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Nigella sativa oil Ameliorate Gonadotoxic effect of Lead Acetate Induced Testicular Damage in Adult Wistar Rats

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ABSTRACT

There is growing concern about the increasing levels of lead acetate in the environment, primarily due to unsafe mining and natural resource extraction. *Nigella sativa* oil, a natural herbal product, is used to treat many diseases. Its pharmacological constituents have demonstrated antioxidant, anti-inflammatory, antimicrobial, and antiparasitic properties. This study investigated the protective effects of *Nigella sativa* oil on lead acetate-induced testicular damage in adult male Wistar rats. A total of forty-two rats were divided into six groups, each consisting of seven rats. Group I was administered normal saline equivalent to the volume given to the highest dose of treated rats. Group II received 120 mg/kg of lead acetate, while Group III (positive control) received 120 mg/kg of lead acetate and 160 mg/kg of vitamin C. Groups IV, V, and VI were administered 120 mg/kg of lead acetate followed by 6 mg/kg, 4 mg/kg, and 2 mg/kg of *Nigella sativa* oil, respectively, via oro-gastric intubation for seven days. The lead acetate caused a significant increase ($P<0.001$) in the liver enzymes (AST, ALT, and ALP). Histopathology examination showed testicular tissue damage in the lead acetate group. However, there is a great improvement in the morphology of the testicular tissue in the treated groups, even at the minimal doses. The changes in the level of biomarkers and improvement in the histomorphological appearance of the testis suggested that consumption of *Nigella sativa* oil does have a protective effect against lead acetate-induced testicular damage.

INTRODUCTION

Exposure to lead acetate in the environment and the workplace is still a major public health issue, especially in developing countries, because lead and its compounds are very toxic and have many severe adverse health effects in humans [1]. This has been compounded by the fact that in the recent past, lead poisoning has claimed the lives of hundreds of people in Nigeria. The concentration of lead acetate in the environment has increased over the years, and the main causes of this are unsafe mining activities and the extraction of natural resources that are not closely monitored [2]. The sources of lead exposure vary from industrial emissions, water contamination, lead-based paint, battery manufacturing, and electronic waste disposed of improperly. Industrial activities have been recognized as the major source of environmental lead pollution in most areas of the world. Numerous research studies have established the adverse health impacts of lead on the hematopoietic system, bone marrow, liver, kidneys, and nervous system. Long-term effects include severe neurological disorders, renal impairment, anemia, and other systemic toxicities. As the search for natural products that can help alleviate the symptoms of lead poisoning continues, *Nigella sativa*, also known as black seed or black cumin, has emerged due to its numerous pharmacological activities. *Nigella sativa* is a small shrub that is a member of the Ranunculaceae family and is found in different parts of the world. It has large fruits containing many seeds that have been used traditionally in medicine for centuries [2].

Current scientific studies reveal that different parts of *Nigella sativa* have numerous bioactivities. The seeds and oil fractions have been found to possess potent antibacterial, antioxidant, anti-inflammatory, and antidiabetic activities. Also, its ingredients, including thymoquinone, have been found to improve cognitive function and memory and have potential cancer-fighting properties. *Nigella sativa* is also used in hepatoprotective and nephroprotective actions and has been found to have protective effects against heavy metal and

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environmental pollutants-induced liver and kidney toxicity [2]. Moreover, herbal medicine uses *Nigella sativa* oil orally and topically to treat different diseases. A number of studies have been conducted to establish the pharmacological suitability of the plant, and it has been determined that it has analgesic, antimicrobial, antiparasitic, and immune system enhancement properties. Due to the numerous medicinal effects of *Nigella sativa*, it is currently being investigated as a possible protective agent against lead toxicity and as a natural way of treating heavy metal poisoning and its complications.

