Life Sciences 226 (2019) 193–201

Contents lists available at ScienceDirect

Life Sciences

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

Protective effect of diallyl sulfide against lead-mediated oxidative damage, apoptosis and down-regulation of CYP19 gene expression in rat testes

Eman Hassan[a,*], Khaled Kahilo[b], Tarek Kamal[b], Mahmoud El-Neweshy[c], Marwa Hassan[d]

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

ARTICLE INFO

Keywords:
Lead
Diallyl sulfide
Male infertility
Oxidative stress
Aromatase P450 gene
Caspase-3

ABSTRACT

Aims: The present study aimed to investigate the potential therapeutic effect of diallyl sulfide (DAS), a natural component of garlic (*Allium sativum*), in the improvement of lead (Pb)-induced testicular toxicity and its underlying mechanisms.

Materials and methods: Thirty-two male albino rats were randomly divided into control, PbAc (20 mg lead acetate/kg bwt, orally), DAS (200 mg/kg bwt, orally), and PbAc + DAS groups for 49 successive days. The investigation based on the following criteria: Paired testes and epididymides weights, epididymal sperm analysis, level of serum sex hormones (Testosterone and17β-estradiol (E2)), aromatase (CYP19) expression, Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) enzyme, reduced glutathione (GSH), testicular histopathology, spermatogenesis score and apoptosis detection (Caspase-3 immunoexpression).

Key findings: Pb caused significant decline in epididymal sperm count and motility, testes and epididymes weights, spermatogenesis score and serum testosterone and1E2, as well as a significant decrease in SOD and GSH level, and a significant elevation of MDA and NO compared to a control group. In addition, Pb induced significant downregulation of CYP19 gene expression, increase of Caspase-3 immunoreactivity, various testicular degenerative and necrotic changes. Whereas, co-treatment of rats with DAS improved sperm analysis, and testicular histology and antioxidative status. Furthermore, DAS co-administration regulated testicular CYP19 and Caspase-3 expressions.

Significance: Collectively, DAS seemed to be a promising agent for protection against Pb-induced testicular toxicity through antioxidative properties, beside regulation of testicular apoptosis and aromatase expression.

1. Introduction

Lead (Pb) is one of the most widespread environmental pollutant that causes severe damage in different organs including kidney, brain, liver and testis [1]. Nowadays, Pb-induced reproductive toxicity remains a matter of public health concern especially in industrial countries. Previous studies have shown that Pb has deleterious effects on male fertility and causes severe damage of testicular tissue and testicular function impairment [2,3]. Exposure to Pb negatively affects semen quality including, sperm count, viability, morphology, and DNA integrity leading to functional disorder [4]. In spite of the fact that; Pb is a non-redox metal, oxidative stress is considered as an important underlying mechanism of its toxicity through overproduction of reactive oxygen species (ROS) and depletion of antioxidants [5,6]. In addition, Pb toxicity exerted steroidogenesis malfunction via suppression of aromatase P450 gene, which impair gonadotrophins and testosterone hormone level [7].

Aromatase P450 (CYP19) is the key enzyme of estrogen biosynthesis in testicular tissue via irreversibly conversion of androgens into estrogens [8]. O'donnell et al. [9] reviewed the importance of estrogen for spermatogenesis. CYP 19 is one of the potential targets of endocrine-disrupting chemicals (EDC). EDC has the capacity to modulate both aromatase's expression and function, consequently, they can modify the rate of estrogen-related biological process [10].

Abbreviations: ANOVA, One-way analysis of variance; cDNA, Complementary DNA; CYP19, Aromatase P450; DAS, Diallyl sulfide; E2, 17β-estradiol; DMRT, Duncan's multiple range test; ELISA, Enzyme linked immunosorbant assay; GSH, Reduced glutathione; 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

* Corresponding author.
E-mail address: dremanhassan77@gmail.com (E. Hassan).

https://doi.org/10.1016/j.lfs.2019.04.020
Received 20 February 2019; Received in revised form 27 March 2019; Accepted 7 April 2019
Available online 12 April 2019

Previous reports reviewed the critical role of natural antioxidant against heavy metal toxicity [11,12]. Medicinal plants, contain flavonoids compounds, play an effective role against heavy metal-induced reproductive toxicity as antioxidants, altering cellular signal transduction, binding to sex hormone receptors, and modulating the activity of detoxifying enzymes [13]. Garlic, *Allium sativum*, is one of the oldest global medical herbs due to its numerous biological activities. It has plentiful amount of antioxidants, flavonoids and sulfur containing compounds that utilized in detoxification systems [14]. Diallyl sulfide (DAS), the most bioactive sulfur component in garlic, protect against environmental toxicants via stimulating hemeoxygenase-1 (HO-1), an enzyme has crucial role in cell defense system against oxidative stress [15,16]. Furthermore, DAS has prophylactic effect against chemical carcinogenesis, and mutagenesis [17–19] and alleviates cisplatin- induced nephrotoxicity in rats [20]. Pervious investigations indicated that DAS has an alleviating effect on cadmium-induced testicular steroidogenesis impairment [21], and cyclophosphamide-induced testicular spermatotoxicity and oxidative stress [22] in rats; attenuated carbon ion irradiation-induced apoptosis in mouse testis [23]. Despite of various investigations has been deduced the biological significance of DAS, its therapeutic effectiveness on Pb-induced testicular toxicity have yet to be published. However, the mechanism through which DAS exerts its testicular activity remains elusive.

2. Materials and methods

2.1. Chemicals and kits

Lead acetate (PbAc) trihydrate (purity ≥99.99%, Molecular Weight 379.33) and DAS (purity 80%, Molecular Weight 146.27) were supplied from Sigma-Aldrich Chemicals (St. Louis, Mo, USA). Polyclonal rabbit anti-Caspase 3 was from Thermo Fisher Scientific In. (1:100; Cat: CPP32). All other chemicals and reagents were of analytical grade and acquired from standard commercial suppliers.

2.2. Experimental animals and animal welfare

Thirty-two male Swiss albino rats (200–250 g, bwt – aged 4–6 months) were housed in metal cages and were supplied with soft-wood chips for bedding. All animals were given standard rat diet and fresh drinking water ad libitum and were housed at 22 ± 2 °C with 12 h light/dark cycles through the period of the experiment. Rats remained two weeks without any treatment for acclimatization before the beginning of the experiment. Experiment was done in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Local Ethics Committee of Medical Experimental Research Center of Mansoura University approved the experimental protocol.

2.3. Experimental design

Following the acclimatization period, rats were randomly assigned into four groups ($n = 8$/each) and the experiment continued to 49 days. Control group received daily corn oil, the vehicle used for DAS (1.0 ml/kg bwt) via gastric tube. PbAc group received PbAc (20 mg/kg bwt) dissolved in distilled water via gastric tube once daily [24]. DAS group was administrated daily with DAS (200 mg/kg bwt) dissolved in corn oil (1.0 ml/kg bwt) via gastric tube, the dose of DAS adjusted according to Szutowski et al. [25]. PbAc + DAS group received PbAc plus DAS, 30 min before, at the same regime of the PbAc and DAS groups, respectively.

2.4. Samples collection and processing

After 24 h of the last treatment, blood samples were collected from all rats from the retro-orbital venous plexus under sodium pentobarbital anesthesia. Serum was separated by centrifugation, after which all serum samples was kept frozen at −20 °C until analysis of testosterone and estradiol level. Rats were sacrificed immediately by cervical decapitation; then the testes and cauda epididymes were removed quickly to be weighed. For each rat, right testis was immediately immersed in liquid nitrogen and kept at −80 °C; one part was used for subsequent analysis CYP19 expression by quantitative real-time PCR (qPCR), while the other part was used for oxidative and antioxidant assay. Left testis was fixed in buffered formalin 10% for at least 48 h for the subsequent histopathological and immunocytochemical assessments.

