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RESEARCH ARTICLE

Exploring the Protective Effects of Garlic Essential Oil Against Lead Nitrate-induced Reproductive Toxicity in Swiss Albino Mice

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Abstract:

Background: Lead (Pb) is a well-known environmental toxin that adversely affects male reproductive health. Chronic lead exposure can impair testicular function, sperm quality, and hormonal balance, necessitates the exploration of potential protective agents. **Aim:** This study investigates the protective effects of Garlic Bulb Essential Oil (GBEO) against lead nitrate-induced reproductive toxicity in Swiss albino male mice. **Methods:** Male Swiss albino mice were orally administered lead nitrate at a dose of 50 mg/kg body weight, dissolved in distilled water. Two doses of GBEO - 50 mg/kg (low) and 80 mg/kg (high) - were administered orally with olive oil as a carrier, alongside a standard drug, silymarin, was taken for comparison. The study evaluated reproductive organ weights, sperm parameters, testosterone levels, and histological changes in testicular tissue. **Results:** Lead nitrate exposure significantly reduced the weight of the testes and epididymis, decreased sperm count and motility, altered testosterone levels, increased sperm abnormalities, and disrupted testicular structure. GBEO supplementation reversed these effects in a dose-dependent manner. Both low and high doses of GBEO improved reproductive organ weights, sperm parameters, and testosterone levels. Histological analysis revealed structural recovery, with the high dose nearly restoring normal testicular architecture. **Conclusion:** GBEO demonstrates potential as a protective agent against lead-induced male reproductive toxicity. Future studies should focus on optimizing GBEO dosage for therapeutic applications.

Major Findings: GBEO effectively mitigated lead nitrate-induced reproductive toxicity in Swiss albino male mice in a dose-dependent manner. It improved reproductive organ weights, sperm quality, and testosterone levels while restoring testicular structure, with the higher dose showing near-complete normalization. These findings highlight GBEO's potential as a protective agent against lead-induced male reproductive dysfunction.

Keywords: Dose-dependent Effect, Garlic Essential Oil, Lead Nitrate, Reproductive Toxicity, Spermatogenesis

1. Introduction

Male infertility is a growing concern worldwide, affecting approximately 10-15 % of couples globally[1]. Researchers widely acknowledge that exposure to various heavy metal in the environment and workplace significantly contributes to this issue[2]. Lead (Pb), a common heavy metal, poses a particular threat to male reproductive health as it can accumulate in the testes and disrupt sperm production processes[3]. Exposure to Lead can occur through various routes, such as inhaling contaminated air, consuming tainted food or water, or absorbing it through the skin[4]. Certain occupations like battery manufacturing, Lead smelting, and paint production pose higher risks. However, even low and prolonged exposure to Lead from sources like Lead-based paint dust, polluted soil, and certain food additives can harm male fertility[5].

The testes, responsible for sperm production and testosterone secretion, are particularly vulnerable to Lead poisoning[6]. Studies have demonstrated that Lead exposure can disrupt sperm production by interfering with cell growth, differentiation, and maturation in the seminiferous tubules, leading to reduced sperm

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production[7]. Additionally, Lead can affect sperm movement and energy metabolism, increase the number of abnormally shaped sperm (teratospermia), and cause structural damage to the seminiferous tubules[8]. It can also interfere with Leydig cells, which produce testosterone, leading to abnormal testosterone levels, affecting libido, erectile function, and sperm production[9]. These adverse effects on sperm quality and testicular function ultimately result in male infertility[10]. Finding effective ways to counteract the harmful effects of Lead exposure on male fertility is crucial. Natural products with antioxidant and chelating properties show promise in this regard. Garlic (*Allium sativum*), known for its health benefits, has been used in traditional medicine for centuries[11]. GBEO, a concentrated extract of garlic cloves, possesses potent antioxidant and chelating properties[12].

Antioxidants function by neutralizing harmful molecules called free radicals, which contribute to oxidative stress and cell damage. Lead exposure induces oxidative stress in the testes, and antioxidants may help to protect sperm and testicular tissue from this damage[13]. Chelating agents, on the other hand, bind to metal ions like Lead, aiding in their removal from the body and potentially reducing their accumulation in the testes[14]. Based on these findings, this study investigates the potential protective effects of garlic essential oil against Lead nitrate-induced reproductive toxicity in Swiss albino male mice. The hypothesis is that supplementation with GBEO can alleviate the harmful effects of Lead nitrate exposure on testicular weight, sperm parameters, testicular morphology, and hormone levels. This research aims to enhance our understanding of the impact of environmental Lead exposure on male fertility and explore the potential use of natural products like garlic essential oil as a protective measure.

2. Materials and Methods

2.1 Chemical Compound Bought

All the chemicals used throughout the study were sourced from Thermo Fisher. The selection of these chemicals was according to their characteristics and their capacity to facilitate the goals of the research. To ensure purity and quality, we strictly followed supplier-provided quality control procedures and verified certificates of analysis for each compound. We diligently consulted and followed the Safety Data Sheets (SDS) provided by the suppliers, implementing established laboratory safety practices. The selection and acquisition of chemical used seamlessly aligned with our research objectives, ultimately contributing significantly to the study's success.

