EFFICACY OF *Nigella sativa* OIL TO RELIEVE EFFECTS OF THE LEAD MONOXIDE TOXICITY ON TESTICULAR EFFICIENCY AND SEXUAL BEHAVIOUR DISORDERS IN ALBINO RATS

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ABSTRACT

The prevalence of testicular toxicity induced via contamination with heavy metals is increasing worldwide, and a close connection between testicular toxicity and lead has recently been identified. The present study demonstrated the possible therapeutic and protection properties of *Nigella sativa* oil (NSO) against dangerous testicular toxicity and sexual behaviour disorders in rat models induced by lead monoxide (PbO) food poisoning. A total of 24 male rats were used and divided into four groups, consisting of Group 1: Control (1 mL of distilled water); Group 2: PbO (80 mg/kg); Group 3: NSO (0.5 mL/rat); and Group 4: (NSO+PbO) 0.5 mL/rat of NSO, followed by PbO (80 mg/kg/day) respectively for 60 days. The testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17 beta-hydroxysteroid dehydrogenase activity (17β-HSD)**,** sperm parameters, and sexual behaviour (copulation behaviour test) were measured. The results showed that sex hormone levels were significantly decreased (p<0.05) in rats exposed to PbO, while the levels of these hormones were significantly higher in rats exposed to NSO and NSO+PbO. The sperm parameters (numbers, concentrations, motilities, and abnormality) were lower in rats exposed to PbO compared to those in the control, NSO, and NSO+PbO groups that showed significant increases. Sperm abnormality and 17β-HSD activity showed a significant increase at p<0.05 in the group exposed to the PbO compared to those of the control, NSO, and NSO+PbO groups. The results also significantly suppressed sexual behaviour in rats exposed to PbO compared to those in the control, NSO, and NSO+PbO groups, rats were administered NSO+PbO showed improvement in sexual behaviour parameters compared to PbO groups. PbO toxicity was obvious in the reproductive tract through severe histopathological changes. The impact of lead on tissues was partially mitigated by NSO. The study showed protective effects of *N. sativa* oil against changes in sex hormones, sperm characteristics, and sexual behaviour in male rats exposed to lead monoxide-induced testicular toxicity.

Key words: Lead monoxide, *Nigella sativa* oil, sexual behaviour, sex hormones, sperm parameters, testicular toxicity

INTRODUCTION

Nigella sativa oil (commonly known as black seed) is a medicinal plant belonging to the botanical family (Ranunculaceae), and it is used in the Middle East as a medicine for treating different diseases, such as rheumatism, gastrointestinal problems, respiratory complications, and reproductive system dysfunctions. NSO contains essential and fixed oils with unsaturated fatty acids, alkaloids, proteins, and saponin (Mosbah *et al*., 2016). Thymoquinone is the main element of the essential oil, bioactive component, its role promotes immunity, cell survival, and energy metabolism, and it has multiple medicinal hallmarks such as antioxidant components, anti-inflammatory, analgesic, anti-tumour, antiparasitic, antiulcer, anti-asthmatic, neuroprotective effects (depression & Alzheimer's disease), antiparasitic, antitoxin activities, hypoglycemic properties, hypolipidemic properties and renal protective (Yaman & Balikci, 2010; De Silva & Alcorn, 2019; Yimer *et al*., 2019).

Despite the significant advances in understanding the mechanisms by which the role of NSO improves testicular performance (sperm viability & motility) and sexual hormones (Darand *et al*., 2019), as well as its beneficial effects on aphrodisiac properties and fertility enhancement (Gali-Muhtasib *et al*., 2006; Hala & Wahb, 2011; Ahmad *et al*., 2013; Assi *et al*., 2016), there are still some unexplored or poorly understood aspects that point to NSO's protective effect against the dangerous toxicity of heavy metals on the reproductive system. This study highlights some theories suggesting that daily consumption of NSO plays a key role in the protection against disrupters of the seminiferous epithelium of testis, damage to the important regulation steps in spermatogenesis,

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infertile and dysfunction the sexual behaviour induced by heavy metal contamination (Yimer *et al*., 2019).

No reverse side effects were reported in using NS oil (5 mL/day) for six months in functional dyspeptic patients, with some simple or mild adverse effects observed, such as bloating, nausea, and burning sensation (Tavakkoli *et al*., 2017). In other studies carried out on Iranian infertile men to estimate the effects of NS oil (5 mL/day) on abnormal sperm quality, were no observed severe side effects (Kolahdooz *et al*., 2014). Dirjomuljono *et al*. (2008) found the NS extract safety (1,080 mg/day) for treating patients with tonsillopharyngitis. In another study that investigated the anticestodals impacts of NS powdered seeds (40 mg/kg body weight) on children infected with cestodes, were no reported dangerous side effects (Tavakkoli *et al*., 2017).