MATERIALS AND METHODS

Ethical Approval and Chemicals Used. The Animal Care and Use Committee (ACUC), Faculty of Health Sciences, Bauchi State University, Gadau, approved this study. (Ref. Number: BASUG/FHS/ACUC/2019/018). A pure (100% Natural) *Nigella sativa* oil (Hermani International KEPZ, Kerachi-Pakistan). The Lead acetate (BDH Chemical Ltd Poole England, 29021) was obtained from the chemistry department, Bauchi State University, Gadau. The vitamin C (Care Industrial Ltd Debo, Lagos, VC 491).

Animal Husbandry

Adult Wistar rats (both sexes) were obtained from the National Veterinary Research Institute Vom Jos, Plateau State of Nigeria. Animal handling was performed in accordance with ACUC guidelines. The animals were kept for 2 weeks in the Human Anatomy Department, Faculty of Basic Health Science, Bauchi State University, Gadau. The rats were sheltered and caged under a uniform husbandry condition at room temperature, with a 12-hour light/dark cycle. The animal cages were cleaned daily, and rats were fed with a standard laboratory diet (Bendel feeds, Ilorin) and drinking water *ad libitum*.

Experimental Design

Acute Toxicity

The lead acetate acute toxicity was performed following the Up and Down procedure as described in the guideline for testing of chemicals. Four rats were used and administered 10 mg/kg, 50 mg/kg, 250 mg/kg, and 1250 mg/kg-1 body weight of lead acetate, respectively at different times as described. The animals were observed for 48-72 hours for neurological, behavioral changes, and mortality. Similarly, a total number of five adult, female, non-pregnant rats were randomly selected for the *Nigella sativa oil* acute toxicity following the same method. The rats were administered a single oral dose of 10 mg/kg, 20 ml/kg, 40 ml/kg, 80 ml/kg and 160 mg/kg body weight of *Nigella sativa* oil orally, respectively, following the standard protocol for Up and Down procedure. The rats were observed individually at least once during the first 30 minutes after administration and periodically for 72 hours. Toxicological effects were assessed on the basis of mortality.

Experimental Protocol

A total number of forty-two rats were divided into six groups of seven rats per group. Group I rats (normal control) were administered normal saline equivalent to the volume administered to the highest dose of treated rats. Group II (negative control) was administered 120 mg/kg of lead acetate, while and group III (positive control) received 120 mg/kg of lead acetate and 160 mg/kg of vitamin C. Groups IV, V, and VI were administered 120 mg/kg of lead acetate followed by 6 mg/kg, 4 mg/kg, 2 mg/kg of *Nigella sativa* oil respectively via oro-gastric intubation for a period of seven days. All the controls and treated groups were studied in parallel with the administration done between 0900 hr and 0100 hr daily.

After the administration, the rats were allowed free access to feed and clean water. The body weights were recorded daily using an electronic balance (OHAUS AX150/E). Clinical signs such as weakness, poor response to physical activities, and fluctuation in convulsion of the rat was observed throughout the study.

Animal Euthanization and Sample Collection

To eliminate perception of pain, the rats were deeply anesthetized using chloroform. The rats were disinfected with 70% ethanol, and the abdominal skin was incised to expose the abdominal cavity. Blood samples (2ml-5ml) were collected through cardiac puncture from each rat. The blood was dispensed into a specimen container (Lithium heparin) and subjected to further treatment for determination of the liver biomarkers following the standard procedure. The liver tissues were excised and fixed in 10% formalin for histopathological assessment.

Tissue Processing, Staining and Microscopic Examination

Tissue processing was performed according to standard paraffin embedded procedure. The testicular tissues were cut into thin sections and placed in a tissue cassette with a label for processing (dehydration, clearing and infiltration) using an automatic tissue processor (LUPETEC, PT09 TS) (**Fig. 1**). Tissues were embedded using an embedding machine (BIOBASE, BK CPII) and the samples were sectioned at seven µm using a rotary microtome (BIOBASE, BK-MT268M). Serial sections of the tissues were collected and mounted onto microscope slides (Hecate, 7105) and slides were labelled accordingly and dried at 40 ºC on a hot plate. The Hematoxylin and eosin (H and E) (TissuePro Technology, H08- 500R) stain was performed following the manufacturer's protocol to assess the general morphology of the liver. Micrographs of the liver tissues were captured using a bright field light microscope (BIOBASE, BMB-300M) with CCD digital camera attached to the microscope. Three images were obtained from each slide and assessed for any histopathological conditions.