2.2 Experimental Plant

This study utilized fresh *A. sativum* bulbs sourced from Banasthali Vidhyapith, Rajasthan, as the primary experimental plant material. These meticulously selected bulbs were used for the extraction of essential oil via the hydrodistillation technique. This technique involves careful heating and subsequent condensation of a water-plant component solution. This enables the precise extraction of valuable essential oils from the garlic bulbs.

2.3 Hydro-distillation Technique

The essential oil was extracted from fresh garlic bulbs (*A. sativum*) using the reliable hydrodistillation technique. Locally sourced Garlic bulbs from Banasthali Vidhyapith, were meticulously prepared for optimal extraction. Garlic bulbs were finely chopped and homogenized with distilled water using a domestic blender for one min at medium speed. This homogenate was then allowed to macerate for one hour to **enhanc**e the extraction process. The hydrodistillation employed a vertical distillation apparatus. The garlic homogenate underwent a three-hour heating period with controlled temperature, and the generated vapor was condensed and separated through an automatic oil/water separator, a low-pressure pipe water system maintained the optimal cooling throughout the process[15]. To ensure precise results, each extraction was executed in triplicate[16]. After extraction, the essential oil was carefully dried with anhydrous sodium sulfate to remove any remaining moisture. The dried oil was then stored in sealed tubes at 4°C, protected from light, ensuring its preservation and stability for future research and applications. This comprehensive methodology guarantees the reliable extraction and conservation of essential oils from fresh garlic bulbs thus contributes valuable knowledge to the field of natural product chemistry.

2.4 Experimental Animal

For experiment purpose male Swiss albino mice (*Mus musculus*), aged 2-3 months and weighing 20-30 grams were sourced from Lala Lajpat Rai University of Veterinary & Animal Science, Hissar, India. The mice were housed in a controlled environment within a pathogen-free facility, maintaining a stable temperature of 23-25°C and humidity of 50-55%. Cages equipped with solid floors and appropriate substrate ensured hygiene and comfort. The animal received standard diet and free access to distilled water throughout the study. Prior to the experiment, the mice underwent a week-long acclimatization period to adapt to the research environment and ensure their physiological readiness. Institutional Animal Ethics Committee approval was obtained under reference number BV/IAEC/January/2020/10. This study adhered to all guidelines and regulations concerning the use of animals in research to maintain ethical standards. Veterinary care was readily available, and all measures were taken to minimize discomfort or distress to the animals.

2.5 Study Design

This meticulously designed experiment investigated the potential protective effects of *A. sativum* (garlic) bulb essential oil against Lead nitrate-induced toxicity in Swiss albino mice. Lead nitrate, a known environmental toxin, was administered orally at a dose of 50 mg/kg body weight[17], using distilled water as the solvent. Two doses of garlic essential oil: 50 mg/kg (low) and 80 mg/kg (high), were decided on the basis of previous study[16], administered orally with olive oil used as the vehicle. Animals were weighed prior to study The control group (Group I) received no treatment, while Group II served as the Lead nitrate-exposed group. Group III received Lead nitrate along with the low dose of garlic oil (GBEO), and Group IV received Lead nitrate along with the high dose of GBEO. Group V was treated with Lead nitrate in combination with the standard drug, Silymarin, and Group VI received Lead nitrate in combination with olive oil. Each group consisted of six Swiss albino mice. The total treatment duration was of 30 days. Lead nitrate administration spanned 30 days in group II to group VI animals whereas the garlic oil (Both doses), standard drug and vehicle oil started from the 12th day up to the end of the experiment (Table I). After completion of the experiment, animals were fasted overnight then these animals were sacrificed by cervical dislocation and their testes were collected, weighed, washed with 0.9% saline to remove excess blood, and preserved in PBS buffer (pH: 7.4) for experimental purpose.

2.6 Tissue Homogenate Preparation

Swiss albino mice testes tissue was meticulously collected and cleaned in pre-chilled PBS on ice. After determining the required tissue weight, a homogenization buffer (100 mg tissue per 900 **µl** buffer of PBS) was prepared. Tissue homogenization

Table 1. Treatment composition and administration methodology in the experiment design

Group	Treatment	Lead nitrate dose	Garlic bulb oil dose	Vehicle	Number of mice	Treatment duration
I	None	NA	NA	Double distilled water	6 Animals in each group	One month
II	Lead Nitrate	50 mg/kg (30 days)	NA	Double distilled water		
III	LN + GBEO Low dose	50 mg/kg (30 days)	50 mg/kg (from 12th day)	Olive oil		
IV	LN + GBEO High dose	50 mg/kg (30 days)	80 mg/kg (from 12th day)	Olive oil		
V	LN + Silimarin (started from 12th day)	50 mg/kg (30 days)	NA	Double distilled water		
VI	LN + Olive oil (started from 12th day)	50 mg/kg (30 days)	NA	NA		

was achieved using a Dounce homogenizer. Sequential homogenization with large and small-clearance pestles ensured proper tissue disruption, while maintaining the sample at 2°**C** -8°C in an ice bath. The process continued until the desired smoothness was attained. Centrifugation at 13,000xg for 2 min separated the cellular components. The supernatant was carefully aspirated to preserve the integrity of the cell pellet[16,18].

