Gonadal health benefits of black cumin (*Nigella sativa*) seed oil on chronic lead-exposed male Wistar rats

Afrina Mustari[1], *, Mahabub Alam[1], Samia Rashid[1], A.G.M.Sofi Uddin Mahamud[2], Mohammad Alam Miah[1], Emdadul Haque Chowdhury[3]

[1]Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensigh-2202, Bangladesh
[2]ABEx Bio-Research Center, East Azampur, Dhaka-1230, Bangladesh
[3]Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensigh-2202, Bangladesh

*Corresponding author
Afrina Mustari
Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensigh-2202, Bangladesh.
Email: afrina.mustari@bau.edu.bd

ABSTRACT

Lead (Pb) is a highly toxic heavy metal that can cause harm even at extremely low concentrations and damage various organs, including those of reproduction. Medical herbs like *Nigella sativa* include antioxidants that have been shown to enhance spermatogenesis and male fertility. This research aimed to investigate the gonadal health benefits of *Nigella sativa* seed oil (NSO) as an antidote in male rats exposed to chronic Pb toxicity. Forty rats were divided into four groups: a control group, a group exposed to Pb acetate (75 mg/kg body weight), a group given NSO (1ml/kg feed), and a group exposed to Pb acetate (75 mg/kg body weight) along with NSO (1ml/kg feed). Pb was administered through water, while NSO with fed for 16 weeks. Rats were euthanized, blood was obtained, and serum was prepared to perform hormonal study. Sperm concentration and motility along with testis weight and diameter were measured. The testes were collected for histology. The findings indicated a significant drop in serum concentrations of testosterone and thyroxin (T4) in Pb-exposed rats, whereas NSO demonstrated a remarkable ability to restore these hormone levels. Moreover, Pb exposure led to a notable reduction in sperm motility, sperm concentration, testis weight, and testis diameter, accompanied by seminiferous tubule degeneration. However, the administration of NSO effectively counteracted the adverse effects induced by chronic Pb exposure in rats. Together, these findings revealed the gonadoprotective effects of NSO against lead toxicity, suggesting its preventive as well as therapeutic potential in preserving the testicular function of rats exposed to chronic Pb toxicity.

INTRODUCTION

Lead (Pb) is widely recognized as one of the most perilous toxic heavy metals that possess the potential to inflict severe harm on nearly all essential organ systems, encompassing the neurological, renal, and reproductive systems [1]. As per the World Health Organization (WHO) estimates, Pb exposure results in roughly 1 million fatalities and 21.7 million disability-adjusted life years (DALYs) worldwide each year. Moreover, it is linked to idiopathic intellectual disability (accounting for 30% of cases), cardiovascular disease (approximately 4.6%), and chronic kidney diseases (around 3%)[2]. Numerous industrial chemicals are well-documented for their detrimental effects on human reproductive health [3], with a particular focus on the adverse outcomes associated with the workplace as well as atmospheric contact with heavy metals such as Pb [4, 5]. Exposure to inorganic Pb in male health is linked to decreased semen quality, impacting spermatogenesis and fertility. Elevated Pb levels in seminal plasma can also reduce sperm count [6]. Moreover, Pb exposure can adversely affect semen quality parameters, such as sperm density, motility, and morphology [7]. Epidemiological studies have indicated that male employees having blood Pb

concentrations between 10 and over 40 μg/dl may face an elevated risk of experiencing infertility [8].

It is important to note that Pb poisoning can affect not only humans but also domestic animals, ranging from livestock to pet animals [1]. Animals can get intoxicated from Pb-contaminated feed and water, primarily from soil, industrial pollution, agricultural practices, and feed processing. Notably, Pb in poultry feed often comes from bone, blood meals, and tannery waste, leading to poisoning, reduced performance, and animal fatalities [9]. Acute Pb poisoning in animals can result in a 100% fatality rate, while chronic exposure affects the male reproductive system by causing testicular damage, atrophy of Leydig cells, and semen abnormalities like azoospermia, asthenozoospermia, teratozoospermia, and morphologically abnormal sperm via leading to reduced fructose and succinic dehydrogenase levels [10]. For instance, Pb exposure leads to reduced sperm counts within the epididymis along with ceased sperm production [11]. Additionally, microscopic examinations have revealed noteworthy alterations in the morphological structure of testicular tissues and reduced germ cell populations [12]. Moreover, mice exposed to Pb exhibited degeneration of the seminiferous tubule [13].

Herbal medications, which have been used for therapeutic purposes since antiquity and are often free of side effects, are becoming increasingly popular over synthetic treatments due to their natural origin and the fact that nature supplies the primary resources for human health [14]. The alcoholic extract of *Nigella sativa* has been found to improve reproductive health, including body weight gain, reproductive parameters (e.g. seminiferous tubules, spermatogonia, spermatocytes, spermatids, Sertoli and Leydig cells, Leydig cell diameter, and epididymal caudal region epithelial cell height), and testosterone along with follicle-stimulating hormone (FSH) levels [15]. In rams, black cumin seed extract increased ejaculation volume, sperm activity, and motility, improving accessory gland production [16], while in male rats, this improved histomorphological functions of testicles while decreasing sperm abnormalities [17]. However, the toxic effect of Pb on reproduction has already been revealed but mitigation of toxic effects by natural herbs like *Nigella sativa* seed oil (NSO) is still unclear in Wistar male rats. Therefore, the present study aimed to reveal the effects of NSO on the testicular morphology, hormonal assay, and histological changes in chronic Pb-exposed male Wister rats.