Fig. 1. Shows testis tissues placed in formalin after the sacrifice.

Statistical Analysis

Numerical data obtained from the study were analyzed using SSPS (Version 25). The difference in the significance level between the control and treated groups was determined using analysis of variance (ANOVA) and the probability values of $P \leq 0.05$ were considered statistically significant. The results were expressed as mean ± SEM.

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RESULTS

Table 1 presents the descriptive statistics from the one-sample t-test analysis comparing the experimental and control groups. The experimental group showed greater variability in body weight and related parameters, with a higher standard deviation in both weight gain and weight loss measurements. The survival rate, difference in body weight, and weight change indicators (gain and loss) are presented with their respective standard errors. **Table 2** presents the results of the one-sample t-tests for weight-related parameters across experimental rats. The t-values were high, and the confidence intervals were narrow, indicating that there were significant mean differences in body weight, weight gain, and weight loss. These values indicate that there was a change in body mass and other related indices during the treatment period. **Table 3** shows the mean body weights of rats in the different treatment groups. The negative control rats that received lead acetate alone had the highest mean weight, whereas the rats that were treated with different doses of *Nigella sativa* oil had lower mean weights. The 4 mg/kg *Nigella sativa* group had the most moderate weight restoration among the treatment groups.

Table 1. Summary statistics of body weight and related parameters in experimental and control rats.

One-Sample Statistics	Mean	Std. Deviation	Std. Error Mean
Experimental Group	3.00	1.430	.213
Control Group	1.00	.000[a]	.000
weight of rats in Grams	80.508	36.169	5.167
Survival rate	1.09	.293	.040
Difference in body weight	1.22	.422	.060
Significance in weight loss/gain	1.37	.668	.095
weight gain	1.13	.343	.056
weight loss	1.09	.302	.091

Note: One sample statistics in the t-test showing all the variables and their mean statistics.

Table 2. One-sample t-test results for weight parameters across experimental groups.

One-Sample Test					
	T	df	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
Experimental Group	13.837	44	2.950	2.52	3.38
weight of rats in Grams	15.571	48	80.458	70.069	90.847
Difference in body weight	19.502	48	1.174	1.05	1.30
Significance in weight loss/gain	13.815	48	1.317	1.13	1.51
weight gain	19.463	37	1.082	.97	1.19
weight loss	11.450	10	1.041	.84	1.24

Note: Significant increase in body weight of rats at p-value of 0.05(p>0.05).

Table 3. Mean body weight of rats in control and treatment groups

Study Groups	Mean	Std. Deviation
Negative control Group (lead)	107.900	56.808
Positive Control Group (Vit. C and lead)	73.287	39.323
lead and 6mg/kg *Nigella sativa* oil	72.111	35.538
lead and 4mg/kg *Nigella sativa* oil	86.900	23.041
lead and 2mg/kg *Nigella sativa* oil	77.386	16.302

Note: Weight of rats in the treatment group showed a significant increase in lead (only) group compared to the other groups.

Light Microscopic Examination Using Hematoxylin and Eosin (H&E Staining)