2.5. Sperm analysis

Cauda epididymis was anatomized, instantly minced in 5 ml of physiological saline, and incubated at 37 °C for 30 min. Sperm count was estimated using Neubauer hemocytometer slide according to Yokoi et al. [26]. While, sperm motility was evaluated and expressed as a rate of motile and non-motile sperms according to method described by Aly and Azhar [27].

2.6. Measurement of serum sex hormones levels

Serum testosterone level was determined by enzyme linked immunosorbent assay (ELISA) using a commercial kits (Diagnostic System Laboratories Inc., Webster, USA) according to the guidelines. All of the samples were run at the same time to avoid inter-assay variation. E2 concentration was assessed by radioimmunoassay (RIA) using Spectra Estradiol RIA kit (Orion Diagnostica, Oy, Espoo, Finland) according to the guidelines.

2.7. Evaluation of Cyp19 expression level by qPCR

Four testicular samples from each group were analyzed in triplicates. Total RNA was extracted from iced testes tissue using RNA Purification Kit (Qiagen, Hilden, Germany). Total RNA integrity was determined through denaturation of agarose electrophoresis; the proportion of 260/280 was used to survey the purity. Superscript II Reverse Transcriptase was utilized to synthesize the first chain of cDNA according to the manufacturer's guidelines (Thermo Scientific, California, USA). The synthesized cDNA was utilized as a template to evaluate Cyp19 relative expression using Step One Plus reverse transcriptase-polymerase chain reaction (RT- PCR) System (Applied Biosystem, USA). The primers (Forward primer:/5 TGGAACCTGCCCC CAGGACC -/3; Reverse primer: /5- CCACGATGCGCCTTGAGCCA -/3) and B-actin as reference gene (Forward primer: /5-AGGGAAATCGTG CGTGAC-/3; Reverse primer: /5- CGCTCATTGCCGATAGTG-/3) were used. The synthesized cDNA was amplified using 2× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA) following the manufacturer's protocol and gene-specific primers. Briefly, The PCR mixture was carried out in a 25 µl that contains the following: 1 µl of cDNA template, 12.5 µl of 2× Maxima SYBR Green, preparation of 1 µl each of forward and reverse primer, and 8.5 µl of nuclease-free water. The thermal cycler condition used during real-time PCR were 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 Monga et al. [28]. At the final of each extension, SYBR Green fluorescence was assessed. The values calculated from the individual standard curves used to quantify cDNA of each gene. All values were calculated using the $2 - \Delta\Delta Ct$ method [29].

2.8. Oxidative stress and antioxidant capacity assay

In testicular homogenate, superoxide dismutase (SOD) and reduced glutathione (GSH) level were estimated according to methods described by Nishikimi et al. [30] and Beutler [31], respectively. In addition, the concentration of malondialdehyde (MDA), the lipid peroxidation (LPO)

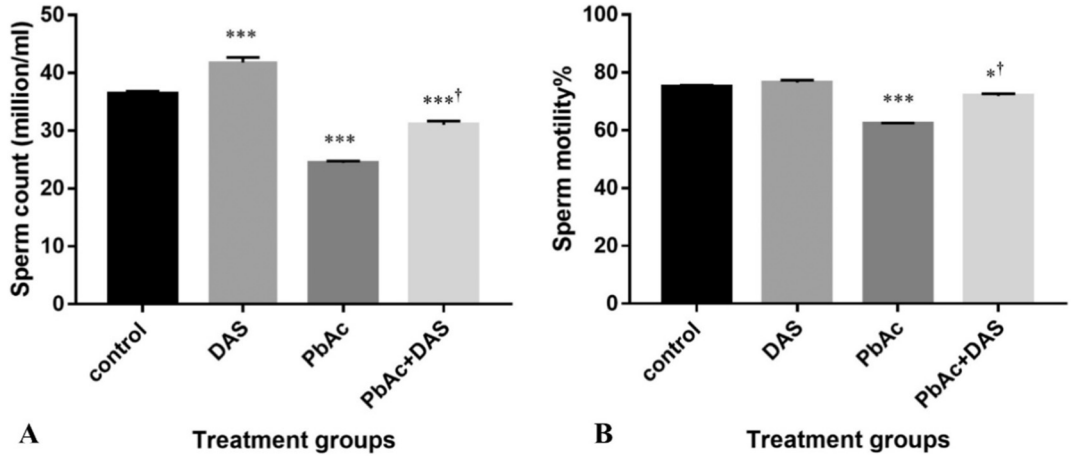

Fig. 1. Effect of DAS treatment on PbAc- induced toxic changes in sperm count (A) and motility (B) in male rats. Data are expressed as the mean ± SEM. *Significance at $p < 0.05$; ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.0001$ versus PbAc group as positive control (Unpaired *t*-test).

biomarker and nitric oxide (NO) as total nitrite/nitrate were measured using the methods of Mabrouk and Ben [32] and Aydın et al. [33], respectively.

2.9. Histopathological examination

Fixed testicular tissue specimens were processed using the conventional paraffin-embedding techniques including the dehydration through ascending grades of ethanol, clearing in three changes of xylene ended by embedding in paraffin wax at 65 °C. Five- μm thick sections were obtained then stained with hematoxylin and eosin according to Culling [34] and finally examined under light microscope for evaluation of the histopathological changes and spermatogenesis.

2.10. Spermatogenesis evaluation based on Johnsen's score (J score)

Seminiferous tubules (STs) organisation and the spermatogenic activity were graded using Johnsen's Scoring System [35]. Briefly, five different sections of testicular tissues stained with H&E per each rat were evaluated at X400 magnification under a light microscope. Twenty randomly selected STs in each cross-section were evaluated and scored from 1 to 10 according to the organisation and the spermatogenesis, as follows: 10 = normally organised tubules with full spermatogenesis; 9 = disorganised tubular epithelium with many late spermatids; 8 = only a few late spermatids; 7 = many early spermatids and absence of late spermatids; 6 = only a few early spermatids; 5 = many spermatocytes, but, no spermatids or spermatozoa; 4 = arrest of spermatogenesis at the primary spermatocyte stage; 3 = only spermatogonia; 2 = only Sertoli cells and no germ cells; and 1 = tubular sclerosis and no seminiferous epithelial cells is present. The mean score was determined for each rat in all groups.

2.11. Immunohistochemical assessment of Caspase-3 expression in testes

Briefly, four-μm-thick paraffin sections were prepared and were deparaffinized by xylene and rehydrated in graded alcohols and washed by distilled water. The prepared sections were boiled in 10 mM citrate buffer (pH 6.0) for 20 min at 95 °C followed by cooling at room temperature for 20 min. After washing with distilled water, endogenous peroxidase deactivated by 3% H2O2 in absolute methanol for 30 min at 4 °C. After washing with PBS, the sections were incubated with 10% normal blocking serum for 60 min at room temperature then incubated overnight at 4 °C with primary antibody (Polyclonal rabbit anti-Caspase 3). After washing with PBS, the sections were incubated for 60 min with biotin-conjugated goat anti-rabbit IgG antiserum (Histofine kit, Nichirei Corporation), then washed in PBS and subsequent incubation for 30 min with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation). The streptavidin-biotin complex was visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB)-H2O2 solution, pH 7.0, for 3 min. The sections were washed in distilled water followed by counterstain with Mayer's hematoxylin. The obtained sections examined at 100× magnification under light microscope (Leica DM500) and ten micrographs from different fields per section were captured using connected digital camera (Leica EC3, Leica, Germany). The area percentage of Caspase-3 immunoreactivity was measured using the image J software (freely available public domain image processing software).