MATERIALS AND METHODS

Ethical Approval

The Animal Welfare and Experimentation Ethics Committee at Bangladesh Agricultural University, Mymensingh, granted approval for the animal care, management, and experimental procedures under reference number AWEEC/BAU/2023 (42).

Experimental site and layout

The research was carried out at the Department of Physiology, Bangladesh Agricultural University, Mymensingh, over 16 weeks. Forty male albino Wistar rats weighing between 270-280 g were sourced from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B) in Dhaka. The animals were kept in a meticulously controlled environment, ensuring precise regulation of factors such as temperature, humidity, ventilation, and lighting. Rats were randomly divided into four groups of 10

rats each. Group A served as a control group that received standard rat chow only. Group B rats were administered 75 mg Pb acetate/kg body weight (BW) in the drinking water whereas group C rats were given 1 mL NSO/kg feed. Lastly, Group D rats were subjected to combined treatment, receiving daily doses of 75 mg Pb acetate/kg BW and 1 mL NSO/kg feed. All the experimental treatments were given for 16 weeks. Pb acetate and NSO were purchased from "Sweety Scientific Traders Ltd", Mymensingh, Bangladesh, and the doses of NSO and Pb was selected according to results obtained from our earlier research [18, 19].

Blood collection and serum preparation

After the 16-week experiment, the rats were subjected to an overnight fast. Subsequently, each rat was placed individually in a sealed chamber containing cotton soaked with diethyl ether. Once unconscious, the rats were removed from the chamber, and blood samples were collected directly from the heart using a sterile syringe. Approximately 3-3.5 ml of blood had been withdrawn and the tubes containing the blood were gently slanted and left at normal temperature for 60 minutes. Afterward, the blood clot had been carefully removed from the inner tube wall, and then given some time for settling, following which the serum was obtained. This serum was then subjected to centrifugation at 3000 rpm for 15 minutes to get clean serum, which was subsequently kept at -20°C till required.

Hormonal assay

At the Institute of Nuclear Medicine & Allied Sciences (INMAS; Mymensingh Medical College, Mymensingh, Bangladesh), serum levels of T_4 and testosterone were measured using a radioimmunoassay kit (Berthold, Bad Wildbad, Germany) in accordance with the standard protocol [20], with an assay sensitivity of 0.10 nmol (0.0288 ng/ml). For testosterone, the inter-assay coefficient of variation was 12.2%.

Assessment of sperm physiological characteristics along with testicular parameters

After sacrifice, the testis was checked for weight by using an electronic balance. The testis diameter was measured by using slide calipers. The epididymis was taken out to test sperm motility and concentration. In brief, a small amount of the diluted mixture was put on a previously warmed slide, and an outer cover slip was placed on it. The percentage (%) of sperm motility had been established. Both the epididymal sperm collection and sperm computation had been completed [21]. To do this, the rat's cauda epididymis was removed in a Petri dish and then minced with the help of scissors. The macerated epididymis was put into a test tube containing 4 milliliters of prewarmed (37 °C) normal saline. After that, 5-10 minutes were given so that the sperm could come out. Finally, the sperm was estimated using Neubauer's chamber and a microscope's high-power objective.

Histopathological analysis

Testicles were obtained from each group of rats, and thorough removal of blood was achieved through perfusion with phosphate-buffered saline. These collected testicles were then placed in 10% neutral buffered formalin and preserved for a duration of 15 days. After this preservation period, the properly fixed tissues were subjected to

processing, sectioning, and staining (hematoxylin and eosin) in collaboration with the Department of Pathology at Bangladesh Agricultural University, Mymensingh. This followed the established protocol as outlined by [22]. The stained slides were examined using an Olympus Photomicroscope (Model CX43) at the Department of Physiology, BAU, Mymensingh, and photographs of these slides were taken.

Johnsen scores were used to assess spermatogenesis in seminiferous tubules [23]. At a magnification of 40X, twenty cross-sectional segments of seminiferous tubules were examined in every sample and ranked from 1 to 10. 10: Indicates the presence of perfect tubules and complete sperm production; 9: Multiple sperm cells present but with disorganized sperm production; 8: Few sperm cells present; 7: No sperm cells present but numerous immature sperm cells (spermatids) present; 6: Few immature sperm cells present; 5: Neither sperm cells nor immature sperm cells present but numerous early-stage sperm cells (spermatocytes) present; 4: Few early-stage sperm cells present; 3: Only early-stage sperm cells (spermatogonia) present; 2: No germ cells (cells that give rise to sperm) present; 1: Neither germ cells nor Sertoli cells (cells that support sperm development) present.