The testes of Group I (control rats) showed typical testicular structure in their histological analysis. The seminiferous tubules took an oval or rounded form while being encircled by a thin basement membrane. Spermatogenic cells arranged themselves according to spermatogenesis stages throughout the tubules. The seminiferous tubules contained spermatogonia followed by primary spermatocytes and secondary spermatocytes and rounded and elongated spermatids before reaching the lumen where mature spermatozoa resided. The seminiferous tubules were enveloped by a thin connective tissue sheath containing myoid cells (**Fig. 2**). The histological sections from Group II (lead-induced rats) showed severe degenerative changes. The seminiferous tubules showed widespread necrosis of spermatogenic cells together with vascular congestion. The tissue presented severe interstitial edema together with dilated interstitial blood vessels. Some of the samples showed total necrosis of the seminiferous tubules (**Fig. 2**). Group III (lead combined with vitamin C) showed a partial protective effect. The sections from this group showed some seminiferous tubules had mild necrosis, moderate interstitial blood vessel congestion, and mild interstitial edema (**Fig. 3**). Group IV (lead combined with 6 mg Nigella sativa oil) showed seminiferous tubules that were mostly normal. The seminiferous tubules had only minor congestion of the interstitial blood vessels while maintaining their typical structure (**Fig. 4**). Group V (lead combined with 4 mg Nigella sativa oil) showed testicular histology that matched the control group. The testes maintained their normal structure and architecture with very few pathological changes (**Fig. 5**). Group VI (lead combined with 2 mg Nigella sativa oil) presented histological findings that were almost normal, thus showing a protective effect similar to Group V (**Fig. 6**).

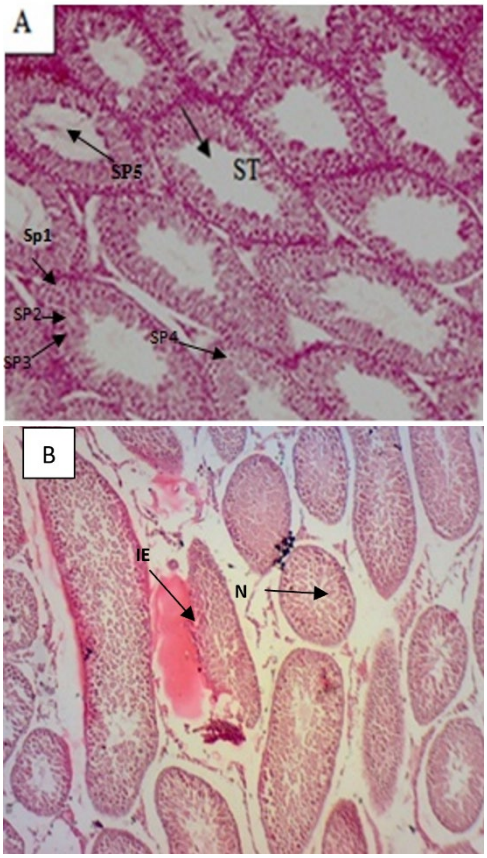

Fig. 2. (A): Transverse section, H and E micrograph of a testis treated with normal saline and feeds; control group showing seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP1), primary spermatocytes (SP2), secondary spermatocytes (SP3), spermatids (SP4) and spermatozoa (SP5). (B): is a testis treated with mainly lead acetate known as the toxicity group at 120 mg/kg, showing necrosis of spermatogenic cells (N) in the seminiferous tubulesd congestion of interstitial blood vessels. Magnification (x40).

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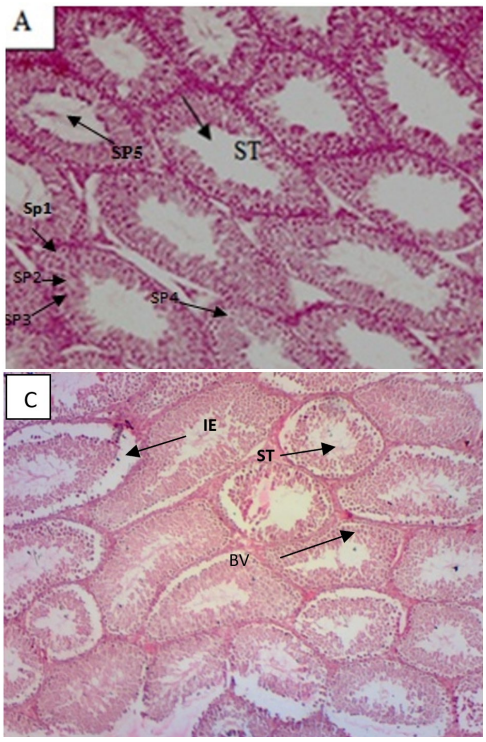

Fig. 3. (A)Transverse section, H&E photomicrograph of testis treated with normal saline (control group) showing seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP1), primary spermatocytes (SP2), secondary spermatocytes (SP3), spermatids (SP4) and spermatozoa (SP5) and (B) testis treated with 120 mg pb+160 mg/kg vit C; showing showed mild necrosis of some seminiferous tubules as well as congestion of the interstitial blood vessels and mild interstitial edema as well as congestion of blood vessels. Magnification (x100).