2.12. Statistical analysis

Using GraphPad prism version 7.0 for windows (GraphPad Software Inc., San Diego, USA); Significance of each treated group versus the control group (as negative control) was determined by one-way analysis of variance (ANOVA) followed with Dunnett's multiple comparison test. The effect of DAS co-administration was compared to PbAc group (as positive control) using unpaired *t*-test. Mean values were considered statistically significant when $p < 0.05$.

3. Results

3.1. Sperm parameters

As shown in Fig. 1A and B. Epididymal sperm analysis in PbAc-treated group revealed a significant reduction in sperm count and motility (24.44 ± 0.30 million/ml and 62.26 ± 0.26%, respectively) compared to those mean values in control group (36.38 ± 0.43 million/ml and 74.94 ± 0.63%, respectively). However, the co-administration of DAS significantly improved sperm count and motility compared to PbAc group (31.04 ± 0.64 and 71.86 ± 0.72, respectively), although not to the level of control group. Supplementation of DAS alone could increase sperm number (41.81 ± 0.88 million/ml), but had no effect on sperm motility.

3.2. Testes and epididymes weights

As shown in Fig. 2(A and B), PbAc-treated rats shown a significant decrease in their testes and epididymis weights (0.46 ± 0.05 g and 0.35 ± 0.05 g, respectively) as compared to control rats (2.69 ± 0.13 g and 0.54 ± 0.01 g, respectively). However, compared to PbAc group, rats treated with PbAc plus DAS revealed a significant improvement of their testes weight (2.21 ± 0.22 g) to the level of

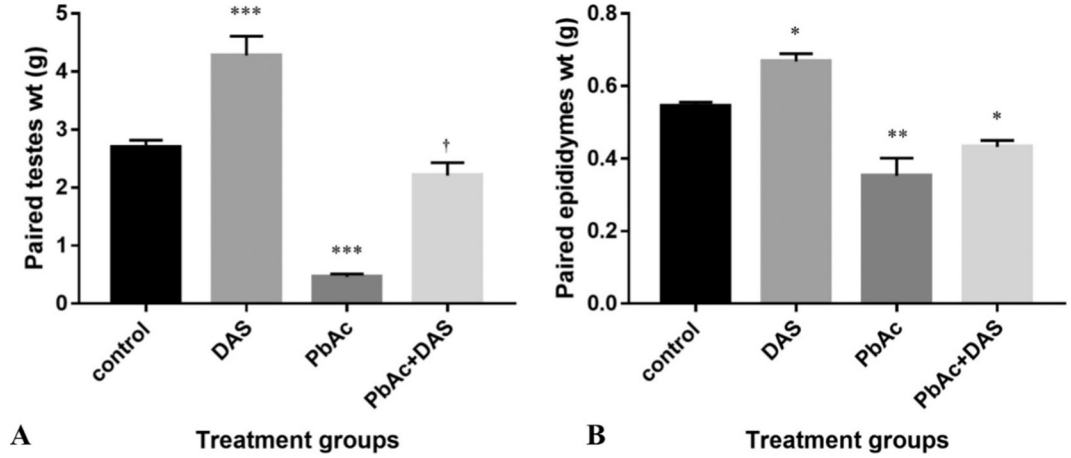

Fig. 2. Effect of DAS treatment on PbAc- induced toxic changes in the weights of paired testes (A) and epididymes (B) in male rats. Data are expressed as the mean ± SEM. *Significance at $p < 0.05$; **Significance at $p < 0.01$; ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.001$ versus PbAc group as positive control (Unpaired *t*-test).

control group, but not showed change in their epididymes weight (0.43 ± 0.02 g). Interestingly, oral DAS administration significantly raised the weights of testes and epididymes (4.27 ± 0.33 g and 0. ± 0.21 g, respectively) compared to control group.

3.3. Serum sex hormones (testosterone and E2)

A significant reduction in both testosterone and E2 levels were noticed in PbAc-treated rats (1.63 ± 0.01 and 16.34 ± 0.41 ng/l, respectively) compared to control group (3.89 ± 0.36, 22.81 ± 0.78 ng/l, respectively). Versus PbAc group, serum testosterone and E2 levels were significantly restored to the level of control group (3.17 ± 0.20 and 21.76 ± 0.83 ng/l, respectively) following co-administration of DAS. Meanwhile, rats received DAS alone showed significant increase in serum testsestorne and E2 levels (4.34 ± 0.42 and 26.65 ± 0.41 ng/l, respectively) compared to control group. These results were presented in Fig. 3(A and B).

3.4. Cyp19 gene expression

A significant downregulation in the level of cyp19 gene expression was detected in PbAc-treated rat testis (0.36 ± 0.02) compared to control group (0.82 ± 0.01). Whereas, co-treatment with DAS significantly upregulated the level of cyp19 gene expression compared to PbAc group (0.72 ± 0.01), albeit not reach the control mean value. Interestingly, Administration of DAS alone significantly upregulated the level of cyp19 gene expression (0.93 ± 0.03) compared to control groups (Fig. 4).

3.5. Testicular oxidative parameters

It is obvious from the data displayed in Table 1 that, versus control group, the testicular SOD and GSH levels in PbAc-treated rats were significantly lowered. On the contrary, the testicular level of LPO biomarkers (MDA and NO), was significantly increased in rats exposed to PbAc compared to control group. Whereas, compared to PbAc group, significantly elevated SOD level, decreased MDA and NO levels were noticed after oral DAS co-administration versus PbAc group, although not to the level of control group. Interestingly, DAS group shown significant elevation in the SOD and GSH levels compared to control group.

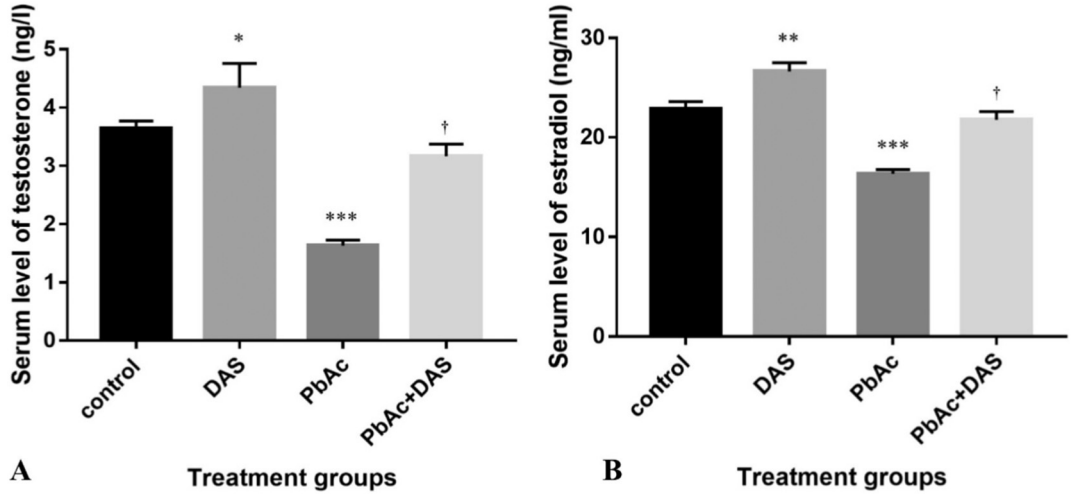

Fig. 3. Effect of DAS treatment on PbAc- induced toxic changes in serum testosterone (A) and estradiol (B) levels in male rats. Data are expressed as the mean ± SEM. **Significance at $p < 0.01$; ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.001$ versus PbAc group as positive control (Unpaired *t*-test).