Statistical analysis

Initially, laboratory data were recorded using Microsoft Excel 2016 and then imported into GraphPad Prism 5 for a thorough analysis. To evaluate mean values, standard error of the mean (SEM), and determine statistical significance (p-value), we conducted a Bonferroni multiple comparison test across various parameters. Given the utilization of multiple comparisons, we calculated and adjusted the P-values to significant levels of 0.001, 0.01, and 0.05. For the comparative analysis of hormonal, sperm and testicular parameters in rats corresponding to fed supplementation, an Analysis of Variance (ANOVA) was employed.

RESULTS

Effect of NSO and Pb on testosterone and thyroxin concentration

The results of the hormonal assay, specifically measuring testosterone levels, revealed a noteworthy impact of Pb treatment. The serum testosterone level was markedly reduced to 1.15 ± 0.25 ng/mL, a significant decrease ($p \leq 0.001$) when compared to the control group of rats, where it averaged 2.59 ± 0.17 ng/mL (Figure 1A). Interestingly, the serum testosterone level demonstrated resilience following supplementation with NSO, with levels reaching 3.10 ± 0.10 ng/mL, and in the NSO singly supplemented group, where levels reached 3.85 ± 0.05 ng/mL (Figure 1B).

Furthermore, the influence of Pb treatment extended beyond testosterone, impacting serum T_4 levels. Notably, T_4 levels significantly declined after Pb exposure but exhibited a notable increase following NSO supplementation.
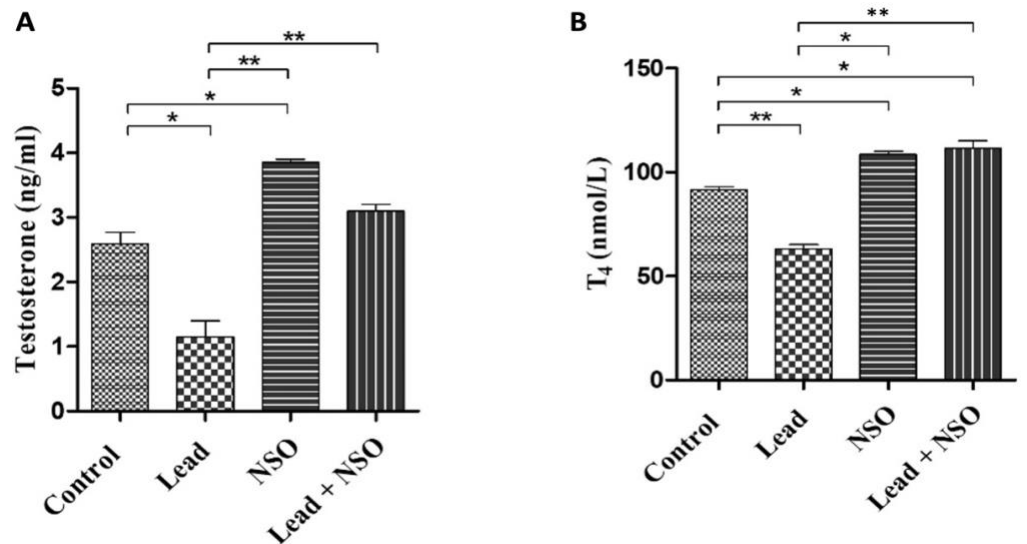
Figure 1. Effect of Pb and *NSO* (NSO) on testosterone and thyroxin (T_4) concentration. Rats were kept untreated or treated with Pb and Pb plus NSO and the testosterone and thyroxin (T_4) concentrations were tested. Data indicate mean ± SEM (n=10). One-way ANOVA with Bonferroni multiple comparison test. * p≤0.05 and ** p≤0.01