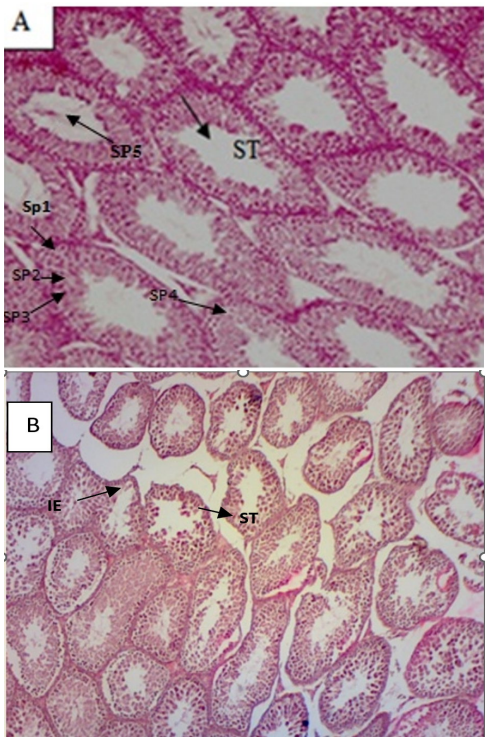

Fig. 4. (A) Transverse section, H&E Photomicrograph of a testis treated with N/Saline, (d) liver treated with 120 mg/kg P + 6mg/kg *Nigella sativa oil* showing seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP1), primary spermatocytes (SP2), secondary spermatocytes (SP3), spermatids (SP4) and spermatozoa (SP5); (B) treatment group 4. revealed mild congestion of the interstitial blood vessels and the seminiferous tubules appeared approximately normal. Magnification (x 40).

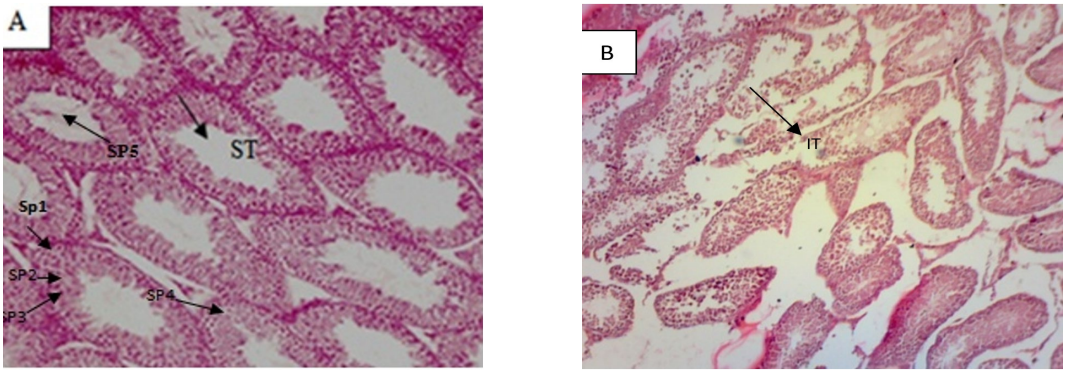

Fig. 5. (A) Transverse section, H&E Photomicrograph of a liver treated with normal saline, showing seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP1), primary spermatocytes (SP2), secondary spermatocytes (SP3), spermatids (SP4) and spermatozoa (SP5) (B) Liver treated with 120 mg/kg Pb + 4mg/kg *Nigella sativa oil* showing more or less normal histological appearance of testis and interstitial tissue. Magnification (x40).