2.7 Testicular Parameters

2.7.1 Testes and Epididymis Weight

In this study, the weight of testes and epididymis of the animal model was taken by using analytical balances, which are commonly preferred in laboratory settings for their exceptional sensitivity and precision.

2.7.2 Sperm Concentration

The methodology for assessing sperm count was conducted according to the previously described guidelines[19], employing an upgraded Neubauer hemocytometer. Initially, epididymal sperm were collected in 1 ml of normal saline and then transferred to a sterile Petri dish, where they were thoroughly mixed. Subsequently, the mixture underwent a 5-min incubation at 37°C to aid in the separation of sperm within the suspension[20]. Following this incubation period, the sperm suspension was appropriately diluted. Approximately 100 **µ**L of the diluted sperm suspension was then loaded onto the hemocytometer for sperm counting under a light microscope at a 40X magnification. The resulting sperm count values were adjusted by the dilution factor and recorded as millions per milliliter (10^6/mL)[21].

2.7.3 Sperm Motility

The sperm motility assessment protocol involves collecting sperm from the cauda epididymis of mice. After euthanization, the right epididymis is dissected within a min and a half and placed in a pre-warmed dish containing a specific solution. The epididymis is then punctured to release sperm, followed by a three-min "swim-out" period. A small sample is then taken from the sperm cloud and placed on a pre-heated slide for analysis using an automated system[22]. This system assesses sperm motility at five different fields on the slide to provide a more accurate average value. It's crucial to maintain the temperature of the dish between 36-37°C to avoid damaging the sperm during the analysis[21].

2.7.4 Testosterone Concentration

Testosterone concentration was measured using the ELISA kit provided by Calbiotech, a Life Science Company, with catalog number TE373S.

2.7.5 Histological Analysis of Testicular Structure

Swiss albino mice were euthanized using an approved method, and their testes were meticulously dissected under a dissecting microscope, adhering to the previously described guidelines[23]. The collected tissues were then immersed in Bouin's solution for the recommended duration as per the manufacturer's instructions to ensure proper fixation. The fixed testicular tissues were placed in tissue cassettes and underwent a series of graded ethanol dehydration and xylene clearing steps. Following this, the tissues were infiltrated with paraffin wax and affixed onto a microtome chuck. Thin transverse sections measuring 3-5 µm were then meticulously cut and placed onto pre-coated glass slides as per the method described[24].

The slides were prepared by deparaffinizing (if necessary) and rehydrating through a descending series of ethanol concentrations. General morphology assessment was conducted using haematoxylin-eosin staining, while periodic acid-Schiff staining was employed to highlight specific structures such as basement membranes. Following staining, the sections underwent dehydration, xylene clearing, and were finally mounted with a coverslip. The stained sections were meticulously examined under a light microscope at various magnifications. Key testicular structures including seminiferous tubules, Leydig cells, interstitial tissue, and blood vessels were identified and compared against reference images or descriptions from literature detailing normal Swiss albino mice testes. Any deviations from normal morphology and potential pathological changes were meticulously documented and subjected to detailed analysis[23].

2.7.6 Sperm Morphology

Initiates with a precise measurement of the caudal portion of the one epididymis, ensuring accuracy down to four decimal places. Subsequently, the tissue was

minced multiple times within a Petri dish containing deionized water, with gentle swirling that aids in the release of cells[25]. Two drops of the resulting mixture were then carefully transferred onto each of two standard glass microscope slides. A delicate application of the edge of a clean glass slide establishes a thin layer of sperm cells on each slide. These slides were then left to air dry before undergoing staining in a 1% eosin solution and being cover-slipped for subsequent microscopic examination. Criteria for defining abnormal sperm morphology included deviations in tail and head structure[26].

2.7.7 Statistical Analysis

The statistical analysis of testicular parameters was performed using one-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test to identify statistically significant differences between experimental groups. Data were expressed as mean ± Standard Error of the Mean (SEM). Statistical significance was evaluated at three thresholds: $p < 0.05$, $p < 0.01$, and $p < 0.001$, indicating varying levels of significance. The analysis was conducted using a statistical software package, ensuring accurate computation of group means and inter-group comparisons. ANOVA was utilized to assess the overall effect of treatments across the experimental groups, while Tukey's post-hoc test determined specific group differences by controlling for Type I error. This approach ensured a robust and reliable interpretation of the data, allowing the identification of significant changes in testicular parameters influenced by the treatments.