Heavy metals are associated with many diseases in both humans and animals, such as immunotoxicity, changed sex hormone balance, impaired reproductive systems (low fertility), mental retardation, changed metabolism, and behavioural abnormalities (Wu *et al*., 2016). Lead monoxide (PbO) is a hazardous metal to humans and different ecosystems, as it is used in various industries such as paints, glass, ceramics, and batteries (Boldyrev, 2018). Humans are exposed to metals mainly by inhaling dust and fumes or by consuming contaminated water and food (Cobbina *et al*., 2015). Food can be contaminated with lead during its processing or through food containers. Soil plays an important role in ecosystems and is the main resource of food and crop production, moreover, the soils are threatened by many pollutants, such as heavy metals are a great concern. heavy metals can reside in the soil for long periods resulting in health and environmental issues, these metals can be slowly accumulated in the human body in different ways like ingestion, inhalation, and dermal contact (Hu *et al*., 2020). Water-related diseases can often be attributed to exposure to industrial waste tailings (like the illegal disposal of acid lead batteries) and lead plumbing, as well as agricultural waste containing increased lead concentrations as a result of anthropogenic activities (Aloke *et al*., 2019).

PbO overexposure causes reproductive toxicity, behavioural abnormalities, and sterility in humans and experimental animals (Rabinowitz, 1998; Farooq *et al*., 2008; Kapitula, 2015) because it overlaps with physiological processes and cell functions, causing damage to spermatogenesis and dysfunction in sex hormone secretion and sexual behaviour (Ara & Usmani, 2015). According to the World Health Organization, haematological toxicity occurs at a lead concentration of 200 micrograms per litre of blood and begins poisoning the reproductive and neurological systems at a lead concentration of 400–500 micrograms per litre of blood (Hussain *et al*., 2019; Wani *et al*., 2019).

Several studies have shown that PbO toxicity targets male fertility and the pituitary-testes axis by inducing infertility, decreasing sex hormones, reducing sperm quality (sperm number, concentration, motility), and altering the morphology of testis tissues (Navas-Acien *et al*., 2007; Sikka & Wang, 2008; Mokhtari & Zanboori, 2011). PbO poisoning has dangerous side effects, so the use of herbs such as NSO to treat lead poisoning is considered to be a very important therapeutic strategy. Therefore, the purpose of this study was to illustrate the protective effects of NSO against the reproductive system toxicity of adult rats induced by lead monoxide exposure.

MATERIALS AND METHODS

Ethical approval

The experimental protocols were approved by the Department of Biology, College of Sciences, University of Babylon (1123/18-3-2020), and the experiment was carried out following approved guidelines and in compliance with ethical standards according to the National Committee for Research Ethics in Science and Technology (NETNT).

Animals model

In this study, 24 adult male Wistar albino rats approximately three months old (weighing 200–220 g) were used, obtained from the animal house in the College of Science, University of Babylon. All rats were housed in cages (six rats per cage) for two weeks at a relative humidity (60 ± 10%), controlled temperature (23 ± 3 °C), and under a 12-h dark and 12-h light cycle, with allowance for water and food *ad libitum*.

Experimental design

After two weeks of acclimatisation, the animals were divided into four groups, with each group containing six rats, as follows:

Group 1: (control group)
The rats were fed a basic diet with orally administered 1 mL of distilled water.
Group 2: (lead monoxide induction group)
The rats were fed lead oxide (80 mg/kg/day) mixed with their diet for 60 days, after determining the registered LD50 half-dose according to Assi (2019).
Group 3: (Nigella sativa oil (NSO) group)
The rats were administered NSO (0.5 mL/rat/day) orally for 60 days. after determining the registered LD50 half-dose by Zaoui *et al*. (2002)
Group 4: (co-treatment of each Nigella sativa oil and lead monoxide (NSO+PbO)
The rats were fed a basal diet mixed with lead monoxide (80 mg/kg/day), followed by 0.5 mL/rat/

day of NSO, orally for 60 days.

Preparation of the lead monoxide and *N. sativa* oil

Lead monoxide powder (PbO) was obtained from the laboratories of the College of Science, University of Babylon, and it was prepared at 80 mg/kg of body weight before being mixed with the basal diet of the rat.

NS oil seed pure (100%) was procured from a local pharmacy which was prepared by steam distillation at the manufactory by Fabriqué par: Hemani International KEPZ (Pakistan).