Effect of NSO and Pb on sperm motility and concentration

The findings indicate that sperm motility in the control group was 82.50 ± 2.50%, but this motility significantly decreased to 34.00 ± 1.00% after exposure to Pb. However, when NSO supplementation was administered alongside Pb exposure, there was a notable improvement in sperm motility, which increased to 64.00 ± 4.00% (Figure 2A). Similarly, sperm concentration experienced a substantial decline after Pb exposure but exhibited a significant increase when NSO supplementation was introduced (Figure 2B).
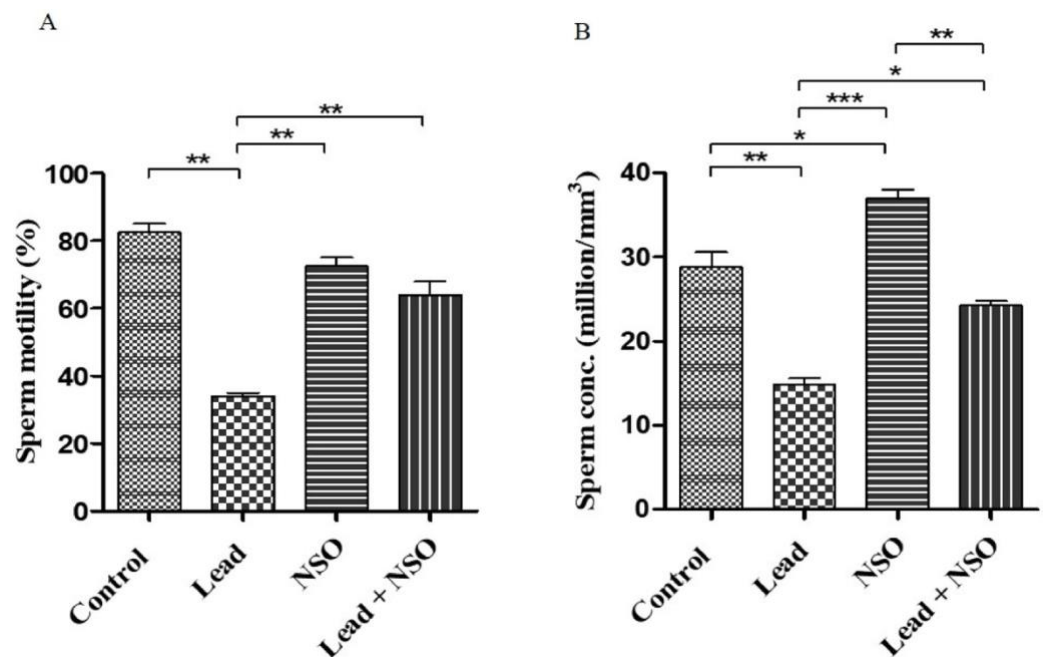

Figure 2. Effect of Pb and *Nigella sativa* oil (NSO) on Sperm motility and concentration. Rats were kept untreated or treated with Pb and Pb plus NSO and the sperm motility and concentration were determined. Data indicate mean ± SEM (n=10). One-way ANOVA with Bonferroni multiple comparison test. * p≤0.05 and ** p≤0.01, *** p≤0.00.

Effect of NSO and Pb on testis weight and diameter

Testis weight was measured during the necropsy. The study findings indicated that in the control group, the mean weight of both the left and right testis was recorded as 1.56 ± 0.02 g. However, in rats treated with Pb, there was a significant decrease in testicular weight. Conversely, Pb-treated rats supplemented with NSO exhibited a notable increase in testicular weight compared to those treated with Pb alone (Figure 3A).

Testis diameter measurements were obtained during necropsy, revealing distinct findings among the experimental groups. In the control group, the mean diameter of both left and right testis was 2.28 ± 0.04 cm. However, significant shrinkage in testicular diameter was observed in Pb-exposed rats. Notably, the Pb-treated rats that received supplementation with NSO exhibited a notably greater testicular diameter than rats that were administered Pb only (Figure 3B).
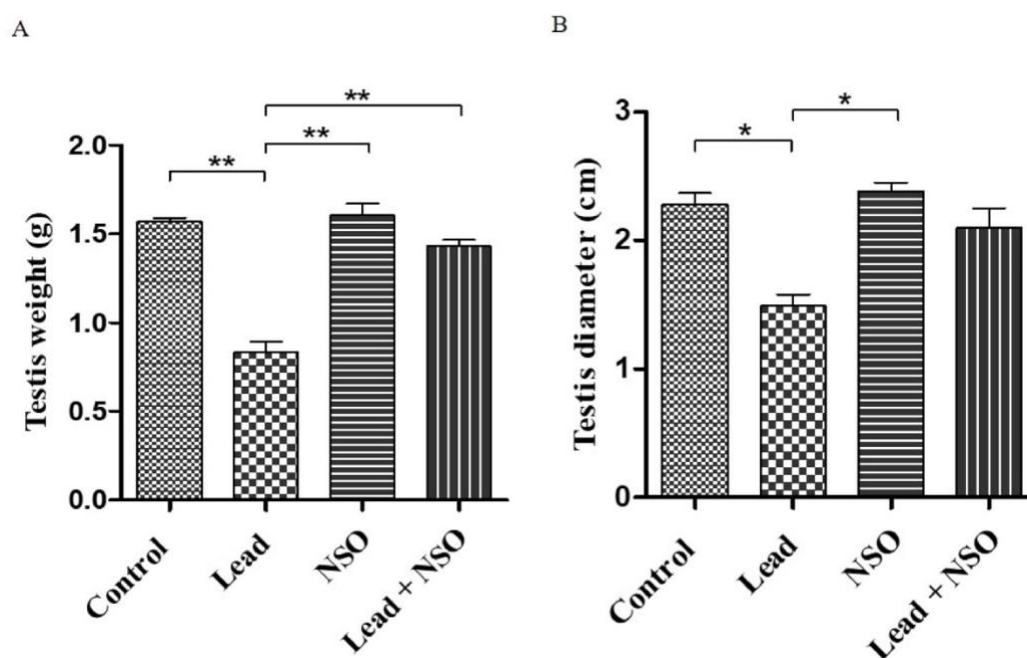

Figure 3. Effect of Pb and *Nigella sativa* oil (NSO) on testis weight (3a) (mean weight of both left and right testis) and diameter (3b). Rats were kept untreated or treated with Pb and Pb plus NSO and the testis weight and diameter were calculated. Data indicate mean ± SEM(n=10). One-way ANOVA with Bonferroni multiple comparison test. * $p \leq 0.05$ and ** $p \leq 0.01$

Effect of NSO and Pb on testicular histopathology

Histological examination of testicular tissue in the control group revealed a normal tissue structure. In contrast, the Pb-treated group exhibited significant degeneration of seminiferous tubules. However, both the group treated with NSO alone and the group treated with both Pb and NSO displayed normal histological characteristics in their testicular tissues (Figure 4).