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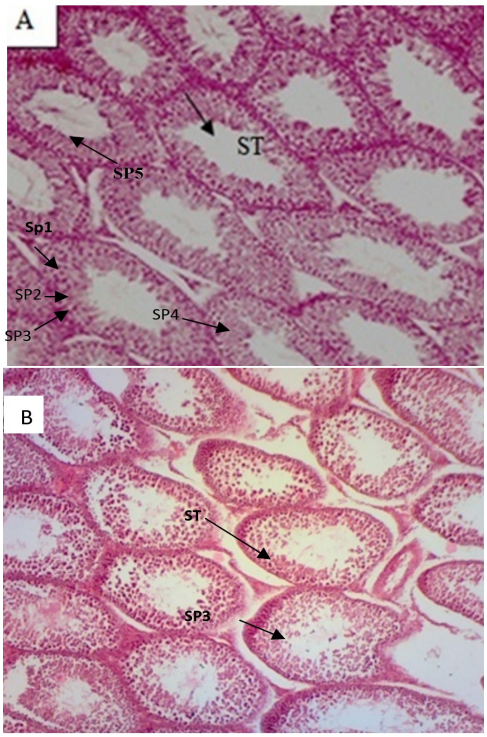

Fig. 6. Transverse section, H&E Photomicrograph of a liver treated with N/Saline, (A) liver treated with 120 mg/kg Pb + 2 mg/kg of *Nigella sativa oil* showing seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP1), primary spermatocytes (SP2), secondary spermatocytes (SP3), spermatids (SP4) and spermatozoa (SP5). (B) Showed group 6 with a more normal histological testicular tissue and normal outline. Magnification (x 40)

DISCUSSION

Heavy metals are widely distributed in the environment. Some of these, such as lead, cadmium, arsenic, and mercury, can adversely affect male reproductive function. Environmental discharge of lead from sources like petroleum products (especially leaded petrol), construction works, paint removal, demolition, vehicle batteries, and car repairs contribute to airborne lead pollution [3] and possibly introduce high concentrations of this potential reproductive toxicant into the environment, which may cause physiological, biological, and histological disorders [4].

Given the potential for severe testicular damage, this study emphasizes the crucial need for protection from lead acetate exposure. Our findings confirm that lead acetate significantly disrupts testicular function in male rats. Although weight gain in treated animals did not reach statistical significance in this study, the negative impact of lead on male fertility is well-established [1,5,6]. These results showed that lead acetate administration significantly decreased the SOD, GPx and increased MDA levels. Lead acetate also decreased the sperm count, motility, viability, and altered histopathological testis (testicular damage, seminiferous tubule necrosis, and spermatid loss) compared to the negative control group. Lead-induced testicular damages have been attributed, at least in part, to toxicant-induced oxidative stress. The results suggest that lead stimulates the formation of ROS, thus causing oxidative damage to various tissues, resulting in loss of membrane functions. Long-term exposure to lead increases MDA or lipid peroxidation and causes inhibition of SOD activity, inducing oxidative damage in testes [7,8]. The toxic effects of lead in biological systems have been linked to increased MDA or lipid peroxidation as an early and sensitive consequence of lead exposure. Lead acetate toxicity generates free radical damage by two separate pathways, including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation and the direct depletion of antioxidant reserves. Also has been reported that the accumulation of lead in the testis is a substantial basis for resulting in spermatogenesis and sperm development to be suppressed. The primary mechanism of the toxic action of lead appears to be a disruption of the hypothalamic control of pituitary hormone secretion.

In this study, we evaluated the effect of 120 mg of lead acetate exposure on the male reproductive system in rats. We found that exposure to lead acetate resulted in necrosis of spermatogenic cells in the seminiferous tubules, congestion of blood vessels, severe interstitial edema as well as congestion of interstitial blood vessels and complete necrosis in the seminiferous tubules in some cases. These results are consistent with the above reports and indicate that lead exposure induced toxicity to the male reproductive system, especially to spermatogenesis, sperm development, and sperm maturation. Furthermore, we evaluated the protective role of *Nigella sativa* oil against the oxidative stress changes in the testicular tissue resulting from the administration of lead acetate in rats.