3. Results

The present study examined the impact of Lead nitrate and various treatments on male reproductive health in different groups. Authors analysed testes weight, epididymal weight, sperm concentration, sperm motility, histoarchitecture, sperm morphology and testosterone concentration.

3.1 Testes Weight

The experimental result provided compelling evidence of variations in testes weight among the groups. Notably, a statistically significant difference ($p<0.001$) was observed between the toxicated group i.e., Group II and the control group (Group I), indicating a harmful effect of Lead nitrate on testes weight. Similarly, significant variations were observed when compared Group II with Groups IV and V, supporting the potential of high-dose GBEO and the standard drug in reversing these effects. Conversely, the non-significant differences in testes weight between Group II, Groups III, and Group VI ($p>0.05$) suggest that low-dose GBEO may not be effective, while olive oil appears to be a promising carrier oil. Interestingly, further analysis revealed statistically significant differences ($p<0.05$) in testes weight when compared Group IV with Group VI and Group III with Group IV. These findings again highlight olive oil as a potentially beneficial carrier oil for high-dose garlic, and suggest that high-dose GBEO is the most effective dosage for reversing changes in testes weight (Figure 1, Graph A).

3.2 Epididymis Weight

Our analysis of epididymis weight revealed intriguing variations across the groups. Notably, a highly significant difference ($p<0.001$) was observed between the Lead nitrate-exposed group (Group II) and the control group (Group I). Interestingly, similar significant variations were found when compared Group II with Groups IV, V i.e., $p<0.001$. Conversely, no significant differences in epididymis weight were observed between Group II and Groups III and VI, nor between Groups III and VI, additionally when Group IV compared with Group V is also non-significant i.e., $p>0.05$). However, the analysis also revealed significant differences ($p<0.05$) in epididymis weight between comparison groups: Group II and Group IV, Group III and Group IV, and Group IV and Group VI, suggesting High dose of Garlic oil may be more effective compared to low dose hence showed comparable curative effect like silimarin, additionally olive oil can be used as carrier oil for GBEO (Figure 1, Graph B).

3.3 Sperm Concentration

In the comparison between Group II and Group I, a significant decrease in sperm count was evident ($p<0.001$), indicating the harmful impact of Lead nitrate. Conversely, significant and highly significant differences emerged at the $p<0.05$ and $p<0.001$ level when compared Group II with Group III and Group