The mean Johnsen score (MJS) of the untreated mice was 9.40 ± 0.19, whereas mice that received Pb had a significant reduction in MJS of 7.6 ± 0.28. The group supplemented with NSO along with Pb had increased the MJS to 9.53 ± 0.19, which clearly indicates the restoring effect of the male reproductive system by NSO on Pb intoxication (Figure 5).
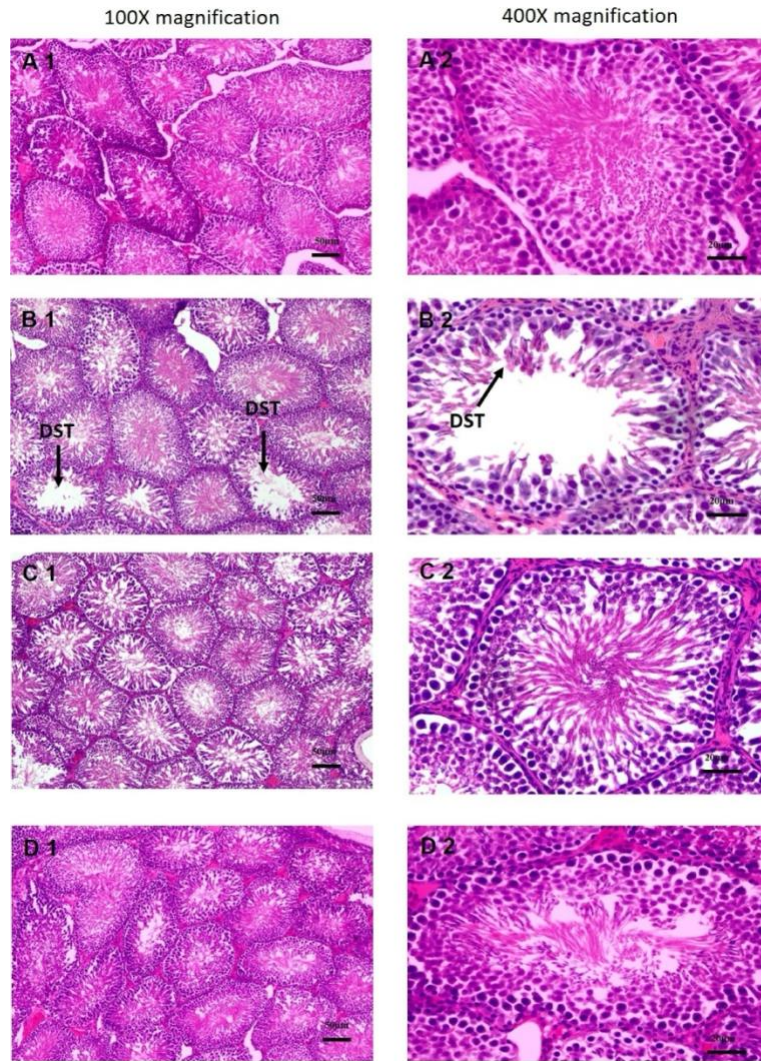
Figure 4. Effects of Pb and *Nigella sativa* oil (NSO) on testis in male rat. Photomicrograph of testis of control (A1-2), Pb (B1-2), NSO (C1-2), and Pb along with NSO (D1-2) treated rats at 100x and 400x magnification. Bar = 50 μm and Bar = 20 μm indicate magnification (a-d). It shows degeneration of the seminiferous tubule (DST) in rats treated with Pb (b).
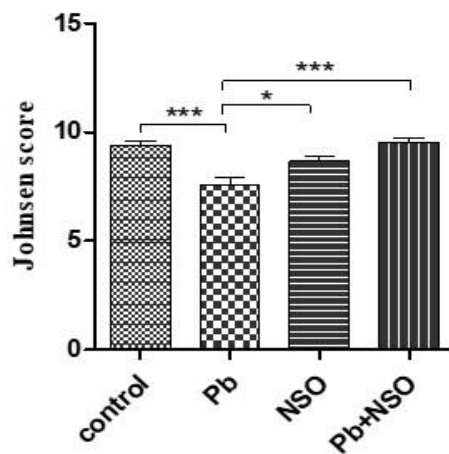

Figure 5. Effect of Pb and NSO on Johnsen score (functionality of seminiferous tubule) in Wistar male rats. Rats were kept untreated or treated with Pb and Pb plus NSO and the Johnsen score was quantified. Data indicate mean ± SEM (n=10). One-way ANOVA with Bonferroni multiple comparison test was performed. * p≤0.05 and *** p≤0.001.