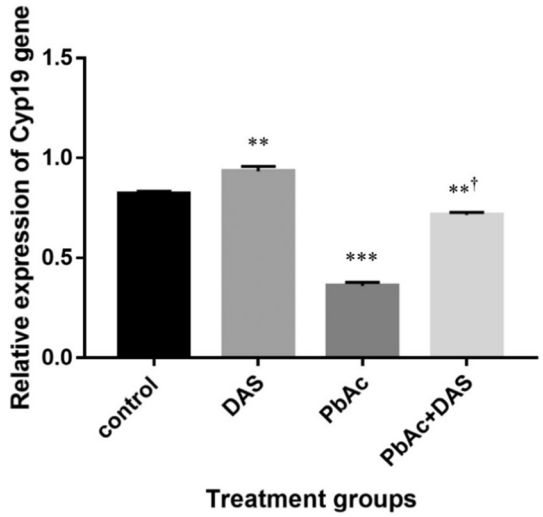

Fig. 4. Effect of DAS treatment on PbAc- induced toxic changes in relative expression of Cyp19 gene in testes of male rats. Data are expressed as the mean ± SEM. **Significance at $p < 0.01$; ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.0001$ versus PbAc group as positive control (Unpaired *t*-test).

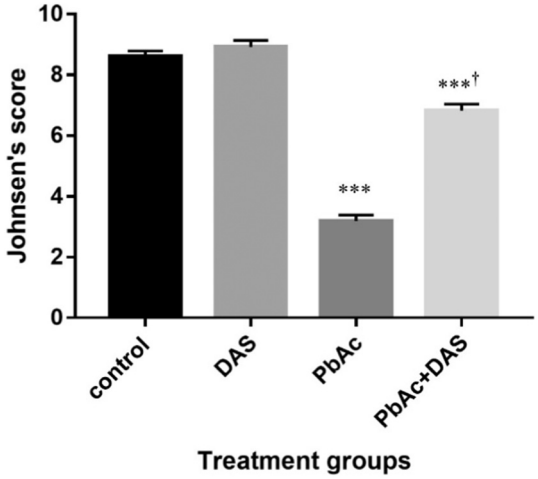

Fig. 5. Effect of DAS treatment on PbAc- induced toxic changes in spermatogenesis (Johnsen's score) in male rats. Data are expressed as the mean ± SEM. ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.0001$ versus PbAc group as positive control (Unpaired *t*-test).

3.6. Spermatogenesis

Spermatogenesis, based on J score, were markedly poor in the PbAc group (3.18 ± 0.20) compared to the control group (8.6 ± 0.19), that was significantly improved by the co- administration of DAS compared to PbAc rats (6.81 ± 0.22), albeit not to the level in the control group. The administration of DAS alone had no effect on the J score (8.92 ± 0.22) as shown in Fig. 5

3.7. Testicular histopathology

Testes of control rats (Fig. 6A) showed compactly arranged STs at different spermatogenic cycle stages separated by interstitial tissue. The most encountered cells in interstitium is Leydig cells. Each ST lined with stratified layers of spermatogenic cells and supporting somatic cells, Sertoli cells. The spermatogenic cells were consist of spermatogonia, which lined next to the basement membrane and then the primary and secondary spermatocytes present in more than one layer, spermatids and mature sperms. Active STs were associated with free sperms in the lumen of some tubules (Fig. 6A). Testes of rats that received only DAS showed enhanced spermatogenic cycle, most of the STs were impacted with spermatids (Fig. 6B). PbAc- treated rats developed severe testicular lesions as shown in Fig. 6C, hence 68% of the STs were affected. Approximately, 23% of the STs exhibited necrosis and hyalinization, while 26% impacted with sloughed epithelium, and 11% showed hypospermatogenesis, 8% exhibited complete cessation of spermatogenesis with sertoli cell–only pattern STs, while the remaining of STs (32%) showed normal structure and contain different spermatogenic cells. The spermatogenesis was significantly improved in most of the STs (79%) in rats that treated with PbAc plus DAS (Fig. 6D), wherein testicular histology nearly like those in the control group, except for a few hyalinized STs (4%), impacted STs (7%) and others showed hypospermatogenesis (10%).

3.8. Testicular Caspase-3 immunoexpression

Testes of PbAc-treated rats exhibited strong Caspase-3 immunopositivity in sertoli cells and different spermatogenic cells of almost STs and to lesser extent in leydig cells (Fig. 7C) compared to control (Fig. 7A) and DAS-treated (Fig. 7B) groups, while in PbAc + DAS group (Fig. 7D) showed a mild Caspase-3 immunopositivity compared to PbAc -treated rats. In addition, comparing to the control treated rats, the testes of PbAc –treated group exhibited significant increase in the area percentage of Caspase-3 immunoreactivity (62.17 ± 3.88) compared to control group (5.88 ± 0.62). Meanwhile, Caspase-3 area percentage (20.17 ± 1.35) was declined, not reached the level of control, after DAS co-treatment. The administration of DAS alone had no significant effect on the area of Caspase-3 immunoreactivity (3.7 ± 0.38) compared to the control group (Fig. 8).

4. Discussion

In many countries, despite strict regulatory authority to diminish environmental Pb toxicity, Pb exposure still an important public health concern especially in developing countries. In the current study, we focus on the reproductive toxicity of Pb and the possible therapeutic effect of dietary DAS supplementation. Our results revealed that Pb-induced reproductive toxicity through induction of testicular oxidative

Table 1
The effect of DAS supplementation against PbAC-induced change in stress-related parameters.

Parameters	Control	DAS	PbAc	PbAc + DAS
SOD (μmol/mg protein)	29.14 ± 0.45	32.16 ± 0.62**	9.76 ± 0.40***	25.23 ± 0.59***†
GSH (mg/g protein)	10.74 ± 0.60	13.8 ± 0.61**	4.04 ± 0.58***	5.96 ± 0.62***
MDA(nmol/mg protein)	31.24 ± 1.26	28.08 ± 0.63	71.74 ± 0.46***	38. 21 ± 0.78***†
NO (nmol/mg protein)	52.26 ± 0.70	49.69 ± 0.64	99.19 ± 0.17***	77.35 ± 1.50***†

Data are expressed as the mean ± SEM. **Significance at $p < 0.01$; ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.0001$ versus PbAc group as positive control (Unpaired *t*-test).
SOD (Superoxide dismutase), MDA (Malondialdehyde), GSH (Reduced glutathione), NO (Nitric oxide).

