. (B) Rats treated with oral thymoquinone. (C) Rats treated with lead acetate: Seminiferous tubules (Sertoli cells and different spermatogenic cells stages and to lesser extent in leydig cells) showed positive staining of Bax. (D) Rats treated with lead acetate and thymoquinone. All micrographs at X400.

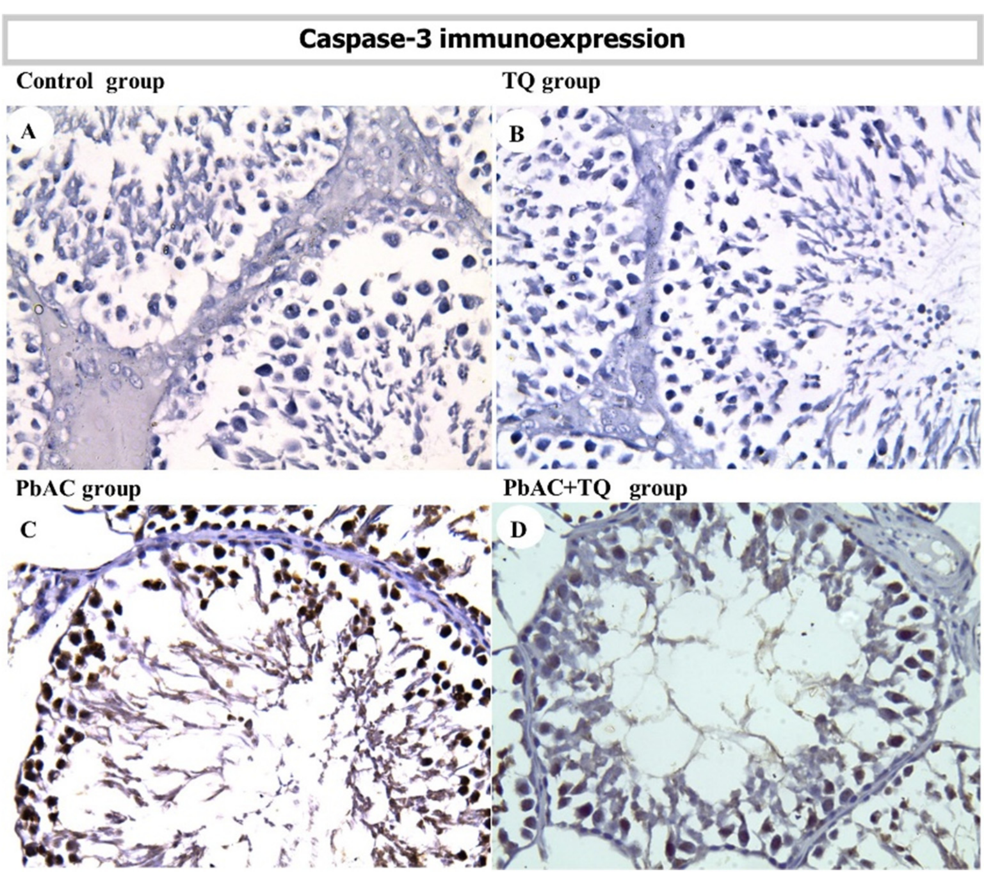

Fig. 6. Immunohistochemical localization of Caspase-3 antigen in testes of the control and treated groups. (A) Control. (B) Rats treated with oral thymoquinone. (C) Rats treated with lead acetate: Seminiferous tubules (Sertoli cells and different spermatogenic cells stages and to lesser extent in leydig cells) showed positive staining of Caspase-3. (D) Rats treated with lead acetate and thymoquinone. All micrographs at X400.

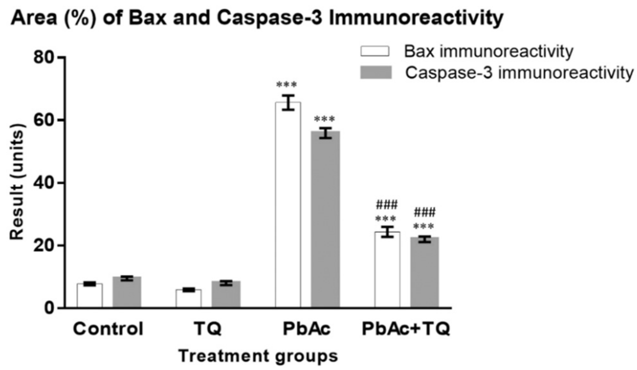

Fig. 7. Alleviating effect of oral thymoquinone administration on testicular apoptosis of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. *** Significance at $p < 0.001$ with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test). ### Significant change at p < 0.0001 with respect to PbAc group as a positive control (Unpaired *t*-test).