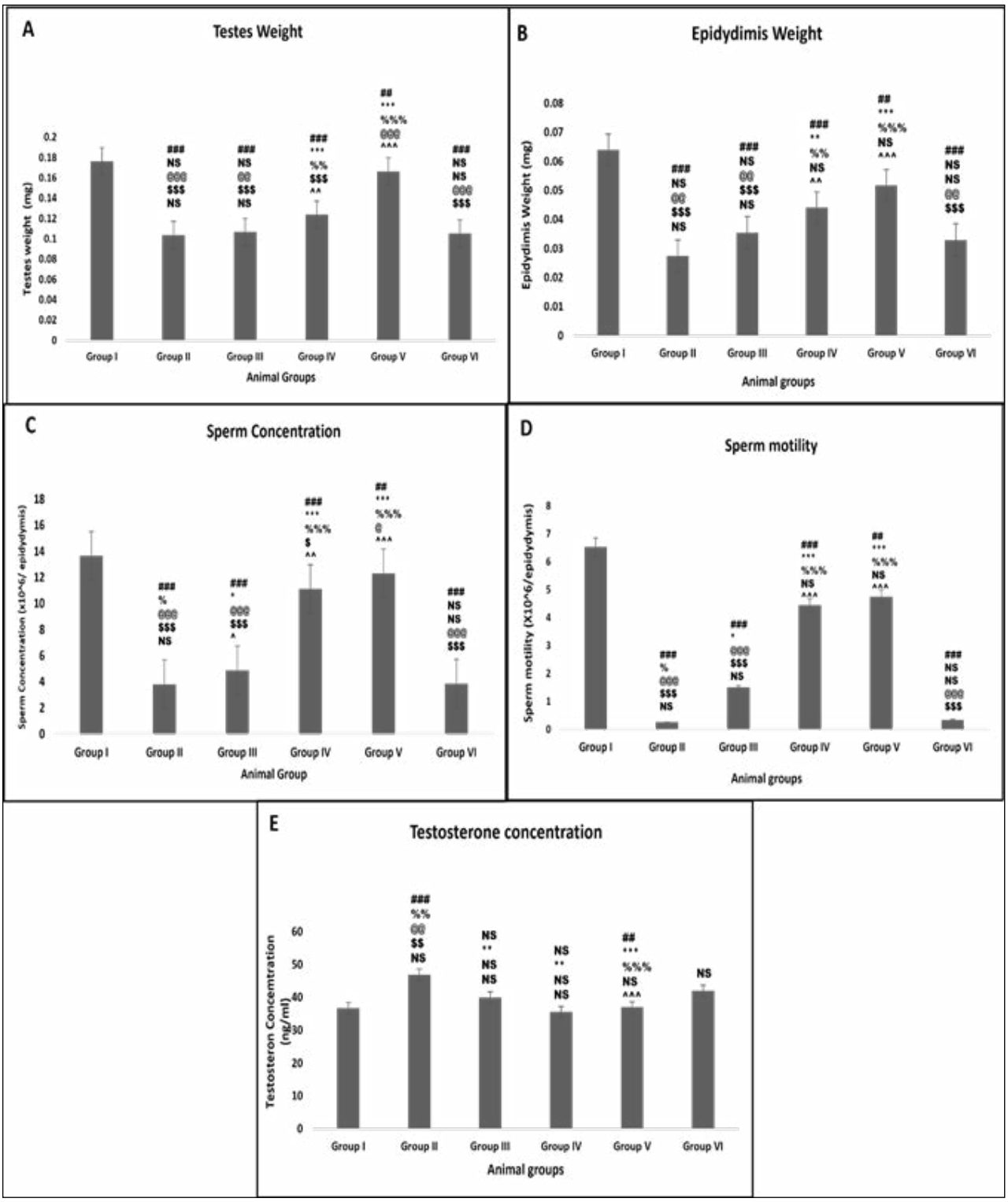


Figure 1. Bar graphs (A-E) show the testicular parameters across the study groups. Data are presented as mean ± SEM (n=6), with statistical significance indicated at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels. NS stands for non-significant. The significance markers are as follows: (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared to Group I), (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to Group II), (%$p < 0.05$, %%$p < 0.01$, %%%$p < 0.001$compared to Group III), (@$p < 0.01$, @@@$p < 0.001$compared to Group IV), ($p < 0.05$, $$$p < 0.01$, $$$p<0.001$ (@$p < 0.05$compared to Group V), and (^$p < 0.05$, ^^$p < 0.01$, ^^^$p < 0.001$ compared to Group VI).

II with Group IV respectively. Moreover, notable distinctions were observed in the comparison of Group II with Group V, where Lead nitrate was administered alongside silymarin ($p<0.001$). However, no significant difference in sperm count was observed when Group II was compared with Group VI ($p>0.05$). This shows minimal curative effects of carrier oil. Additionally, significant differences were discerned between Group III, received Lead nitrate with a low dose of garlic oil, and Group V ($p<0.001$). Furthermore, a marked decrease was observed when compared Group V with Group VI, where Lead nitrate was combined with olive oil ($p<0.001$). Nonetheless, significant disparities noted between Group III and Group IV ($p<0.05$) highlighted the dose dependent effect of GBEO i.e. high dose is more effective. On other hand different comparison groups like Group III and Group VI ($p<0.05$), Group IV and Group V ($p<0.05$), and Group IV and Group VI ($p<0.05$), highlighting the varied effects of different treatments on sperm count (Figure 1, Graph C).

3.4 Sperm Motility

The results regarding sperm motility among the experimental groups showed significant differences. Specifically, Group II had significantly lower sperm motility compared to Group I ($p<0.001$). Moreover, Group II exhibited significant differences compared to Group III ($p<0.05$), Group IV ($p<0.001$), and Group V ($p<0.001$). However, there was no significant difference in sperm motility when compared Group II with Group VI ($p>0.05$). Furthermore, Group III had significant differences compared to Group IV ($p<0.001$) and Group V ($p<0.001$), showing effectiveness of the high dose and silimarin in comparison to low dose, but not with Group VI ($p>0.05$). Notably, Group IV showed significant differences when compared to Group VI ($p<0.001$), while there was no significant distinction between Group IV and Group V ($p>0.05$) highlighting potential of high dose of GBEO similar to standard drug. Additionally, significant differences were observed between Group V and Group VI ($p<0.001$, Figure 1: Graph D).

3.5 Testosterone Concentration

Comparing the testosterone levels between Group II (exposed to Lead nitrate) and Group I (healthy subjects) showed significantly lower levels ($p<0.01$), indicating that Lead exposure leads to an increase in testosterone level. Similarly, significant decreases in testosterone levels were observed when compared Group II with Groups III (Lead nitrate + low dose of garlic oil), IV (Lead nitrate + high dose of garlic oil), and V (Lead nitrate + silymarin), bringing testosterone level almost near to that of the control group. However, no significant difference was found when compared Group II with Group VI (Lead nitrate + olive oil), suggesting that olive oil, as a carrier for garlic oil, does not have a therapeutic effect. Additionally, there were no significant differences in testosterone levels between Groups III, IV, and V ($p>0.05$) showed both GBEO doses are as effective as silimarin. Interestingly, Group III and IV showed significantly lower testosterone levels nearly equal to control group compared to all other treatment groups, highlighting the effectiveness of both the dose of GBEO (Figure 1, Graph E).