Estimate levels of testosterone, FSH, and LH hormones

The rats were anaesthetised and dissected. The blood sample was collected directly from the cardiac puncture and separated into two: the first blood sample was used to obtain serums that were used for the assessment of testosterone, LH, and FSH levels, which were determined using the sandwich-ELISA kit following the manufacturer's instructions (Elabscience company, China). The second blood sample was used for spectrophotometric analysis of blood PbO levels by using an "Atomic Absorption Spectrophotometer" (Daku & Salisu, 2016).

Estimate of steroidogenic enzyme 17 β-HSD activity

The steroidogenic enzyme 17β hydroxysteroid dehydrogenase activity was evaluated in the homogenate testis by a specifically described method (Abarikwu *et al*., 2016). The activity of the enzyme was measured by the addition of NAD to the tissue supernatants at 340 nm against a blank (without NAD). One unit of enzyme activity is the amount causing a change in absorbance of 0.001 per min at 340 nm.

Epididymal total number and sperm concentration

The epididymis was removed directly from the rats after anaesthesia and was put in Petri dishes containing one mL of normal saline (5%). These solutions were stirred, one drop was put on the slide, and a coverslip was put over the droplet. At least 10 microscopic fields were observed at 400x magnification to find the concentration of sperm (N) in 10 fields according to the following equation by Chabuk (2019).

$$\text{Sperm concentration in (1 mL) of the epididymis} = \frac{N}{10} \times 10^6$$

The epididymis was cut using a sharp scalpel. 10 mL of formalin (40%) and 90% of normal saline were then added. The sperm counting was done using a haemocytometer chamber in a small box to slice

count. The number of sperm was extracted according to the equation by Mazen *et al*. (2017):

$$\text{Total number of sperms} = \frac{N}{80} \times 4000 \times 10$$

Sperms motility and abnormality percent

To evaluate the motility, one drop of the epididymis solutions was put on a clean slide to calculate the moving sperm percentage in 10 random fields according to the equation. After that, eosin-nigrosin stains were used to stain the sperms to determine their morphology (Chabuk 2019).

$$\text{Sperms Motility Percentage} = \frac{\text{Number of sperm motile}}{\text{Number of total sperm in the epididymis}} \times 100$$

Sexual behaviour test (copulation testing)

A copulation behaviour test was performed after the treatment period ended. One receptive female was placed with the male in the home cage for 10 min and the behavioural tests were recorded for 10 min (each male rat was introduced to a female rat at a single once test in the day) by a video camera. The scoring output analysis yields a set of parameters by which copulation behaviour is estimated: the number of mounts, mounting latency, number of intromissions, intromission latency, and number of anogenital sniffing of the female, according to the method by Heijkoop *et al*. (2018).

Histopathological examinations

Small testis pieces were fixated using Bouin's solution, then exposed to the histological analysis process, alcohol series, infiltration, embedded in wax paraffin for two hr, and stained with eosin and haematoxylin for examination under a light microscope to estimate the possible alterations in the seminiferous tubules by a pathologist. Seminiferous tubule damage was observed; a four-level grading was used to measure the quantity of the histopathological changes. Grade 1 indicated (-) no change, while Grade 2 showed a mild (+) histopathological change. Grade 3 signified a moderate (++) histopathological change, whereas Grade 4 demonstrated a severe (+++) histopathological change (Kianifard *et al*., 2011).

Statistical analysis

Data is represented as M ± SE, and statistical differences between the groups were analysed using one-way ANOVA and Tukey's posthoc test was done using SPSS version 23. The differences were considered statistically significant at $p<0.05$.

RESULTS

Sex hormones

Figure 1 illustrates data on testosterone, FSH, LH levels, and activity of the 17*β*-HSD enzyme in rats exposed to NSO, PbO, and their combinations for 60 days. Animals treated with PbO exhibited a

significant ($P<0.05$) decrease in testosterone, LH, and FSH levels. However, 17β-HSD activity showed a significant increase ($P<0.05$) compared to that of the control, NSO, and PbO+NSO groups, whereas the levels of these hormones, were improved, notably at ($P<0.05$) in both the NSO and PbO+NSO groups. 17β-HSD activity significantly decreased ($P<0.05$) in the control, NSO, and PbO+NSO groups compared to that of the PbO group.

Spermatozoa characteristics

The changes in spermatozoa properties of rats treated with NSO, PbO, and their combinations are shown in Figure 2. PbO alone caused a significant decrease ($p<0.05$) in total numbers, motility, and concentration of sperm, with a significantly increased abnormal and dead sperm count in comparison to the control, NSO, and PbO+ NSO groups. Meanwhile, treatment with NSO alone induced a significant improvement in spermatid concentration, numbers, and motility and reduced abnormal and dead sperm counts compared to the control and PbO group. The presence of NSO with PbO diminished the toxicity of spermatozoa quality compared to the PbO group. Consequently, prior exposure to NSO was able to block the development of these impacts.