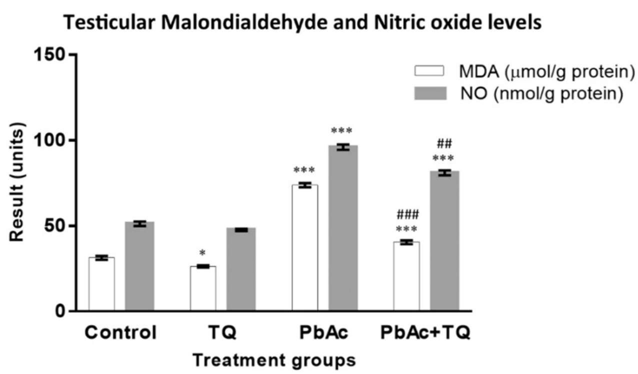

Fig. 8. Effects of oral thymoquinone administration on MDA and NO formation in testes of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. * Significance at p < 0.05; *** Significance at p < 0.001 with respect to the control group as negative a control (ANOVA with Dunnett's multiple comparison test). ## Significant change at p < 0.001; ### Significant change at p < 0.0001 with respect to PbAc group as a positive control (Unpaired *t*-test).

presented in Fig. 8.

3.7. Testicular antioxidative status

PbAc caused significantly ($p < 0.001$) reduction in testicular SOD and GSH compared to the control. On contrary, rats received only TQ showed improved testicular antioxidant values indicated by significant elevation of SOD and GSH compared to control values. Again, TQ treatment prevented the toxic effect of PbAc on the testicular tissues through increase testicular SOD and GSH to control levels (Fig. 9).

3.8. CYP19 gene expression

A significant ($p < 0.001$) downregulation of CPY19 gene expression in testes of PbAc-treated rats compared to control. While rats received TQ alone showed a non-significant change of relative CYP19 gene expression in their testes. Furthermore, administration of TQ with PbAc had no significant effect on PbAc-induced CYP19 gene downregulation in testes. These results are presented in Fig. 10.

3.9. Serum sex hormones levels

PbAc-treated rats showed a significant decline in serum levels of testosterone ($p < 0.01$) and E2 ($p < 0.001$) with respect to control.

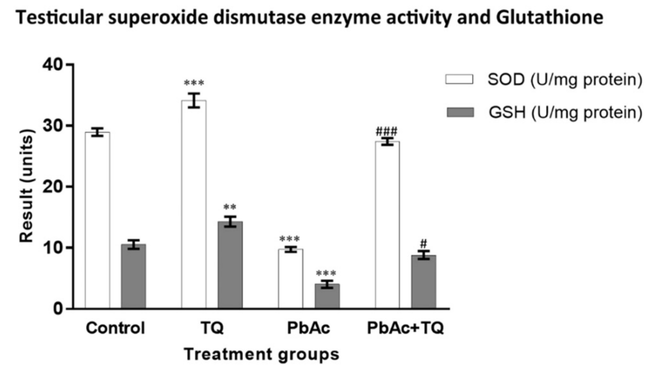

Fig. 9. Effects of oral thymoquinone administration on SOD and GSH levels in testes of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. *** Significance at p < 0.001 with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test). # Significant change at $p < 0.01$; ### Significant change at p < 0.0001 with respect to PbAc group as a positive control (Unpaired *t*-test).

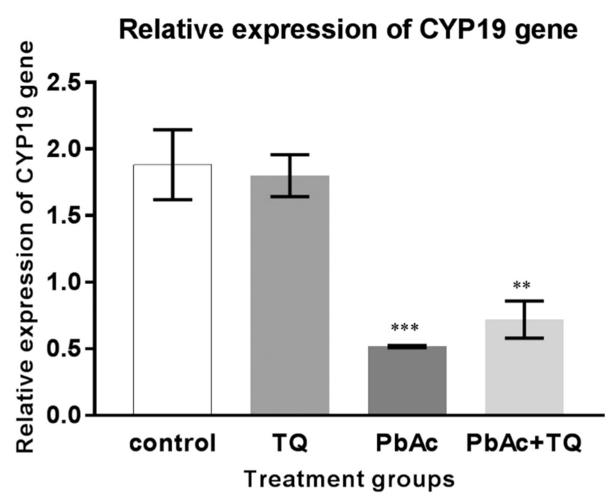

Fig. 10. Effects of oral thymoquinone administration on CYP19 gene expression in testes of male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. ** Significance at p < 0.01; *** Significance at p < 0.001 with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test). # Significant change at $p < 0.005$ with respect to PbAc group as a positive control (Unpaired *t*-test).