3.6 Histology Slides

The analysis of testicular tissue under the microscope uncovered notable histological changes triggered by the various treatments. Control group (Figure 2: labelled A) shows spermatogonia, secondary spermatocyte, primary spermatocyte, spermatid, the tissue displays intact seminiferous tubules containing normal spermatozoa and a complete spermatogenic germ cell series. Additionally, the density of Leydig cells are normal, and there is no apparent disruption in interstitial edema. In contrast, the Lead-exposed group (labelled B) exhibit seminiferous tubule shrinkage, accompanied by degenerative changes in the spermatogenic series, diffuse interstitial edema, and a decrease in Leydig cell density. The low-dose garlic oil treatment group (labelled C) showcase a reversal in seminiferous tubule size, with relatively intact tubules housing few spermatozoa and showing mild interstitial edema. Furthermore, there is a reversal in Leydig cell density. Conversely, the high-dose garlic oil treatment group (labelled D) display nearly normal-sized seminiferous tubules filled with mature spermatozoa and a complete spermatogenic germ cell series. The interstitial edema remained intact, and there is an almost complete reversal in Leydig cell density. The selected standard drug Silymarin treatment group (labelled E) demonstrate persistent tubular shrinkage, despite being filled with mature spermatozoa, and

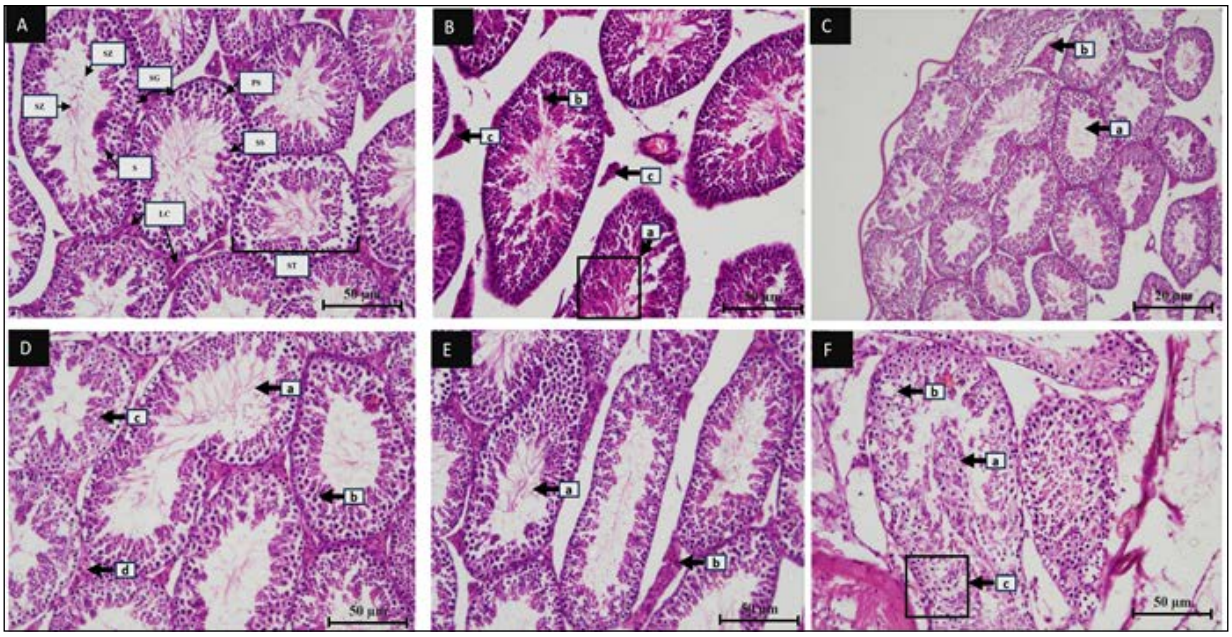


Figure 2. **Image depicts** histological slides of thin transverse sectioned testes; **(A). Control group**: intact (ST) seminiferous tubules with normal (SG) spermatogonia, (PS) primary spermatocyte, (SS) secondary spermatocyte, (S) spermatid, (SZ) spermatozoa, a complete spermatogenic germ cell series, a normal density of (LC) Leydig cells and intact interstitial edema; **(B). Lead-exposed group**: shrinkage of seminiferous tubules was observed, (a) degenerative changes in the spermatogenic series, (b) diffuse interstitial edema, and (c) decreased Leydig cell density. **(C). Low-dose garlic oil treatment group:** exhibited a reversal of seminiferous tubule size, with relatively intact tubules (a) containing few spermatozoa and mild interstitial edema, and (b) reversal of Leydig cell density. **(D). High-dose garlic oil treatment group:** showed nearly normal-sized seminiferous tubules filled with (a) mature spermatozoa and a complete spermatogenic germ cell series, as well as (b) (c) intact interstitial edema and almost complete reversal of (d) Leydig cell density. **(E). Silymarin treatment group:** displayed persistent tubular shrinkage, tubules were filled with (a) mature spermatozoa and exhibited a (b) reversal of Leydig cell density. **(F).** olive oil treatment group presented irregularly shaped tubules with (a) disorganized lumens, (b) vacuolation, and (c) degeneration of spermatocyte layers, along with a notably low number of Leydig cells. Magnification = 40 X. Scale bar = 50 µm (A, B, D, E, F), 20 µm (C).

exhibit a reversal in Leydig cell density, showing high dose and silimarin are almost equally effective. Finally, the olive oil treatment group (labelled F) present irregularly shaped tubules with disorganise lumens, vacuolation, and degeneration of spermatocyte layers. Moreover, there is a notably low number of Leydig cells, showing selected carrier oil has nil effect on recovery of testicular structure. These findings contribute significantly to our understanding of the histological changes induced by different treatments on testicular tissue architecture.