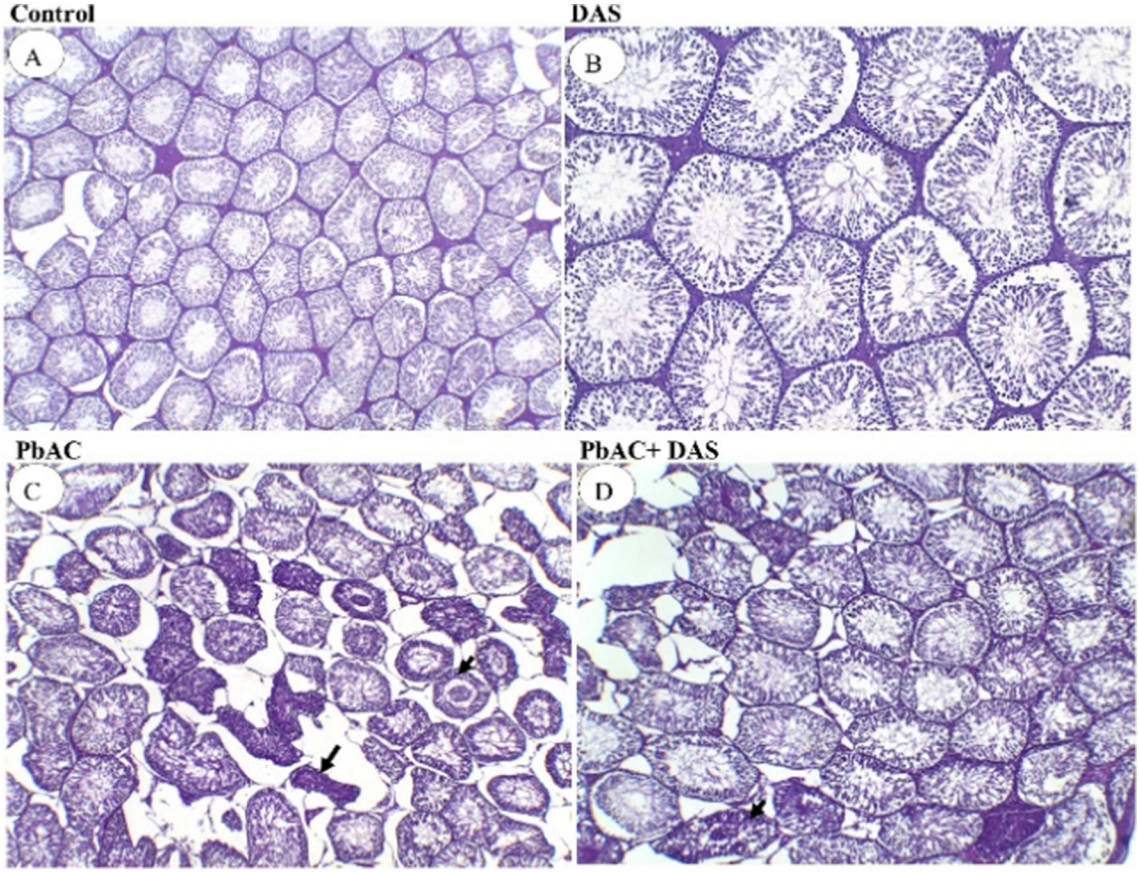

Fig. 6. Representative photomicrograph of testes of the control and treated groups stained with H&E: (A) Control group showed testicular histology (X100), (B) DAS group showed normal testicular histology with improved spermatogenesis (X200). (C) PbAc group showed severe testicular lesions featuring necrosis and hyalinization (long arrow), impacted STs with sloughed germinal epithelium (short arrow) and hypospermatogenesis, while the remaining STs normal (X100). (D) PbAc + DAS group showed few hyalinized and impacted STs (short arrow) while the most STs histologically normal.

stress and apoptosis, disruption of hormonal balance and suppression of CYP19 expression.

Pb resulted in a remarkable reduction in sperm count and motility [4,36]. These changes can be related to hypothalamic-pituitary axis disturbance straightforwardly influencing spermatogenesis [37], as well as delay spermiation throughout spermatogenesis and liberate an immature spermatogenic cells in the seminiferous tubules [38]. These findings agreed with our results concerning with the deteriorated sperm parameters due to PbAc administration.

The current results revealed that the oral administration of DAS attenuated the toxic effect of Pb on semen quality and sex organs weight. Similarly, it was reported that garlic allyl sulfide pretreatment effectively attenuated the Pb-induced poor sperm quality [39]. Furthermore, Asadpour et al. [40] reported that co- treatment with aqueous garlic extract quenching the lethal impacts of PbAc on sperm viability. Various researches have elucidated that the defensive mechanism of garlic extract, indeed allyl sulfides, is attributed to antioxidative activity of garlic via the regulation of cytochrome P450 enzyme [81,82]. Certain studies have deduced the antioxidant power of garlic is retained to polyphenols [41,42]. On contrary, another experimental study found that oral administration of higher doses of aqueous extract of garlic resulted in reduction of sperm count and motility in rat [43].

In the current study, PbAc administration reduced the weights of testes in treated rats that might attributed to the structural damage of STs, apoptosis of the germinal epithelium and withdrawal of the trophic action of testosterone [44]. In addition, epididymes weight was decreased in PbAc-treated rats that might be explained by decreased epididymal sperm count, oxidative stress [45] and lowered testosterone level.

The results of current and other studies showed a remarkable decrease in serum testosterone level in Pb-treated animals [3,4,46]. This result might be explained by leydig cells apoptosis in Pb-exposed rats as detected in our study and previous report [47], inhibition of LH and gonadotrophin-releasing hormone [48], and impaired hypothalamic-pituitary-testicular (HPT) axis activity [4,49,50]. Many studies [51–53] asserted that Pb targets the spermatogenesis and sperms within the epididymis by producing reproductive toxicity rather than acting within the HPT axis. They also suggested that the gonadotoxic effect of Pb including the intra-testicular sites with minimal effects on hormonal levels and no effect on extra-testicular sites. Other studies elucidated that Pb-induced imbalances in the HPT hormonal axis, hence inappropriate level of testosterone release from leydig cells and change the steroid negative feedback loop [4]. Our results showed that DAS could counteract the adverse effects of Pb on serum testsestorne. This effect can be attributed to the ability of DAS to stimulate LH secretion from the pituitary gland by the increased plasma noradrenaline concentration [54].

The physiological role of estrogen in male fertility has been reviewed [55]. Estrogen is clearly involved in negative feedback effect of testosterone on the brain to control of pituitary gonadotrophin secretions, and hence control or interfere with spermatogenesis. Estrogen also plays a functional role in control of germ cells viability/apoptosis, sertoli cells proliferation and leydig cells maturation. Furthermore, estrogen plays an essential role in development of epididymis and efferent ductules. Opinions favoring a role for estrogens, particularly E2, in spermatogenesis have typically come from indirect evidence, including expression of Cyp19 and estrogen receptors in the testis as well as