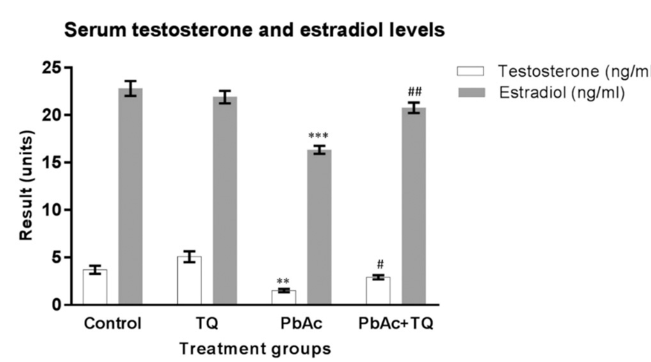

Fig. 11. Effects of oral thymoquinone administration on serum testosterone and estradiol levels in male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. ** Significance at $p < 0.01$; *** Significance at $p < 0.001$ with respect to control the group as a negative control (ANOVA with Dunnett's multiple comparison test). # Significant change at p < 0.01; ## Significant change at p < 0.001 with respect to PbAc group as a positive control (Unpaired *t*-test).

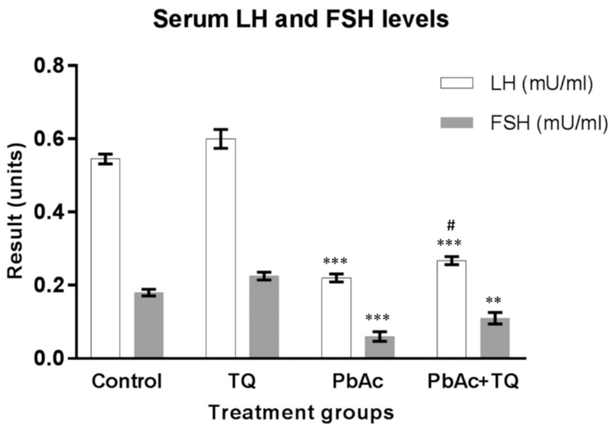

Fig. 12. Effects of oral thymoquinone administration on serum LH and FSH levels in male rats treated with lead acetate (20 mg/kg bwt) for 56 days. Data are expressed as the mean ± SEM. ** Significance at $p < 0.01$; *** Significance at $p < 0.001$ with respect to the control group as a negative control (ANOVA with Dunnett's multiple comparison test). # Significant change at $p < 0.005$ with respect to PbAc group as a positive control (Unpaired *t*-test).

On the other hand, oral administration of TQ only had no effect on serum testosterone and E2 levels. While TQ administration significantly enhanced serum levels of testosterone ($p < 0.001$) and E2 ($p < 0.0001$) compared to PbAc-treated rats (Fig. 11).

Serum LH and FSH levels were significantly ($p < 0.001$) diminished in PbAc-treated rats with respect to control. TQ treatment shortly after PbAc lessen the toxic effect of PbAc on serum LH level by about 22.7%, but not significantly affect serum FSH level. Again, oral administration of TQ only had no effect on serum LH and FSH levels (Fig. 12).

4. Discussion

The testis is one of the main target organs for Pb toxicity [38]. Male reproduction depends mainly on spermatogenesis, the process that take places within STs, and by which the immature germinal epithelium undergo division, differentiation and meiosis to give mature spermatids [39]. Other than sperm production, the testis is involved in sex hormones secretions and feedback on the hypothalamus and pituitary to control the secretions of gonadotropins. In the current study, subchronic Pb exposure exerted their toxic effect on fertility in male rats through impairment of testicular oxidant/antioxidant status, apoptosis, aromatase down-expression, and sex hormones disruption. In addition, our study focused on the therapeutic potential of TQ against Pb-induced testicular and spermotoxicity.

Our data further support previous studies in rats [24,40,41] showed a remarkable reduction in testicular and epididymal weights following chronic Pb exposure. These results can be explained by the parenchymal atrophy and various deteriorated histopathological lesions in testis as well as decreased epididymal sperm concentration. In contrast, Graça and Ramalho-Santos [42] reported a significant increase in testicular weight in acute Pb chloride exposure in mice that associated with interstitial edema. While other studies [43,44] didn't observe any change in testicular weight following Pb toxicity. These controversial results may be attributed to variation in Pb dose, exposure time, route of administration, and animal species and age. TQ succeeded to prevent the toxic effect of Pb on testes and epididymes weights in the current study. The latter result may be explained by the ability of TQ to lessen Pb-induced testicular histological damage and improve sperm count as indicated in our study and Mabrouk [3].