3.7 Sperm Morphology

The analysis of sperm morphology across the experimental groups revealed distinct patterns reflecting the effects of treatments. In the control group (Figure 3: labelled A), sperm cells display typical morphology, with well-formed head, mid, principle, and end piece structures, showing no signs of abnormalities. Conversely, in the Lead-exposed group (labelled B), abnormalities like coiled end pieces, oval-shaped heads, and swollen tail structures are evident, indicating detrimental effects on sperm morphology. In the low-dose garlic oil treatment group (labelled C), while head shows reversal of morphology, some sperm exhibit bent midpieces may affect their movements, though principle and end piece structures are also recovered to their normal structure. In contrast, both the high-dose garlic oil treatment group and the Silymarin treatment group (labelled D) predominantly show normal sperm morphology, characterised by intact head, midpiece, and principle and end piece structures,

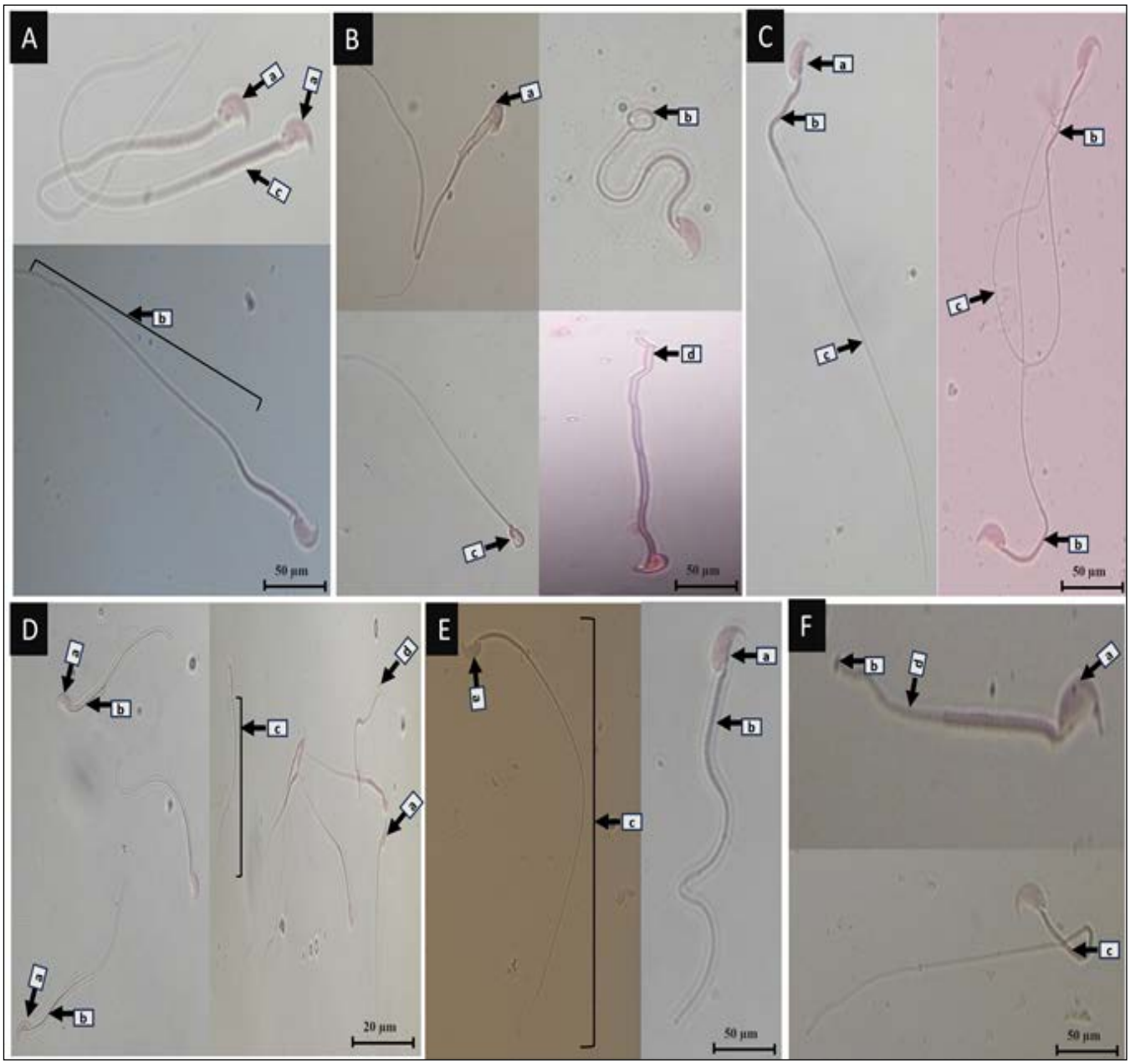


Figure 3. Images showing thin smear of semen stained with eosin: **(A) Control group:** normal sperm cells characterized by a typical (a) head morphology, (b) principle, and end piece structures, exhibiting no abnormalities. **(B) Lead-exposed group:** (a) abnormalities in sperm morphology are evident, including (b) coiled end pieces, (c) oval-shaped heads, and (d) swollen tail structures, indicating adverse effects on sperm caused by Lead exposure**. (C) Low-dose garlic oil treatment**: group sperm cells display (a) normal head morphology, although some exhibit (b) bent midpieces. However, the (c) principle and end piece structures appear unaffected by the low dose of garlic oil treatment. **(D) High-dose garlic oil treatment group**: This image shows sperm cells with (a) normal head morphology and (b) midpieces, as well as (c) intact principle and end piece structures. Coiled end pieces are rarely observed in this group. **(E) Silymarin treatment group**: sperm cells exhibit (a) normal head morphology and (b) midpieces, along with (c) intact principle and end piece structures. Notably, (d) coiled end pieces are rarely seen. **(F) Olive oil treatment group**: The image depicts sperm cells with (a) abnormal morphology, including (b) coiled end pieces, (c) bent midpieces, and (d) swollen tail structures, indicating detrimental effects resulting from olive oil treatment. Magnification: 40 X, Scale bar = 50 µm (A, B, C, E, F), 20 µm (D).

with only occasional instances of coiled end pieces. Conversely, the olive oil treatment group (labelled E) display abnormal sperm morphology, including coiled end pieces, bent midpieces, and swollen tail structures, indicating no curative effects. These findings highlight the diverse impacts of different treatments on sperm morphology, underscoring the potential protective effects of high-dose garlic oil among decided doses of GBEO in comparison of Silymarin against Lead-induced sperm abnormalities.

4. Discussion