Blood lead monoxide concentration

Table 1 shows the values of lead monoxide concentration in the blood of the different groups. A significant decrease ($P\leq0.05$) was recorded in rats from the PbO+NSO, NSO, and control groups compared to the PbO group which showed increased blood lead monoxide concentration. No significantly ($p>0.05$) difference was noted between control and NSO groups.

Sexual Behaviour Testing

Analysis of the parameters of the sexual behaviour (Table 2) showed that rats exposed to lead monoxide had a significant decrease ($P\leq0.05$) in sexual behaviour parameters such as the number of mounts, intromissions, and anogenital sniffing of the female compared to other groups (control, NSO, & PbO+NSO groups). Conversely, *Nigella sativa* caused a significant increase ($P\leq0.05$) in sexual behaviour parameters compared with the PbO+NSO group. Meanwhile, the number of mounts and intromissions among the control group, NSO, and PbO+NSO groups was non-significant. The number of anogenital sniffing parameters showed a significant decrease ($P\leq0.05$) between the control and PbO+NSO groups. PbO administration significantly increased ($P\leq0.05$) in time of mounts and intromissions latency (s) in the male rats that were achieved during the 10 min sexual behaviour recording compared to control, NSO, and PbO+ NSO groups, but after treatment with NSO / or their combinations significantly reduce the time of mounts and intromissions latency (s) compared to
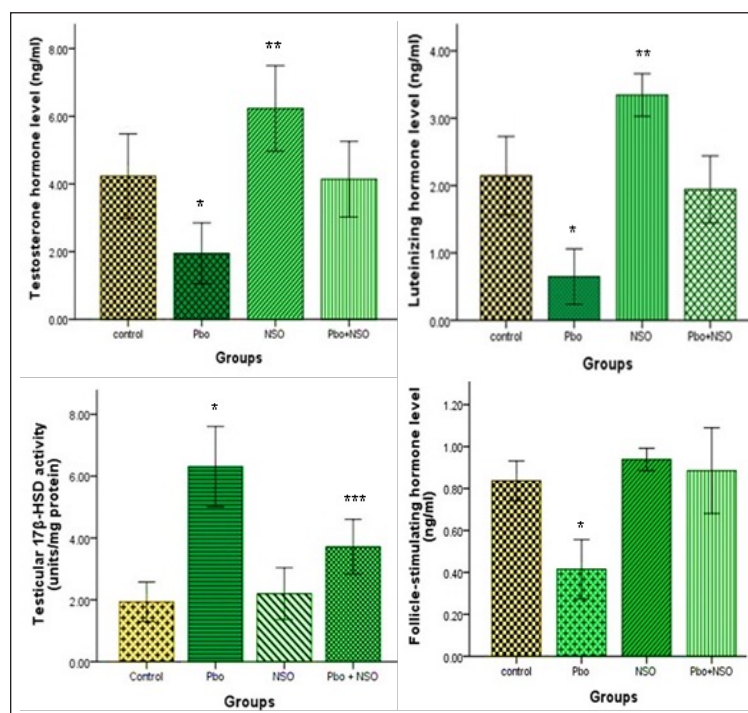

Fig. 1. Alterations in testosterone, FSH, LH levels and activity of 17β-HSD enzyme after rats exposed to PbO, NSO and/or their combination for 60 days. PbO: lead monoxide, NSO: *Nigella sativ*a oil, FSH: follicle stimulating hormone; LH: luteinizing hormone. Data represented as mean ± SE.
*Values differ significantly at the $P\leq0.05$ from PbO group.
**Values differ significantly at the $P\leq0.05$ from NSO group.
***Values differ significantly at the $P\leq0.05$ from PbO + NSO group.

PbO group.