In consistent with previous studies [4,45], our data showed that subchronic Pb exposure causes poor sperm quality as evidenced by a significant decrease of sperm count, motility and viability as well as an increased percentage of abnormal sperms. These obtained results associated with Pb-induced severe degenerative and necrobiotic changes in testicular tissue. Additionally, these results associated with a significant decrease in spermatogenesis score of Pb-treated rats. The harmful effect of PbAc on sperm quality can be attributed to oxidative stress-induced DNA damage of testes and epididymal sperms [46]. Our data proved that treatment with TQ lessen the toxic effects of Pb on sperm count, motility and viability, and neutralized Pb-associated high sperm abnormality. Additionality, TQ improved spermatogenesis score in Pb-treated rats.

Histopathological examination of current Pb-exposed rats testes showed marked degenerative to necrobiotic changes in STs, which may eventually lead to poor spermatogenesis and hypospermia [2,47]. These results indicated that testis is more susceptible to oxidative stress due to the higher polyunsaturated fatty acid content of mammalian germ cells [48]. Our data clearly indicated that TQ has the ability to lessen the testicular structural damage in Pb treated rats [3].

Apoptosis is a well-known mechanism of Pb toxicity in various organs [49–51]. Bax is a proapoptotic member of Bcl-2 that play a key role in regulating the mitochondrial-dependent apoptotic pathway [52]. While Caspases, the cysteine aspartases, especially Caspase-3 play a major role in the execution of apoptosis [53]. Our data revealed that PbAc provoked significant testicular apoptosis via activation of Bax and caspase-3 apoptotic pathways as evident by the strong immunoreactivity of both Bax and Caspase-3 proteins in Sts and interstitial cells. These results are in accordance with those of previous studies [13,14] and may be attributed oxidation-damaged DNA in testis after PbAc exposure [14]. However, the diminished immunoexpression of Bax and Caspase-3 after oral TQ administration was reflecting the anti-apoptotic effect of TQ against PbAc-induced testicular damage. The anti-apoptotic effect of TQ was previously reported against testicular ischemia–reperfusion injury in rats [54] and methotrexate-induced germ cell apoptosis in male mice [55].

Lipid peroxidation is considered as an indicator of oxidative injury and plays a key role in xenobiotic toxicity, because it disrupts cellular membrane causing cellular dysfunction [14,56]. Our data showed a significant increment of testicular MDA in PbAc treated rats. Furthermore, the elevated NO, a pro-oxidative molecule, in PbAc-treated rats can be attributed to the ability of Pb to stimulate inducible nitric oxide synthase [57,58]. In general, low NO concentration promotes cellular survival, proliferation and homeostasis, while high NO concentration generates oxidative stress enhancing cell cycle arrest and apoptosis [59]. Remarkably, oral administration of TQ with PbAc significantly decreased MDA and NO concentration in testicular tissues, thereby indicating the anti-oxidative effect of TQ against PbAc-induced testicular toxicity. The antioxidative effect of TQ can be explained by the ability of TQ to scavenging free radical-mediated oxidative stress [60] and preventing superoxide anion radical-induced lipid peroxidation [61].

PbAc-treated rats exhibited a significant depletion of testicular GSH content, which suggest the ability of Pb to disturb the redox homeostasis of testes. GSH is the most abundant intracellular non-protein thiol which considers as important antioxidant either by direct interaction with ROS or by enzymatic detoxification for ROS [62]. Furthermore, a significant depletion of SOD activity in the testes of PbAc-treated rats which can be explained by the interaction of the active amino acid of SOD with accumulated free radicals [63]. Depletion of antioxidants resulted in an increase of the susceptibility of the cell membrane to lipid peroxidation [63]. Our results proved that, treatment with TQ significantly improved testicular antioxidant status in PbAc-treated rats via neutralization of the adverse effect of PbAc on testicular SOD and GSH content. The anti-oxidative effect of TQ was previously reported in rats against cyclophosphamide-induced cardiotoxicity [64], diabetic nephropathy [65], gentamicin-mediated acute renal failure [66] and ethanol-induced acute gastric damage [67].

At the molecular level, our data and previous investigation [68,69] proven that Pb induced significant downregulation of CYP19 gene expression. CYP19 is the key enzyme of estrogen biosynthesis in testicular tissue via irreversible conversion of androgens into estrogens [70]. Estrogen plays a vital role in spermatogenesis through control of germ cells viability/apoptosis, Sertoli cells proliferation and Leydig cells maturation as reviewed previously [39]. TQ administration had no effect on the toxic change of Pb on testicular CYP19 expression.