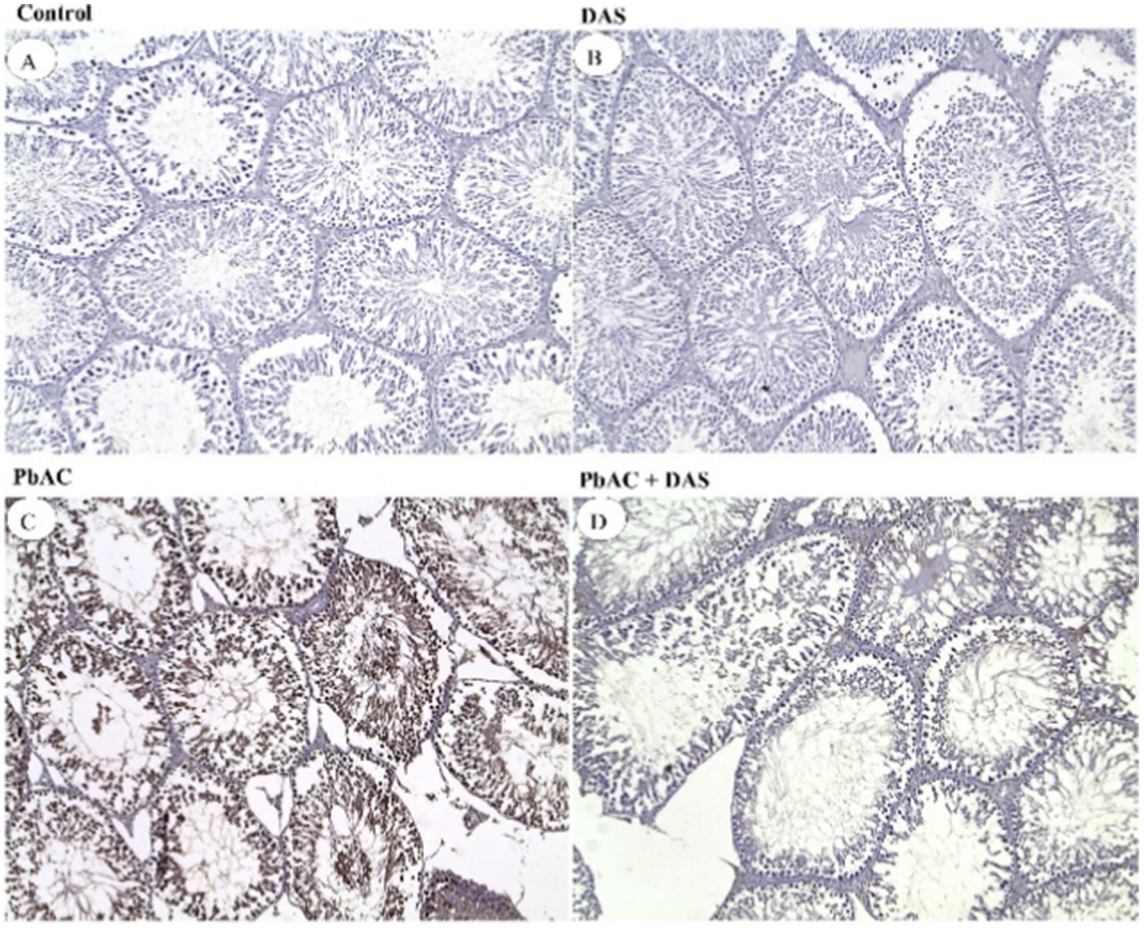

Fig. 7. Representative photomicrograph of Caspase-3 immunoreactivity in testes of the control and treated groups. (A) and (B) control and DAS groups, respectively showed negative immunoreactivity for Caspase-3. (C) The almost STs of PbAc group (Sertoli cells and different spermatogenic cells stages and to lesser extent in leydig cells) showed strong positive immunoreactivity for Caspase-3. (D) The STs of PbAc + DAS group showed moderate positive immunoreactivity for Caspase-3.

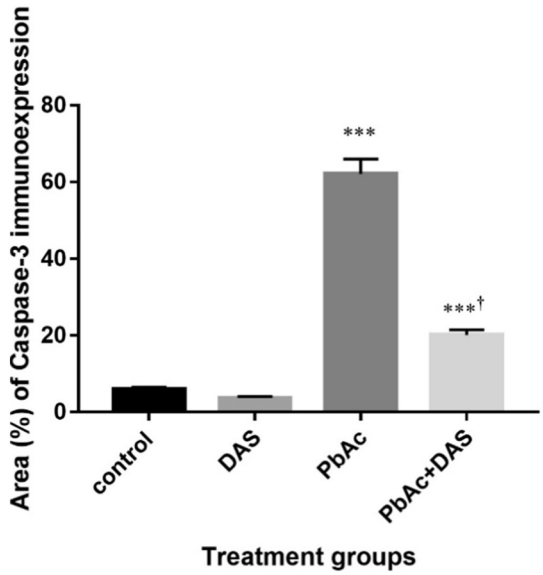

Fig. 8. Effect of DAS treatment on PbAc- induced toxic changes on area (%) of Caspase-3 immunoexpression in testes of male rats. Data are expressed as the mean ± SEM. ***Significance at $p < 0.001$ versus the control group as negative control (ANOVA with Dunnett's multiple comparison test), †Significant change at $p < 0.0001$ versus PbAc group as positive control (Unpaired *t*-test).

application of anti-estrogen treatment [56,57]. In the current study, a significant reduction in serum E2 concentration in PbAc-treated rats was reported; this result partial agreed with the results obtained by [58] who reported severe reproductive disruption accompanied by suppression of circulating estradiol in rats that exposed to Pb during *in utro* stage at gestational day 5. It is therefore possible that Pb indirectly induces infertility through, at least in part, hormonal disturbance. Further studies would be needed to clarify the role of E2 disruption in Pb-induced reproductive toxicity in male. While, administration of DAS could counteract the toxic effect of Pb on serum E2, which can be explained by the ability of DAS to upregulate CYP19 gene expression and decrease germinal cells apoptosis.

The current and previous results suggest that DAS causes disturbance in serum sex hormones level (testsestorne and E2) in healthy animals which can attributed to the ability of garlic supplementation to increase aromatase expression as reported in our study and stimulate LH secretion from the pituitary gland [54]. These findings can consider as undesirable side effect of DAS in normal individuals.

Concerning oxidative stress, in agreement with recent scientific studies [32,59], our results confirmed a remarkable reduction in the antioxidant molecules, SOD and GSH in testicular tissue of rats following the treatment by PbAc. This result may attributed to the direct inhibition effect of Pb on antioxidant level or expression. Additionally, there was a remarkable increase in LPO as shown by the significant increase in MDA content of the testis. The high level of LPO may demonstrate the excess of ROS generation, which adversely affects sperm and cytoplasmic organelle membrane structures via peroxidation of proteins, lipids, and nucleotides, in a manner changing the sperm

motility. Additionally, Rahman et al. [60] concluded that DAS could ameliorate the testicular toxicity via suppression of oxidative stress due to their abilities to scavenge free radicals. These results uncover the therapeutic impact of DAS described by an enhanced level of cellular antioxidants and diminished LPO overproduction in rat testis, in combating the testicular toxicity of Pb.

The downregulation of Cyp19 gene expression in Pb- exposed animals is reported in the current study and previous studies [61,62]. Taupeau et al. [63] stated that Pb reduces messenger RNA and protein levels of cytochrome P450 aromatase, hence decrease its activity. There are controversial results regarding the effect of garlic allyl sulfides on Cyp19 gene expression. In the present study, DAS induced higher expression of Cyp19 and this agreed with Zeng and Xie [64] who recorded the same impact on CYP2B1 gene. In the same way, Wu et al. [65] observed that, garlic sulfides elevated both the CYP2B1 protein content and mRNA levels. On the other hand, another investigation demonstrated that, garlic allyl sulfides showed differential modulation on rat CYP2B1 [66]. This inconsistency might be because of (i) distinctive garlic component may have various effect and they may work synergistically, (ii) variances in both doses and time course (since cells can adjust to the prolonged exposure via altering either absorption rate or metabolism), (iii) species variety, since the hepatic metabolism of chemical differ among species [67].

Histopathological finding and Johnson's score of spermatogenesis support the biochemical and molecular findings. These findings showed that the degenerative changes observed in rats testes exposed to Pb are in parallel with pervious results [24,59,68,69]. Moreover, the present data agreed with Akinola et al. [70]. Further studies indicated that Pb particles are capable of crossing the blood-testis barrier to applying its harmful consequences for spermatocytes, spermatids, and spermatozoa [71,72]. The reversibility of such lesion is plausible after withdrawal of Pb treatment and this is because of the relative abundance of spermatogenic stem cells in the testis of Pb-treated rats. DAS supplementation with PbAc markedly restored abnormal STs with low spermatogenesis score. These results are predictable with the results of previous studies on the effect of DAS against the toxic effects of Pb on different organs histopathology [73–76].

Apoptosis is a physiological programmed cell death and can occurs in testis within a physiological limit to remove damaged cells during the spermatogenesis, but the excessive apoptosis rate adversely affects the male fertility function [77]. The detection of activated cysteine proteases (Caspases) is the hallmark of the early and late apoptotic signal pathways via the immunostaining [78]. Recent researches reported that PbAC-induced testicular toxicity in rats via the promotion of testicular germinal cells apoptosis via increased expression of Caspase-3 [79,80]. The mitochondrial-dependent apoptotic pathway is the major pathway for PbAc-induced apoptosis [81]. The observed results of immunocytochemistry in PbAc-treated animals revealed marked apoptosis of different testicular cells. However, the anti-apoptotic effect of DAS against Pb was reported in PbAc + DAS group.