Figure 3 shows the effect of PbO, NSO, and/or their combinations for 60 days of administration on the sexual behaviour performance of rats. Rats in the PbO group showed less frequent sexual behaviour compared to those in the control, NSO, and PbO+NSO groups. Meanwhile, rats exposed to NSO showed more frequent sexual behaviour. This behaviour is usually measured by *the sum of the number of mounts, intromissions, and anogenital sniffing.*
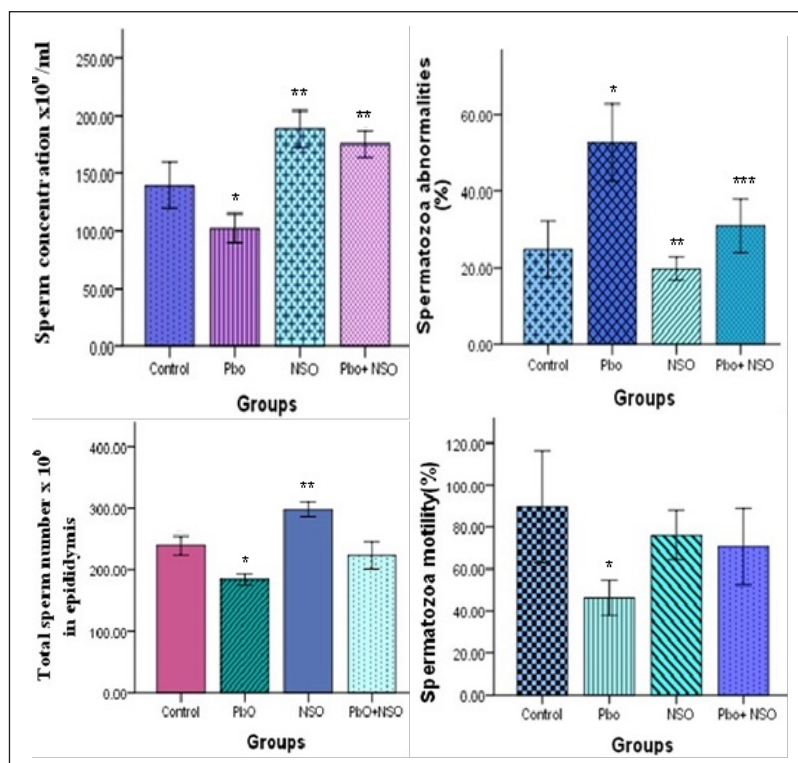

Fig. 2. Effect of PbO, NSO or their combination on sperm parameters of rats for 60 days. (total sperm number, sperms concentration, spermatozoa motility and spermatozoa abnormalities). PbO: lead monoxide, NSO: *Nigella sativa* oil.
* Values differ significantly at the $P \leq 0.05$ from PbO group.
**Values differ significantly at the $P \leq 0.05$ from NSO group.
***Values differ significantly at the $P \leq 0.05$ from PbO + NSO group.

Table 1. Lead monoxide concentration in the blood after rats exposed to PbO, NSO or their combination for 60 days.

Parameters/Groups	Control	PbO	NSO	Pbo+NSO	Sig.
lead monoxide levels (µg/dL)	10.38 ± 0.59	61.78 ±3.08*	6.56 ± 0.82	15.43±0.62**	0.000

Data are mean ± S.E
*The mean is significantly different at the $P \leq 0.05$ as compared to controls, NSO and PbO+NSO groups.
** The mean is significantly different at the $P \leq 0.05$ as compared to the NSO group.

Table 2. The changes in sexual behaviour parameters of males rats exposed to PbO, NSO and/or their combination for 60 days. PbO: lead monoxide, NSO: *Nigella sativa* oil.

Parameters/Groups	Control	PbO	NSO	PbO+NSO
Number of mounts	22.33±5.47**	5.33±1.75*	25.00±3.58	19.50±2.43**
Number of intromission	17.50±4.04**	4.00±1.41*	20.33±4.68	13.00±5.51**
Number of anogenital sniffing of the female	34.17±5.78	8.67±2.80 *	41.17±8.23	25.33±6.50**
Latency of mounts (s)	153.29±4.85	369.51±3.83*	129.95±5.14#	168.28±7.09
Latency of intromissions (s)	356.46±14.94	501.86±33.46*	324.79±12.77	374.80±30.05

Values are mean ± S.D.
*The mean is significantly different at the $P \leq 0.05$ as compared to controls, NSO and Pbo+NSO groups.
** The mean is significantly different at the $P \leq 0.05$ as compared to PbO and NSO groups.
\# The mean is significantly different at the $P \leq 0.05$ as compared to the control, Pbo and Pbo+NSO groups.
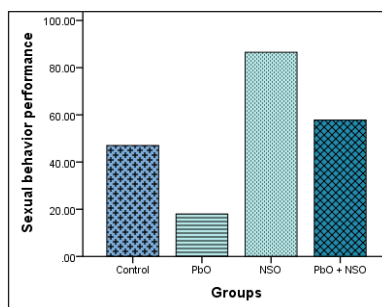
Fig. 3. Sexual behaviour performance for 10 min of males rats exposed to PbO, NSO and/or their combination for 60 days. PbO: lead monoxide, NSO: *Nigella sativa* oil. PbO**:** lead monoxide, NSO: *Nigella sativa* oil,

Values are (Mean ± S.D).
* Values differ significantly at the $P \leq 0.05$ from PbO group.
Values differ significantly at the $P \leq 0.05$ from NSO group. **
Values differ significantly at the $P \leq 0.05$ from PbO + NSO group.