A remarkable decrease in serum testosterone level in Pb-treated animals has been previously reported [71–73]. This effect could be due to the ability of Pb to decrease pituitary secretion of LH and FSH [9,74], enhance Leydig cells apoptosis [75] and impaired hypothalamic-pituitary-testicular (HPT) axis activity [9,72,76]. It has been proposed that Pb targets the spermatogenesis and sperms within the epididymis rather than acting within the HPT axis to exert its toxic effect on male reproduction [77–79]. Whereas, treatment with TQ significantly naturalized the toxic effect of Pb on serum testosterone level, which may be attributed to the ability of TQ to enhance LH release which stimulates Leydig cell production of testosterone [80] and/or diminish Leydig cells degenerative changes and apoptosis as indicated in our data and another investigation [68]. Furthermore, PbAc caused a significant decrease in the serum level of E2 of treated rats. It is, therefore, possible that Pb indirectly induces reproductive toxicity through, at least in part, E2 hormonal disturbance. The role of E2 disruption in Pb-induced male reproductive toxicity is poorly understood and need further investigations. TQ administration had the ability to prevent the toxic effect of Pb on serum E2 level. This effect can be explained by the direct enhancement role of TQ on the testicular oxidant-antioxidant system. However, this action of TQ on sex hormonal level requires further studies.