However, *Nigella sativa* oil (6, 4, 2 mg/kg body weight) administered to rats showed no significant changes in terms of body weight increase. This is in agreement with work done by [9], who reported that oral administration of alcoholic extract of *Nigella sativa* with doses of 200 and 400 mg/kg body weight in male rats for 60 days did not affect the body weight of male rats. In contradiction, oral administration of 0.5 and 1.5 mg/kg body weight of alcoholic extract of *Nigella sativa* for 53 days on rats increased body weight [10]. It has been observed that diabetic male Wistar rats given 2% *Nigella sativa* for 30 days had increased body weight [11]. Body weight increment of animals treated with *Nigella sativa* could be due to the abundance of primary nutritional factors such as amino acid, glutamic acid, and other essential amino acids in the black seed

Nigella sativa oil possesses antioxidant properties and acts as a free radical scavenger. Antioxidant properties of *Nigella sativa* oil was shown in hyperlipidemic rats, where damage caused by hyperlipidemia-induced free radicals on nephrons was ameliorated by *Nigella sativa* oil supplementation [12]. Besides that, administering Nigella sativa oil increased high-density lipoprotein (HDL) levels in rats [13]. HDL is one of the five major groups of lipoproteins, and it is the densest because it contains the highest proportion of protein in lipids. Since HDL is the most abundant lipoprotein in tissue fluid, HDL might counteract the effects of lipid peroxidation in the tissue fluid and may possibly protect cell membranes [14]. HDL also possesses antioxidant properties, and its concentration was increased in rats treated with Nigella sativa oil, suggesting an improvement in antioxidant activities [15].

Nigella sativa administered orally on normal and hyperlipidemic rats for 2 months showed a significant decrease in cholesterol, triglycerides, and LDL levels, while HDL level was increased. Reductions in lipid concentrations might be caused by the hypolipidemic effects of unsaturated fatty acids (oleic and linoleic acids) contained in *Nigella sativa* oil. Improvements were also reported in terms of reproductive efficiency, seminal vesicle weight, testosterone level, sperm motility, and sperm quality of *Nigella sativa*-treated rats. These

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beneficial outcomes on fertility observed in hyperlipidemic rats might be due to the antioxidant and hypolipidemic properties of *Nigella sativa* oil [13]. In a different study, similar findings of favorable effects of *Nigella sativa* oil on abnormal sperm parameters have also been reported. It may be due to the unsaturated fatty acids content of *Nigella sativa* oil [16]. Further stated the hypothesis supported by research findings on the correlation between unsaturated fatty acid supplementation and enhanced sperm count, motility, and normal morphology in infertile men.

Male Wistar rats being fed orally with *Nigella sativa* oil for 60 consecutive days showed a decrease in the number of abnormal sperm, which might be due to a reduction in lipid peroxidation. The positive effects on sperm quality could be attributed to the antioxidant property of *Nigella sativa* oil that inhibits excessive free radical generations [17]. This finding could be attributed to the effects of *Nigella sativa* on oxidative phosphorylation enzymes [18]. It was suggested that dietary supplementation of powdered *Nigella sativa* could inhibit oxidative stress caused by oxidized corn oil in rats [18].

The positive effects of *Nigella sativa* on male fertility are closely associated with the chemical composition of this plant [9]. Phytochemical analysis carried out on the seeds of *Nigella sativa* by [16] showed the presence of high concentrations of unsaturated fatty acids such as linoleic acid (55.6 %), oleic acid (23.4 %), palmitic acid (12.5 %), stearic acid (3.4 %), and others. This was further supported by rats fed with oils rich in polyunsaturated fatty acids (PUFAs), which showed improved reproductive functions. The unsaturated fatty acids were found to be responsible for stimulating 17 β-hydroxysteroid dehydrogenase activities, an important enzyme in the testosterone biosynthesis pathway [19]. Testosterone is responsible for the regulation of the male reproductive organ.