Testicular Histopathology

Testis histopathological alterations exhibited severe tubular damage (necrosis in germinal epithelial layers and vacuolar degeneration), tubular desquamation, the disappearance of spermatozoa in the lumen of the tubule, and a low index of spermatogenesis in PbO-treated rats (Figures 4 & 5). Meanwhile, NSO and PbO+NSO reduced PbO-induced testicular changes (Figures 6, 7, & Table 3). Rats in the control group did not show any changes in testis tissue (Figure 8).

DISCUSSION

Previous studies recorded the beneficial effects of *N. sativa* oil on the principal structural and functional characteristics of the reproductive systems, sperm fertility, and quality, but did not indicate the protective role of NSO against poisoning by heavy metals such as lead monoxide, which cause deleterious impacts on the testicular tissues, reproduction, and fertility in male rats and humans (Assi *et al*., 2016). This study showed decreased levels of LH, FSH, and testosterone hormones in rats chronically exposed to lead monoxide, perhaps due to the direct effect of this metal on the hypothalamus and pituitary gland. Thus, it causes FSH and LH hormone secretion impairment (Assi *et al*., 2016), reduces testosterone hormone secretion from Leydig cells, and leads to decreased spermatozoa quality (Assi, 2019). Lead can also cause high angiotensin II levels and cause impairment in the sodium-potassium pumps. Angiotensin II is connected to the receptors of LH in the membranes of Leydig cells and causes suppression of adenylate cycle activities and cAMP construction, leading to a decreased level of testosterone hormone (Leung & Sernia, 2003). Although PbO increases the levels of a steroidal 17*β*-HSD enzyme that aids in testosterone synthesis, it is rapidly metabolised and does not cause testosterone biosynthesis. NSO reduces 17*β*-HSD and prevents its metabolism. Thus, NSO improves the steroidogenesis and spermatogenesis processes and, consequently, testosterone production by increasing sperm proliferation (Mosbah *et al*., 2016)**.** Since the main functions of the testes are hormone production and spermatogenesis, the toxic effects of lead cause harm to the testes tissue and damage both the hormone production and the spermatogenic process (García-Lestón *et al*., 2010). These results are consistent with Chowdhury (2009), Sharma and Garu (2011), Al-Masri (2015), and Assi *et al*. (2016), which discovered that lead causes toxicity in a male rat's gonadal gland by inducing changes in sperm morphology and motility, as well as a reduction in sex hormones.

The results showed a decrease in sexual behaviour in rats exposed to PbO, which might be due to the aggregation of lead metal in the brain causing changes in the neurotransmitter systems, especially the dopaminergic systems that are essential for a sense of smell and sexual behaviour. Lead reduces the production of the serotonergic and dopaminergic systems (Heijkoop *et al*., 2018) and affects the
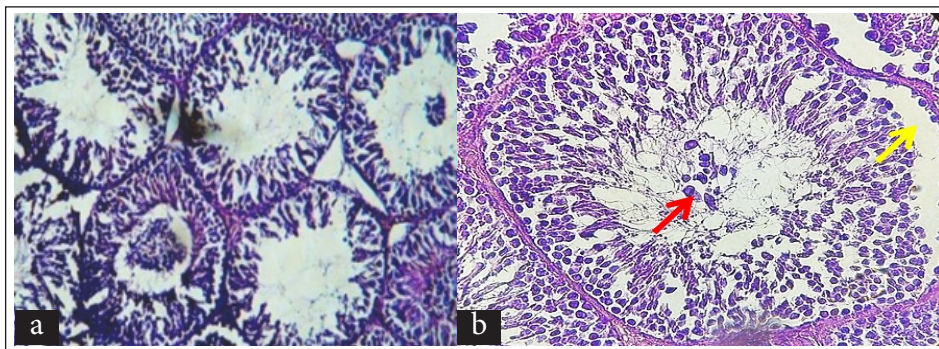