Collectively, DAS can ameliorate the toxic effect of PbAc on rat testes structures and spermatogenesis via the antioxidant and anti-apoptotic properties, and upregulation of aromatase expression.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] D.S. Kacholi, M. Sahu, Levels and health risk assessment of heavy metals in soil, water, and vegetables of Dar es Salaam, Tanzania, Journal of Chemistry 2018 (2018).
[2] N.E. Skakkebaek, E. Rajpert-De Meyts, G.M. Buck Louis, J. Toppari, A.-M. Andersson, M.L. Eisenberg, et al., Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility, Physiol. Rev. 96 (2015) 55–97.
[3] A.L. Wani, A. Ara, J.A. Usmani, Lead toxicity: a review, Interdiscip. Toxicol. 8 (2015) 55–64.
[4] 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.
[5] V. Matović, A. Buha, D. Đukić-Ćosić, Z. Bulat, Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys, Food Chem. Toxicol. 78 (2015) 130–140.
[6] R. Patra, D. Swarup, Effect of Lead on Erythrocytic Antioxidant Defence, Lipid Peroxide Level and Thiol Groups in Calves, (2018).
[7] S. Bhattacharyya, K. Sinha, C Sil P. Cytochrome P450s: mechanisms and biological implications in drug metabolism and its interaction with oxidative stress, Curr. Drug Metab. 15 (2014) 719–742.
[8] 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.
[9] L. O'donnell, K.M. Robertson, M.E. Jones, E.R. Simpson, Estrogen and spermatogenesis, Endocr. Rev. 22 (2001) 289–318.
[10] P. Coumailleau, E. Pellegrini, F. Adrio, N. Diotel, J. Cano-Nicolau, A. Nasri, et al., Aromatase, estrogen receptors and brain development in fish and amphibians, Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 1849 (2015) 152–162.
[11] G. Flora, D. Gupta, A. Tiwari, Toxicity of lead: a review with recent updates, Interdiscip. Toxicol. 5 (2012) 47–58.
[12] J. Pokorný, Natural antioxidants for food use, Trends Food Sci. Technol. 2 (1991) 223–227.
[13] A. Sofowora, E. Ogunbodede, A. Onayade, The role and place of medicinal plants in the strategies for disease prevention, African Journal of Traditional, Complementary and Alternative medicines 10 (2013) 210–229.
[14] P. Shukla, S. Sharma, A. Yadav, V.K. Yadav, Synergistic pharmacological effects of phytochemicals derived from ginger, garlic and tulsi, Int. J. Pharm. Sci. Res. 6 (2015) 4967.
[15] P. Gong, B. Hu, A.I. Cederbaum, Diallyl sulfide induces heme oxygenase-1 through MAPK pathway, Arch. Biochem. Biophys. 432 (2004) 252–260.
[16] N. Nigam, Y. Shukla, Preventive effects of diallyl sulfide on 7, 12-dimethylbenz [a] anthracene induced DNA alkylation damage in mouse skin, Mol. Nutr. Food Res. 51 (2007) 1324–1328.
[17] P. Mellado-García, S. Maisanaba, M. Puerto, A.I. Prieto, R. Marcos, S. Pichardo, et al., In vitro toxicological assessment of an organosulfur compound from Allium extract: cytotoxicity, mutagenicity and genotoxicity studies, Food Chem. Toxicol. 99 (2017) 231–240.
[18] Y. Shan, Z. Wei, L. Tao, S. Wang, F. Zhang, C. Shen, et al., Prophylaxis of diallyl disulfide on skin carcinogenic model via p21-dependent Nrf2 stabilization, Sci. Rep. 6 (2016) 35676.
[19] P. Zhang, M.-L. Noordine, C. Cherbuy, P. Vaugelade, J.M. Pascussi, P.-H. Duée, et al., Different activation patterns of rat xenobiotic metabolism genes by two constituents of garlic, Carcinogenesis 27 (2006) 2090–2095.
[20] A. Elkhoely, R. Kamel, Diallyl sulfide alleviates cisplatin-induced nephrotoxicity in rats via suppressing NF-κB downstream inflammatory proteins and p53/Puma signalling pathway, Clin. Exp. Pharmacol. Physiol. 45 (2018) 591–601.
[21] N.A. Sadik, Effects of diallyl sulfide and zinc on testicular steroidogenesis in cadmium-treated male rats, J. Biochem. Mol. Toxicol. 22 (2008) 345–353.
[22] S.-H. Kim, I.-C. Lee, J.-W. Ko, I.-S. Shin, C. Moon, S.-H. Kim, et al., Mechanism of protection by diallyl disulfide against cyclophosphamide-induced spermatotoxicity and oxidative stress in rats, Molecular & Cellular Toxicology 12 (2016) 301–312.
[23] C.-x. Di, L. Han, H. Zhang, S. Xu, A.-h. Mao, C. Sun, et al., Diallyl disulfide attenuated carbon ion irradiation-induced apoptosis in mouse testis through changing the ratio of Tap73/ΔNp73 via mitochondrial pathway, Sci. Rep. 5 (2015) 16020.
[24] 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.
[25] MaM Szutowski, K. Zalewska, M. Jadczak, M. Marek, In vivo effect of diallyl sulfide and cimetidine on phenacetin metabolism and bioavailability in rat, Acta Biochimica Polonica-English Edition 49 (2002) 249–256.
[26] K. Yokoi, E.O. Uthus, F.H. Nielsen, Nickel deficiency diminishes sperm quantity and movement in rats, Biol. Trace Elem. Res. 93 (2003) 141–153.
[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] 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.
[29] X. Rao, X. Huang, Z. Zhou, X. Lin, An improvement of the 2^ (−delta delta CT) method for quantitative real-time polymerase chain reaction data analysis, Biostatistics, bioinformatics and biomathematics 3 (2013) 71.
[30] 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.
[31] E. Beutler, Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.