This study also discovered that sperm parameters of rats co-administered with nigella lead acetate and *Habbatus sauda* oil showed improvements when comparisons were made against rats treated with only lead or *Habbatus sauda*. [11] reported that the improvements in reproductive function in diabetic male rats were due to the protective effect of *Nigella sativa*. Constituents of *Nigella sativa* seeds, such as proteins, vitamins, minerals, alkaloids, and phenols, were believed to contribute to this protective effect [11] and also found to increased antioxidant activity besides lower measurement of malondialdehyde in the reproductive system of rats treated with *Nigella sativa*.

It also demonstrated the antioxidant properties of black cumin oil, attributed to its active compound, thymoquinone (TQ), which enhanced the scavenger system and led to antitoxic effects. Other researchers also reported that *Nigella sativa* tended to decrease lipid peroxidation product levels and liver enzymes, leading to increased antioxidant defense system activity. Under normal circumstances, ROS would be neutralized by antioxidants present in ejaculatory fluid. Any changes to these circumstances, such as reduction of antioxidant properties or increased ROS production in semen, would, in turn, increase the level of oxidative stress, which would be detrimental to sperm parameters. It was also reported that ROS levels in the semen of infertile patients were elevated. It was also found that the oil of black cumin and its active ingredients, particularly thymoquinone, gave reproducible antioxidant effects by enhancing the oxidant scavenger system. Consequently, this leads to antitoxic effects against detrimental effects induced by several insults and furthermore, indicates that the generation of reactive oxygen species (ROS) as a result of oxidative stress due to any toxic agent was depressed by the antioxidant effects of *Nigella sativa* seeds. The result was consistent with [12], who showed the importance of thymoquinone as a pharmacologically active quinone with antioxidant effect. Clear improvements in testis histological features were observed in rats administered with *Nigella sativa* oil. Thymoquinone (TQ), being a potent antioxidant, has proven its protective effects in studies against nephrotoxicity [12], cardiotoxicity [20], and hepatotoxicity in mice and rats. It also inhibited eicosanoid generation in leucocytes and lipid peroxidation in the ox brain. Another major compound isolated from *Nigella sativa* seed is Nigellone, a carbonyl polymer of TQ. The toxicity of Nigellone is significantly lower than that of TQ. However, its pharmacologic properties are similar to that of TQ. Some of the properties of Nigellone include antioxidant, antispasmodic, and antihistaminic.

CONCLUSION

In conclusion, this study demonstrated that oral administration of lead caused histological damage and disrupted the metabolism of male reproductive organs. However, ameliorative effects were observed with the treatment of vitamin C and *Nigella sativa* oil. *Nigella sativa* oil proved to be effective by improving the redox status, reducing the lead burden, and enhancing semen quality and testicular histology towards normal physiology. The lead dosage used in this study (120 mg/kg) had negative effects on the testes of rats. Conversely, rats treated with *Nigella sativa* oil at dosages of 6 mg/kg, 4 mg/kg, and 2 mg/kg per body weight showed improvements in testicular histological parameters. The findings of this study suggest that the co-administration of lead and *Nigella sativa* oil over a period of seven days can mitigate the harmful effects of lead exposure in rats. Previous research indicates that *Nigella sativa* oil has antioxidant properties, which may counteract the excessive reactive oxygen species (ROS) generated by lead acetate, thereby reducing the severity of damage to sperm and testes. Furthermore, it was observed that the body weight of rats administered both lead and *Nigella sativa* oil did not change significantly. The limitation of the presence study would mostly be the lack of a quantitative evaluation of the blood hormonal level and sperm parameters. In addition, quantitative analysis was also needed for the intensity evaluation of androgen receptors in testicular tissues using an immunofluorescent technique.

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COMPETING INTERESTS

The authors declare no competing interests.

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