Fig. 4. Histopathological analysis of rat testis exposed to Pbo showed seminiferous tubules lumen are empty from spermatozoa and filled with desquamated cells (red), some basement membranes are separated from the overlying germinal epithelial cells (yellow). Hematoxylin and eosin, (a) 100x and (b) 400x.
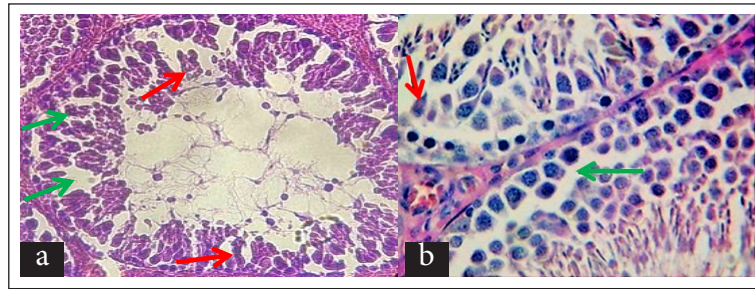
Fig. 5. Histopathological analysis of rat testis exposed to Pbo showed sloughing of the germinal epithelial layers with decayed interstitial space and scattered Sertoli cells (red). necrosis and vacuolar degeneration in the germinal epithelial of seminiferous tubules (green), Hematoxylin and eosin (a) 200x and (b) 400x.
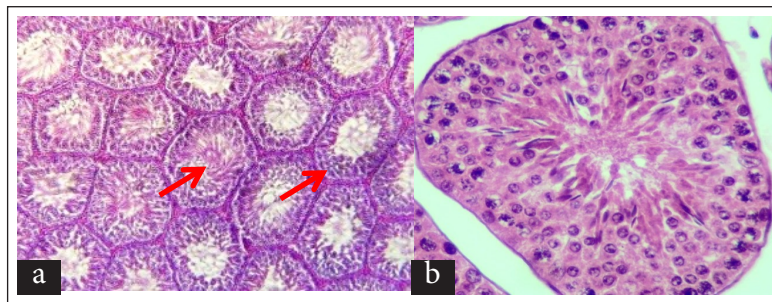

Fig. 6. Histopathological analysis of rat testis treated with NSO optimal spermatogenesis with the appearance increased of sperm maturation in the lumen. Hematoxylin and eosin (a) 100x and (b) 400x.
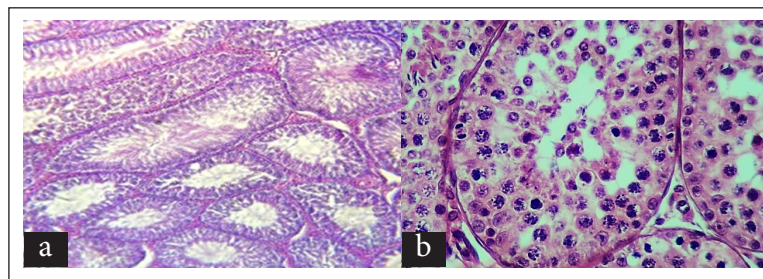

Fig. 7. Histopathological analysis of rat testis treated with Pbo + NSO showed normal appearance and improvement of spermatogenic cells in the seminiferous tubule of rats. Hematoxylin and eosin, (a)100x and (b) 400x.
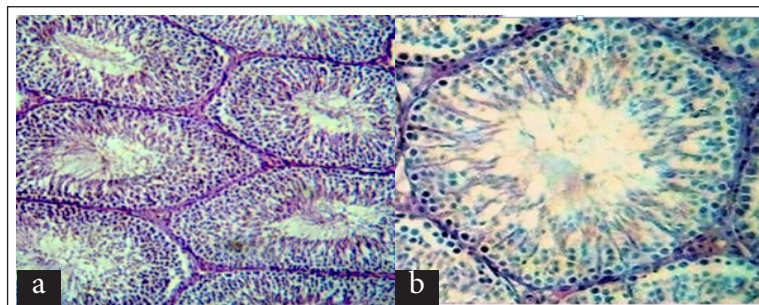

Fig. 8. Histopathological analysis of rat testis in the control group showed normal seminiferous tubules with present spermatozoa in the lumen. Hematoxylin and eosin, (a)100x and (b) 400x.

preoptic area of the rat brain, which is considered the site of gonadal steroids that modulate gonadotropin secretion and sexual behaviour (Naqvi *et al*., 2010).

The production of neurosteroids from gonadal and adrenal glands may influence a male's sexual behaviour through receptors for GABAA existing in the preoptic area of the brain. It is fundamental to sexual attraction, intercourse, erection, and ejaculation (Mokhtari & Zanboori, 2011). Neurosteroids may modify olfactory activities so that they sexually attract males. Considering that lead harms the steroidogenic acute regulatory protein (STAR) and cytochrome enzymes, this enzyme's activities reduce the production of a neurosteroid and lead to the reduction of a fundamental neurosteroid that triggers sexual behaviours, causing a decrease in smell–related behaviours and sexual functions (Yoon *et al*., 2005).