Collectively, TQ might exert its acceptable therapeutic potential against Pb-induced testicular and spermotoxicity via anti-oxidative, anti-apoptotic pathways.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] S.R. Kumar, A.S. Devi, Lead toxicity on male reproductive system and its mechanism: a review, Research Journal of Pharmacy and Technology 11 (2018) 1228–1232.
[2] M.S. El-Neweshy, Y.S. El-Sayed, Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats, Exp. Toxicol. Pathol. 63 (2011) 221–227.
[3] A. Mabrouk, Therapeutic effect of thymoquinone against lead-induced testicular histological damage in male Wistar rats, Andrologia 50 (2018) e13014.
[4] C. Li, K. Zhao, H. Zhang, L. Liu, F. Xiong, K. Wang, et al., Lead exposure reduces sperm quality and DNA integrity in mice, Environ. Toxicol. 33 (2018) 594–602.
[5] R. Patra, D. Swarup, Effect of lead on erythrocytic antioxidant defence, lipid peroxide level and thiol groups in calves, Res. Vet. Sci. 68 (2000) 71–74.
[6] H. Bas, S. Kalender, Antioxidant status, lipid peroxidation and testis-histoarchitecture induced by lead nitrate and mercury chloride in male rats, Braz. Arch. Biol. Technol. 59 (2016).
[7] P.-C. Hsu, M.-Y. Liu, C.-C. Hsu, L.-Y. Chen, Y.L. Guo, Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm, Toxicology 122 (1997) 133–143.
[8] M.F. El-Sayed, S.K. Abdel-Ghafar, M.A. Adly, A.A. Salim, W.M. Abdel-Samei, The ameliorative effects of DMSA and some vitamins against toxicity induced by lead in the testes of albino rats. II, The Journal of Basic & Applied Zoology 71 (2015) 60–65.
[9] Y.S. El-Sayed, M.S. El-Neweshy, Impact of lead toxicity on male rat reproduction at "hormonal and histopathological levels", Toxicological and Environ Chemistry 92 (2010) 765–774.
[10] P. Thomas, I. Khan, Disruption of nongenomic steroid actions on gametes and serotonergic pathways controlling reproductive neuroendocrine function by environmental chemicals. Endocrine Disruptors: Effects on Male and Female Reproductive Systems, (2005), pp. 3–45.
[11] T.J. Evans, Reproductive toxicity and endocrine disruption of potential chemical warfare agents, Handbook of Toxicology of Chemical Warfare Agents, Elsevier, 2015, pp. 599–613.
[12] R.Z. Sokol, H. Okuda, H.M. Nagler, N. Berman, Lead exposure in vivo alters the fertility potential of sperm in vitro, Toxicol. Appl. Pharmacol. 124 (1994) 310–316.
[13] R.A.R. Elgawish, H.M. Abdelrazek, Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats, Toxicol. Rep. 1 (2014) 795–801.
[14] M.A. Dkhil, A.E.A. Moneim, S. Al-Quraishy, Indigofera oblongifolia ameliorates lead acetate-induced testicular oxidative damage and apoptosis in a rat model, Biol. Trace Elem. Res. 173 (2016) 354–361.
[15] Y. Zhang, C. Wang, D. Zhang, Effects of lead on expressions of TGFβ_1 and Caspase-3 in mice testes, Chin. J. Public Health 7 (0) (2006) 62.
[16] S. Darakhshan, A.B. Pour, A.H. Colagar, S. Sisakhtnezhad, Thymoquinone and its therapeutic potentials, Pharmacol. Res. 95 (2015) 138–158.
[17] A. Khan, Antioxidant and anti-inflammatory action of thymoquinone. Molecular and Therapeutic Actions of Thymoquinone, Springer, 2018, pp. 41–56.
[18] A. Mabrouk, I.B.H. Salah, W. Chaieb, H.B. Cheikh, Protective effect of thymoquinone against lead-induced hepatic toxicity in rats, Environ. Sci. Pollut. Res. 23 (2016) 12206–12215.
[19] K. Radad, K. Hassanein, M. Al-Shraim, R. Moldzio, W.-D. Rausch, Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats, Exp. Toxicol. Pathol. 66 (2014) 13–17.
[20] R. Mosbah, Z. Djerrou, A. Mantovani, Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats, Drug Chem. Toxicol. 41 (2018) 206–212.
[21] M. Kanter, Thymoquinone reestablishes spermatogenesis after testicular injury caused by chronic toluene exposure in rats, Toxicol. Ind. Health 27 (2011) 155–166.