DISCUSSION

In the era of the industrial revolution and rapid expansion of agricultural sectors, environmental pollutants comprising toxic heavy elements, including Pb, are indeed entering the food chain resulting in increased exposure to Pb for both humans and animals [24]. Pb toxicity has been linked to male reproductive health reducing sperm quality and fertility in men, potentially leading to reproductive failure [25]. Therefore, this research investigates the detrimental consequences of Pb exposure in experimental animals and assesses whether *Nigella sativa*, as a natural product, may offer protective effects against Pb-induced damage to male fertility.

The findings indicate a notable decrease in serum testosterone (Figure 1A) and T_4 levels (Figure 1B) following Pb exposure, which were subsequently restored to normal levels with NSO supplementation. These results suggest that Pb toxicity likely disrupts the functioning of the pituitary and hypothalamus, leading to impaired luteinizing hormone secretion causing direct damage to the seminiferous tubules in the testes, resulting in reduced testosterone secretion from Leydig cells [26, 27]. Furthermore, research has indicated that Pb exposure in albino rats can impact the steroidogenic activity of the testes, along with the alterations in testosterone and gonadotropin serum levels [28]. Thyroid function often becomes compromised in individuals exposed to Pb [29]. Specifically, a decline in thyroid hormone levels has predominantly been identified through research involving employees at greater exposure limits, typically with mean blood Pb levels exceeding 60 μg/dL. Conversely, at lesser Pb contact limits, the findings showed varying outcomes, with some researches indicating a rise, others showing a decline, and some revealing no significant changes in hormone concentration [30]. Blood Pb levels are shown to be negatively related to total thyroxine levels, which suggests that Pb may lower the number of proteins bound to thyroxine and enhance its clearance from the blood [31]. In our current research context, rats treated with NSO exhibited a marked elevation in both testosterone and thyroxin concentrations (Figure 1). These findings align with the outcomes of previous studies conducted by [32] and [33]. A notable rise in testosterone and thyroxin levels in mice fed with NSO by their antioxidant properties that inhibit ROS generation and enhance the activity of antioxidant enzymes such as superoxide dismutase and glutathione [34]. Following treatment with an alcoholic extract of black cumin seed, there are notable increases in body weight gain, reproductive characteristics (such as the thickness and diameters of seminiferous tubules, the number of spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa, the amount of Leydig cells as well as Sertoli, the diameter of Leydig cells, and the height of epithelial cells that completely filled the epididymal caudal), as well as hormones (such as testosterone and FSH) [35].

In this study, we observed reductions in motility (Figure 2A) and concentrations (Figure 2B), testis weight (Figure 3A), and diameter (Figure 3B) after Pb exposure. Sperm motility, structural integrity, and viability are all significantly impacted by this membrane fluidity [36, 37]. There are several mechanisms through which ROS production can impact sperm motility. One such mechanism by Pb is the potential reduction in phosphorylation of axonemal proteins essential for sperm movement and concentrations [38]. Importantly, this reduced motility may occur without a concurrent drop in mitochondrial membrane potential. Similar outcomes were also observed in investigations examining the impacts of Pb on the weight of testes [39]. Many variables, including an early onset of edema, inflammation, cellular infiltration, and other aspects, can explain this seeming contradiction. Another possibility is the development of Leydig cell hyperplasia [40]. This study observed an increase in the weight and diameter of the testis and the motility and concentrations of sperm in the group treated

with both NSO and Pb. Interestingly, NSO enhanced the rate of reproduction in hyperlipidemic rats [41]. Furthermore, a blend comprising equal proportions of *Eruca sativa*, *Nigella sativa*, and *Raphanus sativus* meals has shown promising effects on semen's physical properties while reducing free radicals in seminal plasma [42]. In addition, when NSO is orally administered daily for up to 60 days, it notably improves the weight, circumference, volume, length, width, and thickness of testicles in comparison to the control. All of the results align with similar observations made in rat studies [32, 43].

This study's findings indicated that 16 weeks of daily oral consumption of Pb had noticeable effects on the testicular structure (Figure 4), suggesting adverse impacts on the process of spermatogenesis. Recent research has also reported comparable or even greater severe alterations in male gonadal tissue in rats and mice exposed to Pb [44, 45]. According to [46], any harm to Sertoli cells can quickly disrupt their paracrine control over other dependent spermatogenic cells, potentially resulting in subsequent cell necrosis, rapid exfoliation into the lumen, and thinning of the seminiferous tubule walls. However, it is important to note that the obvious detrimental effects of Pb on germ cells cannot be dismissed [47]. In the current study, co-treating Pb-exposed rats with NSO effectively protected them from histological changes in the testicles (Figure 4). NSO has demonstrated remarkable potential in preserving testicular health against damage caused by various agents that generate free radicals. For instance, NSO supplementation has been shown to restore spermatogenesis in rats following testicular injury induced by chronic toluene exposure [48]. NSO has also provided protection to experimental mice from testicular tissue damage induced by methotrexate [33] and significantly reversed deviations in semen quality and reproductive characteristics caused by heat stress [49]. The adverse impacts of Pb acetate upon antioxidant defenses, testosterone levels, and spermatogenesis; however, oral NSO treatment mitigated these negative effects [50].