Lead also reduces GnRH levels, which in turn causes a reduction in gonadotropin hormones and testosterone, and ultimately decreases sexual behaviour (Bataineh *et al*., 1998). Therefore, this study used the NSO to assess its effects on lead toxicity. The NSO reduced the harmful effect on the reproductive organs of rats exposed to lead toxicity. These oilseeds have different therapeutic purposes since they contain active compounds including alkaloids such as nigellicine, nigelline, and nigellone, as well as chemical materials like thymoquinone, quinine, and thymohydroquinone. These materials are considered anti-inflammatory, and antimicrobial, and also protect against toxicity (Assi *et al*., 2016). Furthermore, the optimal doses of NSO decreased the deleterious effects of lead on reproductive organs. The exposure of normal rats to NSO showed a rise in sperm production and testosterone levels, indicating that these seeds boost male fertility (Mahdavi *et al*., 2015). This study observed that exposure to NSO with PbO for 60 days led to a reduction in the toxicity of PbO on the gonadal gland, sex hormones, and spermatogenesis. This may be due to NSOs preventing the absorption of lead in the intestines (Darand *et al*., 2019), or maybe due to thymoquinone's remarkable

improvement in the quality of sperm and testicular histology due to the downregulation or inhibition of Bax and caspase-3 apoptotic pathways (Hassan *et al*., 2019). It also promoted sex hormone levels and corrected the sexual hormone imbalance both in vitro and in vivo by upregulating aromatase gene expression and reducing oxidative stress by elevating the levels of antioxidant enzymes. The study proved that NSO had therapeutic effects against PbO toxicity in rats (Wani *et al*., 2022). These results are in line with a previous study, where NSO at 250 mg/kg was reported to increase the level of testosterone in rats exposed to lead acetate (Assi *et al*., 2016).

One of the possible properties of the *N. sativa* seeds is the ability of one or more of their compositions to decrease metal toxicity. Thymoquinone and unsaturated fatty acids can reduce oxidative stress and increase the repair of antioxidant enzymes in the body (Yimer *et al*., 2019). This is thought to be because of the antioxidant properties of NSO that protect normal sperm cells, which are exposed to reactive oxygen species (ROS) (Sangi *et al*., 2019). As a result, NSO scavenges these free radicals created by heavy metals, including weakening the negative impacts of lipid peroxidation and DNA impairment while inhibiting sperm maturation, thus protecting the spermatogenic cell's development (Rahman *et al*., 2013).

The histological analysis showed that exposure of rats to PbO for 60 days induced degeneration in testicular tissues and necrosis, vacuolar degeneration in the germinal epithelial of seminiferous tubules, and several seminiferous tubules composed of fewer spermatogenic layers. Conversely, oral administration of NSO succeeded in preventing the harmful effects of PbO on seminiferous tubules by markedly improving spermatogenesis and sustained sperm quality in rats intoxicated with PbO by restricting oxidative stress. Thymoquinone can protect against testicular tissue-induced damage through an antioxidant mechanism (Mollazadeh & Hosseinzadeh, 2014). This compound inhibits the inflammation process and apoptosis within the testicular tissue by blocking the release of

Table 3. Levels of the histopathological alterations grading in rats testis exposed to PbO, NSO and/or their combination for 60 days. PbO: lead monoxide, NSO: *Nigella sativa* oil

Groups	Seminiferous tubules damage (necrosis and vacuolar degeneration)	Disappear spermatozoa in the tubules lumen	Appear desquamated cells in the lumen	Spermatogenesis index
Control	-	-	-	3.36 ± 0.27
PbO	++++	++++	+++	1.29 ±0.24 *
NSO	-	-	-	4.43 ±0.14
PbO+NSO	++	+++	+	2.49 ±0.17 *

levels grade: (-) no change, (+) mild,(++) moderate,(+++) severe histopathological alterations
* The mean difference is significant at the p<0.05 level at compared to control, NSO groups.

apoptotic mediators and inflammatory cytokines such as cytochrome C and Bax proteins (Wani *et al*., 2022). These findings agree with the remarks that show PbO acts as a spermicidal factor, leading to the suppression of serum reproductive hormone concentration and spermatogenesis in rats. Oral treatment of antioxidant NSO has partly alleviated the effects of lead and induced histological changes in the testis but has not completely prevented them (Mahdavi *et al*., 2015; Assi *et al*., 2016)**.**

CONCLUSION

The current study indicated the role of *N. sativa* oil in reducing the impact of lead monoxide toxicity, which causes damage to the testis and has a serious effect on semen parameters, sexual behaviour, and sex hormone levels, causing histopathological damage to the testis. Meanwhile, co-administration of PbO and NSO completely or partially reversed changes in semen quality and hormone levels, resulting in improved sexual behaviour and reduced histopathological injuries of the testis.

ACKNOWLEDGEMENTS

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