[22] F. Shikhbahaei, A. Ghanbari, Z. Keshtmand, Therapeutic effect of thymoquinone against methotrexate-induced damage on sperm parameters in mice, Int. J. Morphol. 36 (2018) 519–522.
[23] A. Gökçe, S. Oktar, A. Koc, R. Gonenci, F. Yalcinkaya, Z. Yonden, et al., Protective effect of thymoquinone in experimental testicular torsion, Urol. Int. 85 (2010) 461–465.
[24] A. Mabrouk, H. Ben Cheikh, Thymoquinone supplementation ameliorates lead-induced testis function impairment in adult rats, Toxicol. Ind. Health 32 (2016) 1114–1121.
[25] L.D. Russell, R.A. Ettlin, A.P.S. Hikim, E.D. Clegg, Histological and histopathological evaluation of the testis, Int. J. Androl. 16 (1993) 83.
[26] G. Türk, A. Ateşşahin, M. Sönmez, A. Yüce, A.O. Çeribaşi, Lycopene protects against cyclosporine A-induced testicular toxicity in rats, Theriogenology 67 (2007) 778–785.
[27] H.A. Aly, A.S. Azhar, Methoxychlor induced biochemical alterations and disruption of spermatogenesis in adult rats, Reprod. Toxicol. 40 (2013) 8–15.
[28] A. Okamura, M. Kamijima, E. Shibata, K. Ohtani, K. Takagi, J. Ueyama, et al., A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats, Toxicology 213 (2005) 129–137.
[29] C.F.A. Culling, Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques, Butterworth-Heinemann, 2013.
[30] S.G. Johnsen, Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males, Horm Res Paediatr 1 (1970) 2–25.
[31] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to ImageJ: 25 years of image analysis, Nat. Methods 9 (2012) 671.
[32] A. Mabrouk, H.C. Ben, Thymoquinone supplementation reverses lead-induced oxidative stress in adult rat testes, Gen. Physiol. Biophys. 34 (2015) 65–72.
[33] U. Koltuksuz, M.K. Irmak, A. Karaman, E. Uz, A. Var, H. Özyurt, et al., Testicular nitric oxide levels after unilateral testicular torsion/detorsion in rats pretreated with caffeic acid phenethyl ester, Urol. Res. 28 (2000) 360–363.
[34] M. Nishikimi, N.A. Rao, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. 46 (1972) 849–854.
[35] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent, Anal. Biochem. 25 (1968) 192–205.
[36] R. Monga, S. Ghai, T.K. Datta, D. Singh, Tissue-specific promoter methylation and histone modification regulate CYP19 gene expression during folliculogenesis and luteinization in buffalo ovary, Gen. Comp. Endocrinol. 173 (2011) 205–215.
[37] X. Rao, X. Huang, Z. Zhou, X. Lin, An improvement of the 2ˆ (−delta delta CT) method for quantitative real-time polymerase chain reaction data analysis, Biostatistics, bioinformatics and biomathematics 3 (2013) 71.
[38] P.J. Landrigan, P. Boffetta, P. Apostoli, The reproductive toxicity and carcinogenicity of lead: a critical review, Am. J. Ind. Med. 38 (2000) 231–243.
[39] L. O'donnell, K.M. Robertson, M.E. Jones, E.R. Simpson, Estrogen and spermatogenesis, Endocr. Rev. 22 (2001) 289–318.
[40] M. Assi, M. Hezmee, Y. Abba, M. Rajion, H. Wahid, M. Yusof, Assessment of therapeutic effects of Nigella sativa against chronic lead acetate-induced reproductive dysfunction in male Sprague-Dawley rats, Comp Clin Path 26 (2017) 87–97.
[41] M.M. Ommati, A. Jamshidzadeh, R. Heidari, Z. Sun, M.J. Zamiri, F. Khodaei, et al., Carnosine and histidine supplementation blunt lead-induced reproductive toxicity through antioxidative and mitochondria-dependent mechanisms, Biol. Trace Elem. Res. 187 (2019) 151–162.
[42] A. Graça, J. Ramalho-Santos, Effect of lead chloride on spermatogenesis and sperm parameters in mice, Asian journal of andrology 6 (2004) 237–241.
[43] A. Ramah, R.M. EL-shwarby, M. Nabila, E.A. El-shewey, The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil, Benha Veterinary Medical Journal 28 (2015) 43–52.
[44] L. Allouche, M. Hamadouche, A. Touabti, Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats, Exp. Toxicol. Pathol. 61 (2009) 503–510.