CONCLUSIONS

NSO consumption has protective effects on serum testosterone and thyroxine levels and testicular and sperm physiological parameters in chronic Pb-exposed rats, suggesting it may be a useful dietary supplement against Pb-induced reproductive toxicity. Further research is needed to understand protective the cellular level mechanism of NSO against Pb exposure. This could expand knowledge of NSO advantages and may help to its use as a dietary intervention to reduce heavy metal exposure's health risks.

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

AM planned and executed the experiment, monitored it, analyzed the results, and evaluated the final version of the report. In addition to executing the experiment, MA,

SR, and AUM analyzed the data and wrote the initial draft of the paper. MAM and EHC provided significant revisions to the article.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1]	Khan MSH, Mostofa M, et al. Effect of garlic and vitamin b-complex in lead acetate induced toxicities in mice. Bangladesh Journal of Veterinary Medicine. 2008;6:203-10.

[2]	WHO. Almost 1 million people die every year due to lead poisoning, with more children suffering long-term health effects. International Lead Poisoning Prevention Week of Action. Geneva, Switzerland: World Health Organization; 2022.

[3]	Sinclair S. Male infertility: Nutritional and environmental considerations. Alternative Medicine Review. 2000;5:28-38.

[4]	Queiroz EKRd, Waissmann W. Occupational exposure and effects on the male reproductive system. Cadernos de Saúde Pública. 2006;22:485-93.

[5]	Sallmén M, Lindbohm M-L, et al. Paternal exposure to lead and infertility. Epidemiology. 2000;11:148-52.

[6]	Wu H-M, Lin-Tan D-T, et al. Lead level in seminal plasma may affect semen quality for men without occupational exposure to lead. Reproductive Biology and Endocrinology. 2012;10:1-5.

[7]	Jurewicz J, Radwan M, et al. Effects of occupational exposure - is there a link between exposure based on an occupational questionnaire and semen quality? Systems biology in reproductive medicine. 2014;60:227-33.

[8]	Giulioni C, Maurizi V, et al. The influence of lead exposure on male semen parameters: A systematic review and meta-analysis. Reproductive toxicology (Elmsford, NY). 2023;118:108387.

[9]	Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biology and Medicine. 2000;29:927-45.

[10]	Verma R, Vijayalakshmy K, et al. Detrimental impacts of heavy metals on animal reproduction: A review. Journal of Entomology and Zoology Studies. 2018;6:27-30.

[11]	Batra N, Nehru B, et al. Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. Journal of Applied Toxicology. 2001;21:507-12.

[12]	Adhikari N, Sinha N, et al. Lead-induced cell death in testes of young rats. Journal of Applied Toxicology. 2001;21:275-7.

[13]	Graça A, Ramalho-Santos J, et al. Effect of lead chloride on spermatogenesis and sperm parameters in mice. Asian journal of andrology. 2004;6:237-41.

[14]	Bhakta S, Awal A, et al. Herbal contraceptive effect of abrus precatorius, ricinus communis, and syzygium aromaticum on anatomy of the testis of male swiss albino mice. Journal of Advanced Biotechnology and Experimental Therapeutics. 2019;2:36-43.

[15]	Al-Sa'aidi J, Al-Khuzai A, et al. Effect of alcoholic extract of nigella sativa on fertility in male rats. Iraqi Journal of Veterinary Sciences. 2009;23.

[16]	Al-Zamily HAA. The impact of black bean seeds nigella sativa l. In some physiological traits and sperm in male sheep awassi. Collage of Veterinary Medicine. Iraq: Baghdad University; 2008.

[17]	Al-Mayali H. The use of microbial alefittez black seed in reducing the toxicity of cadmium chloride on the reproductive system in male rat's efficiency eggs. College of Education. Iraq: University of Qadisiyah; 2007.

[18]	Mustari A, Sujan KM, et al. Growth performance, hematological parameters and lipid profile of mice treated with black seed oil and vitamin-e. Asian Journal of Medical and Biological Research. 2021;7:132-7.

[19]	Mustari A, Alam M, et al. Remodulatory effects of coral fossil on hematobiochemical parameters and histoaccumulation of specific organs in lead intoxicated broilers. Journal of King Saud University - Science. 2024;36:103071.

[20]	Coyotupa J, Parlow AF, et al. Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. Analytical Letters. 1972;5:329-40.

[21]	Mustari A, Alam M, et al. Retrieval action of zinc and folic acid for the restoration of normal reproductive function in bisphenol-a exposed male albino mice. Veterinarni Medicina. 2022;67:479-86.

[22]	Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. Manual of histologic staining methods of the armed forces institute of pathology1968. p. xii, 258-xii, .

[23]	Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. Hormones. 1970;1:2-25.

[24] Massányi P, Massányi M, et al. Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. Toxics. 2020;8:94.