[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] A. Aydın, Ö. Ercan, S. Taşcıoğlu, A novel method for the spectrophotometric determination of nitrite in water, Talanta 66 (2005) 1181–1186.
[34] C.F.A. Culling, Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques, Butterworth-Heinemann, 2013.
[35] S.G. Johnsen, Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males, Hormone Research in Paediatrics 1 (1970) 2–25.
[36] C. de Angelis, M. Galdiero, C. Pivonello, C. Salzano, D. Gianfrilli, P. Piscitelli, et al., The environment and male reproduction: the effect of cadmium exposure on reproductive system and semen quality and its implication in fertility, Reprod. Toxicol. 73 (2017) 105–127.
[37] A. Sachan, S. Hendrich, Endocrine-Disrupting Chemicals in Food and their Toxicological Implications. Food Toxicology, Apple Academic Press, 2017, pp. 219–270.
[38] M. G Alves, T. R Dias, B. M Silva, P. F Oliveira, Metabolic cooperation in testis as a pharmacological target: from disease to contraception, Curr. Mol. Pharmacol. 7 (2014) 83–95.
[39] S.-H. Kim, I.-C. Lee, H.-S. Baek, C. Moon, S.-H. Kim, J.-C. Kim, Protective effect of diallyl disulfide on cyclophosphamide-induced testicular toxicity in rats, Laboratory animal research 29 (2013) 204–211.
[40] R. Asadpour, A. Shahbazfar, D. Kianifard, M. Azari, N. Zaboli, Comparison of the protective effects of garlic (Allium sativum L) extract, vitamin E and N acetyl cystein on testis structure and sperm quality in rats treated with lead acetate, Revue Med Vet 164 (2013) 27–41.
[41] M. Ouarda, C. Abdennour, Evaluation of the therapeutic efficiency of raw garlic on reproduction of domestic rabbits under lead induced toxicity, Annals of biological research 2 (2011) 389–393.
[42] W. Wang, Y. Sun, In vitro and in vivo antioxidant activities of polyphenol extracted from black garlic, Food Science and Technology 37 (2017) 681–685.
[43] N. Hosseini, A. Khaki, Effect of aqueous extract of garlic (Allium Sativum) on sperms morphology, motility, concentration and its antioxidant activity in rats, Afinidad 80 (2014) 201–204.
[44] M.R. Anjum, P.S. Reddy, Recovery of lead-induced suppressed reproduction in male rats by testosterone, Andrologia 47 (2015) 560–567.
[45] M. Marchlewicz, T. Michalska, B. Wiszniewska, Detection of lead-induced oxidative stress in the rat epididymis by chemiluminescence, Chemosphere 57 (2004) 1553–1562.
[46] M.-y. Huang, Q.-l. Men, X.-g. Meng, X.-x. Fang, M.-z. Tao, Chronic toxic effect of Lead on male testis tissue in adult Pelophylax nigromaculata, Nature Environment and Pollution Technology 16 (2017) 213.
[47] 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.
[48] 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.
[49] K. Doumouchtsis, S. Doumouchtsis, E. Doumouchtsis, D. Perrea, The effect of lead intoxication on endocrine functions, J. Endocrinol. Investig. 32 (2009) 175–183.
[50] 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.
[51] R.Z. Sokol, Hormonal effects of lead acetate in the male rat: mechanism of action, Biol. Reprod. 37 (1987) 1135–1138.
[52] A. Taiwo, S. Ige, O. Babalola, Assessments of possible gonadotoxic effect of lead on experimental male rabbits, Global Veterinaria 5 (2010) 282–286.
[53] 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.
[54] Y. Oi, M. Imafuku, C. Shishido, Y. Kominato, S. Nishimura, K. Iwai, Garlic supplementation increases testicular testosterone and decreases plasma corticosterone in rats fed a high protein diet, J. Nutr. 131 (2001) 2150–2156.
[55] E.R. Simpson, M.E. Jones, L. O'Donnell, K.M. Robertson, Estrogen and Spermatogenesis, Endocr. Rev. 22 (2001) 289–318.
[56] S. Carreau, C. de Vienne, I. Galeraud-Denis, Aromatase and estrogens in man reproduction: a review and latest advances, Advances in medical sciences 53 (2008) 139–144.
[57] R.A. Hess, Estrogen in the adult male reproductive tract: a review, Reprod. Biol. Endocrinol. 1 (2003) 52.
[58] M.J. Ronis, T.M. Badger, S.J. Shema, P.K. Roberson, F. Shaikh, Reproductive toxicity and growth effects in rats exposed to lead at different periods during development, Toxicol. Appl. Pharmacol. 136 (1996) 361–371.
[59] P. Hasanein, F. Fazeli, M. Parviz, M. Roghani, Ferulic acid prevents lead-induced testicular oxidative stress and suppressed spermatogenesis in rats, Andrologia 50 (2018) e12798.
[60] M.A. Rahman, Y. Gong, S. Kumar, In vitro evaluation of structural analogs of diallyl sulfide as novel CYP2E1 inhibitors for their protective effect against xenobiotic-induced toxicity and HIV replication, Toxicol. Lett. 292 (2018) 31–38.
[61] 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.
[62] K. Cheshenko, F. Pakdel, H. Segner, O. Kah, R.I. Eggen, Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish, Gen. Comp. Endocrinol. 155 (2008) 31–62.
[63] C. Taupeau, J. Poupon, D. Treton, A. Brosse, Y. Richard, V. Machelon, Lead reduces messenger RNA and protein levels of cytochrome P450 aromatase and estrogen receptor β in human ovarian granulosa cells, Biol. Reprod. 68 (2003) 1982–1988.
[64] T. Zeng, K.-Q. Xie, The differential modulation on cytochrome P450 enzymes by garlic components, Food Reviews International 26 (2010) 353–363.
[65] C.-C. Wu, L.-Y. Sheen, H.-W. Chen, W.-W. Kuo, S.-J. Tsai, C.-K. Lii, Differential effects of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats, J. Agric. Food Chem. 50 (2002) 378–383.
[66] C.-K. Lii, C.-W. Tsai, C.-C. Wu, Garlic allyl sulfides display differential modulation of rat cytochrome P450 2B1 and the placental form glutathione S-transferase in various organs, J. Agric. Food Chem. 54 (2006) 5191–5196.
[67] M.J. Graham, B.G. Lake, Induction of drug metabolism: species differences and toxicological relevance, Toxicology 254 (2008) 184–191.
[68] 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.
[69] A. Mabrouk, Therapeutic effect of thymoquinone against lead-induced testicular histological damage in male Wistar rats, Andrologia 50 (6) (2018) e13014, , https://doi.org/10.1111/and.13014.
[70] O.B. Akinola, A.O. Oyewopo, S.A. Biliaminu, S.A. Aremu, S.T. Afolayan, G.O. Sanni, Testicular histomorphometry and semen quality of adult Wistar rats following juvenile oral lead intoxication, Eur. J. Anat. 19 (2015) 65–72.
[71] N. Naha, B. Manna, Mechanism of lead induced effects on human spermatozoa after occupational exposure, Kathmandu University medical journal (KUMJ) 5 (2007) 85–94.
[72] M. Vigeh, D.R. Smith, P.-C. Hsu, How does lead induce male infertility? Iranian journal of reproductive medicine 9 (2011) 1.
[73] A. Jarad, Protective effect of garlic against lead acetate toxicity in some biochemical and histopathological parameters in rats, Al-Anbar Journal of Veterinary Sciences 5 (2012) 108–114.
[74] J. Ram, P. Moteriya, S. Chanda, Phytochemical screening and reported biological activities of some medicinal plants of Gujarat region, Journal of Pharmacognosy and Phytochemistry 4 (2015).
[75] H.A. Saleh, G.A. El-Aziz, H.N. Mustafa, A.H.A. Saleh, A.O. Mal, A.H.S. Deifalla, et al., Protective effect of garlic extract against maternal and foetal cerebellar damage induced by lead administration during pregnancy in rats, Folia Morphol. (Warsz) 77 (2018) 1–15.
[76] A. Sharma, V. Sharma, L. Kansal, Amelioration of lead-induced hepatotoxicity by Allium sativum extracts in Swiss albino mice, Libyan journal of Medicine 5 (2010) 4621.
[77] J.H. Richburg, The relevance of spontaneous-and chemically-induced alterations in testicular germ cell apoptosis to toxicology, Toxicol. Lett. 112 (2000) 79–86.
[78] G.S. Choudhary, S. Al-Harbi, A. Almasan, Caspase-3 activation is a critical determinant of genotoxic stress-induced apoptosis, Apoptosis and Cancer: Springer (2015) 1–9.
[79] 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.
[80] 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.
[81] J. Xu, L.-D. Ji, L.-H. Xu, Lead-induced apoptosis in PC 12 cells: involvement of p53, Bcl-2 family and caspase-3, Toxicol. Lett. 166 (2006) 160–167.
[82] G. El-Akabawy, N.M. El-Sherif, Protective role of garlic oil against oxidative damage induced by furan exposure from weaning through adulthood in adult rat testis, Acta Histochem. 118 (5) (2016) 456–463.
[83] Y.C. Hsieh, H.P. Yu, T. Suzuki, M.A. Choudhry, M.G. Schwacha, K.I. Bland, I.H. Chaudry, Upregulation of mitochondrial respiratory complex IV by estrogen receptor-ß is critical for inhibiting mitochondrial apoptotic signaling and restoring cardiac functions following trauma–hemorrhage, J. Mol. Cell. Cardiol. 41 (3) (2006) 511–521.