[45] Vallverdú-Coll Nr, Mougeot Fo, M.E. Ortiz-Santaliestra, C. Castaño, Santiago-Moreno Jn, R. Mateo, Effects of lead exposure on sperm quality and reproductive success in an avian model, Environ Sci Technol 50 (2016) 12484–12492.
[46] T.R. Kumar, K. Doreswamy, B. Shrilatha, Oxidative stress associated DNA damage in testis of mice: induction of abnormal sperms and effects on fertility, Mutation Research/Genetic Toxicology and Environmental Mutagenesis 513 (2002) 103–111.
[47] M. Almansour, Histological alterations induced by lead in the testes of the quail Coturnix coturnix, Res J Environ Toxicol 3 (2009) 24–30.
[48] N.E. Furland, S.R. Zanetti, G.M. Oresti, E.N. Maldonado, M.I. Aveldaño, Ceramides and sphingomyelins with high proportions of very long-chain polyunsaturated fatty acids in mammalian germ cells, J. Biol. Chem. 282 (2007) 18141–18150.
[49] C.-M. Liu, Y.-L. Zheng, J. Lu, Z.-F. Zhang, S.-H. Fan, D.-M. Wu, et al., Quercetin protects rat liver against lead-induced oxidative stress and apoptosis, Environ. Toxicol. Pharmacol. 29 (2010) 158–166.
[50] S. Dewanjee, T.K. Dua, R. Khanra, S. Das, S. Barma, S. Joardar, et al., Water spinach, Ipomoea aquatica (Convolvulaceae), ameliorates lead toxicity by inhibiting oxidative stress and apoptosis, PLoS One 10 (2015) e0139831.
[51] X. Jin, Z. Xu, X. Zhao, M. Chen, S. Xu, The antagonistic effect of selenium on lead-induced apoptosis via mitochondrial dynamics pathway in the chicken kidney, Chemosphere 180 (2017) 259–266.
[52] Proapoptotic B. BAK: a requisite gateway to mitochondrial dysfunction and death Wei, Michael C.; Zong, Wei-Xing; Cheng, Emily H. Y.
[53] A. Degterev, M. Boyce, J. Yuan, A decade of caspases, Oncogene 22 (2003) 8543.
[54] M. Erboga, C. Aktas, O. Kurt, R. Uygur, V. Caglar, B. Turan, et al., Protective effects of thymoquinone on experimental testicular ischaemia–reperfusion injury: an apoptotic, proliferative and biochemical study, Andrologia 48 (2016) 222–230.
[55] F. Sheikhbahaei, M. Khazaei, A. Rabzia, K. Mansouri, A. Ghanbari, Protective effects of thymoquinone against methotrexate-induced germ cell apoptosis in male mice, International journal of fertility & sterility 9 (2016) 541.
[56] A.F. Khafaga, Y.S. El-Sayed, All-trans-retinoic acid ameliorates doxorubicin-induced cardiotoxicity: in vivo potential involvement of oxidative stress, inflammation, and apoptosis via caspase-3 and p53 down-expression, Naunyn Schmiedeberg's Arch. Pharmacol. 391 (2018) 59–70.
[57] H. Wang, S. Li, X. Teng, The antagonistic effect of selenium on lead-induced inflammatory factors and heat shock proteins mRNA expression in chicken livers, Biol. Trace Elem. Res. 171 (2016) 437–444.
[58] N.D. Vaziri, Y. Ding, Z. Ni, Nitric oxide synthase expression in the course of lead-induced hypertension, Hypertension 34 (1999) 558–562.
[59] D.D. Thomas, L.A. Ridnour, J.S. Isenberg, W. Flores-Santana, C.H. Switzer, S. Donzelli, et al., The chemical biology of nitric oxide: implications in cellular signaling, Free Radic. Biol. Med. 45 (2008) 18–31.
[60] M.A. Mansour, M.N. Nagi, A.S. El-Khatib, A.M. Al-Bekairi, Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action, Cell Biochem. Funct. 20 (2002) 143–151.
[61] M.N. Nagi, M.A. Mansour, Protective effect of thymoquinone against doxorubicin–induced cardiotoxicity in rats: a possible mechanism of protection, Pharmacol. Res. 41 (2000) 283–289.
[62] R.A. Jacob, The integrated antioxidant system, Nutr. Res. 15 (1995) 755–766.
[63] M. Bazrgar, I. Goudarzi, T. Lashkarbolouki, M.E. Salmani, Melatonin ameliorates oxidative damage induced by maternal lead exposure in rat pups, Physiol. Behav. 151 (2015) 178–188.
[64] M.N. Nagi, O.A. Al-Shabanah, M.M. Hafez, M.M. Sayed-Ahmed, Thymoquinone supplementation attenuates cyclophosphamide-induced cardiotoxicity in rats, J. Biochem. Mol. Toxicol. 25 (2011) 135–142.
[65] A. Sayed, Thymoquinone and proanthocyanidin attenuation of diabetic nephropathy in rats, Eur. Rev. Med. Pharmacol. Sci. 16 (2012) 808–815.
[66] S. Samarghandian, M. Azimi-Nezhad, H. Mehrad-Majd, S.R. Mirhafez, Thymoquinone ameliorates acute renal failure in gentamicin-treated adult male rats, Pharmacology 96 (2015) 112–117.
[67] S.O. Arslan, E. Gelir, F. Armutcu, O. Coskun, A. Gurel, H. Sayan, et al., The protective effect of thymoquinone on ethanol-induced acute gastric damage in the rat, Nutr. Res. 25 (2005) 673–680.
[68] S. Carreau, S. Wolczynski, I. Galeraud-Denis, Aromatase, oestrogens and human male reproduction, Philosophical Transactions of the Royal Society B: Biological Sciences 365 (2010) 1571–1579.
[69] E. Hassan, K. Kahilo, T. Kamal, M. El-Neweshy, M. Hassan, Protective effect of diallyl sulfide against lead-mediated oxidative damage, apoptosis and down-regulation of CYP19 gene expression in rat testes, Life Sci. 226 (2019) 193–201.
[70] E.R. Simpson, M.S. Mahendroo, G.D. Means, M.W. Kilgore, M.M. Hinshelwood, S. Graham-Lorence, et al., Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis, Endocr. Rev. 15 (1994) 342–355.
[71] Huang M-y, Men Q-l, Meng X-g, Fang X-x, Tao M-z, Chronic toxic effect of lead on male testis tissue in adult Pelophylax nigromaculata, Nature Environment and Pollution Technology 16 (2017) 213.
[72] J. Gandhi, R.J. Hernandez, A. Chen, N.L. Smith, Y.R. Sheynkin, G. Joshi, et al., Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning, Zygote 25 (2017) 103–110.
[73] A.L. Wani, A. Ara, J.A. Usmani, Lead toxicity: a review, Interdiscip. Toxicol. 8 (2015) 55–64.
[74] D. Klein, H. Okuda, R.Z. Sokol, S. Kamyab, Wan Y-JY, Effects of toxic levels of lead on gene regulation in the male axis: increase in messenger ribonucleic acids and intracellular stores of gonadotrophs within the central nervous system1, Biol. Reprod. 50 (1994) 802–811.
[75] X. He, J. Wu, L. Yuan, F. Lin, J. Yi, J. Li, et al., Lead induces apoptosis in mouse TM3 Leydig cells through the Fas/FasL death receptor pathway, Environ. Toxicol. Pharmacol. 56 (2017) 99–105.
[76] K. Doumouchtsis, S. Doumouchtsis, E. Doumouchtsis, D. Perrea, The effect of lead intoxication on endocrine functions, J. Endocrinol. Investig. 32 (2009) 175–183.
[77] R.Z. Sokol, Hormonal effects of lead acetate in the male rat: mechanism of action, Biol. Reprod. 37 (1987) 1135–1138.
[78] S.A. Wadi, G. Ahmad, Effects of lead on the male reproductive system in mice, Journal of Toxicology and Environmental Health Part A 56 (1999) 513–521.
[79] A. Taiwo, S. Ige, O. Babalola, Assessments of possible gonadotoxic effect of lead on experimental male rabbits, Global Veterinaria 5 (2010) 282–286.
[80] S. Haseena, M. Aithal, K.K. Das, S.H. Saheb, Effect of thymoquinone on testosterone and lh levels in sterptozotocine induced diabetic male albino rats, Journal of Pharmaceutical Sciences and Research 7 (2015) 554.