[25] Abdel-Emam RA, Ahmed EA. Ameliorative effect of l-carnitine on chronic lead-induced reproductive toxicity in male rats. Veterinary medicine and science. 2021;7:1426-35.

[26] Ahmad I, Sabir M, et al. Study of the effects of lead poisoning on the testes in albino rats. Pakistan Journal of Medical Research. 2003;42:97-101.

[27] Janicki B, Cygan-Szczegielniak D. Zn and pb concentration in seminal plasma in reference to selected parameters of semiological assessment of bull semen. Folia biologica. 2008;56:97-101.

[28] Biswas N, Ghosh P. Effect of lead on male gonadal activity in albino rats. Kathmandu University Medical Journal. 2004;2:43-6.

[29] Bledsoe ML, Pinkerton LE, et al. Thyroxine and free thyroxine levels in workers occupationally exposed to inorganic lead. Environmental Health Insights. 2011;5:EHI. S7193.

[30] Atsdr U. Toxicological profile for lead (atlanta, ga: Us department of health and human services, agency for toxic substances and disease registry (atsdr), public health service). US EPA Air quality criteria for lead. 2007.

[31] Rahimpour F, Abdollahi O, et al. Evaluation the effect of serum lead levels on thyroid function in battery industry workers. Indian journal of occupational and environmental medicine. 2023;27:120-5.

[32] Mohammad MA, Mohamad M, et al. Effects of black seeds (nigella sativa) on spermatogenesis and fertility of male albino rats. Research Journal of Medicine and Medical Sciences. 2009;4:386-90.

[33] Gökçe A, Oktar S, et al. Protective effect of thymoquinone in experimental testicular torsion. Urologia internationalis. 2010;85:461-5.

[34] Mustari A, Nooruzzaman M, et al. Promoting action of vitamin e and black seed oil on reproductive hormones and organ histoarchitecture of swiss albino mice. Veterinary Medicine Science. 2022;8:710-8.

[35] Assi MA, Hezmee MNM, et al. Prophylactic effect of nigella sativa against lead acetate induced changes in spermiogram, reproductive hormones and gonadal histology of rats. Journal of Veterinary world. 2016;9:1305.

[36] Baumber J, Ball BA, et al. The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. Journal of andrology. 2000;21:895-902.

[37] Wathes DC, Abayasekara DR, et al. Polyunsaturated fatty acids in male and female reproduction. Biology of reproduction. 2007;77:190-201.

[38] Oliveira H, Spanò M, et al. Lead chloride affects sperm motility and acrosome reaction in mice: Lead affects mice sperm motility and acrosome reaction. Cell biology and toxicology. 2009;25:341-53.

[39] Marchlewicz M, Michalska T, et al. Detection of lead-induced oxidative stress in the rat epididymis by chemiluminescence. Chemosphere. 2004;57:1553-62.

[40] Gorbel F, Boujelbene M, et al. Cytotoxic effects of lead on the endocrine and exocrine sexual function of pubescent male and female rats. Demonstration of apoptotic activity. Comptes rendus biologies. 2002;325:927-40.

[41] Bashandy AS. Effect of fixed oil of nigella sativa on male fertility in normal and hyperlipidemic rats. International Journal of Pharmacology. 2007;3:27-33.

[42] El-Tahomi M, El-Nattat W, et al. The beneficial effects of nigella sativa, raphanus sativus and eruca sativa seed cakes to improve male rabbit fertility, immunity and production Journal of American Science. 2010;6:1247-55.

[43] Mansour SW, Sangi S, et al. Sensibility of male rats fertility against olive oil, nigella sativa oil and pomegranate extract. Asian Pacific journal of tropical biomedicine. 2013;3:563-8.

[44] Aithamadouche N, Sadi N, et al. The protective effect of vitamin e against genotoxicity of lead acetate intraperitoneal administration in male rat. Notulae Scientia Biologicae. 2013;5:412-9.

[45] El-Nager D, Aldahmash B. Effect of corn oil, flax seed oil and black seed oil on testicular damage induced by lead acetate in albino mice: A histological study. Pakistan Journal of Zoology. 2013;45:1083-9.

[46] Creasy DM. Pathogenesis of male reproductive toxicity. Toxicologic pathology. 2001;29:64-76.

[47] Adhikari N, Sinha N, et al. Effect of lead on sertoli-germ cell coculture of rat. Toxicology letters. 2000;116:45-9.

[48] Kanter M. Thymoquinone reestablishes spermatogenesis after testicular injury caused by chronic toluene exposure in rats. Toxicology and industrial health. 2011;27:155-66.

[49] Al-Zahrani S, Mohany M, et al. Thymoquinone and vitamin e supplementation improve the reproductive characteristics of heat stressed male mice. Journal of Medicinal Plants Research. 2012;6:493-9.

[50] Assi M, Hezmee M, et al. Assessment of therapeutic effects of nigella sativa against chronic lead acetate-induced reproductive dysfunction in male sprague-dawley rats. Journal of Comparative Clinical Pathology. 2017;26:87-97.