This section examines the diverse therapeutic effects of GBEO on male reproductive function in animal model exposed to Lead nitrate. The results consistently show that as the dosage of GBEO increases, improvements are noticeable in different aspects, indicating that GBEO could be a promising treatment for Lead-induced male infertility. Lead exposure is well-known for causing male infertility by disrupting sperm production and hormonal balance through direct interference with key cellular and molecular processes. Primarily, Lead exposure leads to an imbalance in oxidative stress and antioxidant defense systems, as well as disruption of ionic homeostasis, notably calcium (Ca^{2+}) signalling. Lead mimics calcium ions due to its similar ionic radius, interfering with Ca^{2+}-dependent processes like spermatogenesis, steroidogenesis, and cellular metabolism. In the testes, Lead preferentially accumulates in Leydig cells, impaired testosterone synthesis and alter the microenvironment essential for sperm production and maturation[2]. The significant reduction in testes and epididymis weight in the Lead-exposed group (Group II) compared to the control group (Group I) reflects the severe damage inflicted on testicular architecture. Lead nitrate directly disrupts mitochondrial function by inhibiting critical enzymes in the Electron Transport Chain (ETC), including complex IV (cytochrome c oxidase), leading to reduced ATP production and increased ROS generation[6,10]. This causes lipid peroxidation of the mitochondrial and plasma membranes, triggering apoptosis of germ cells and a decline in sperm production. The histological findings of seminiferous tubule shrinkage, Leydig cell depletion, and interstitial edema further corroborate the toxic impact on spermatogenesis and testosterone production[17].

This study emphasizes how GBEO can counteract these harmful effects. The enhancements seen in sperm quality, such as concentration, motility, and shape, are consistent with previous research[27,28], who also found protective effects of garlic extract in Lead-exposed animals and infertile men, respectively. One significant finding is that the effectiveness of GBEO varies with dosage. Both low and high doses show improvements, but the high dose consistently has a stronger effect. This trend is noticeable in testicular and epididymal weights, as well as in sperm quality, except for testosterone levels, which was normalized by both doses. Similar dose-dependent trends have been reported by various researcher who observed increase in organ weight and normalization of testosterone level with garlic extract. The dose-dependency observed in this study supports the need for further research to determine the optimal GBEO dosages for therapy[29]. There is also an increase in testosterone levels in the Lead-treated group, which can be explained by Lead's inhibition of aromatase cytochrome P-450, an enzyme involved in converting androgens into Estradiol[30]. This increase also may be explained by a compensatory overactivation of steroidogenic acute regulatory (STAR) protein activity early in the exposure. Lead is known to increase the expression of star, which facilitates cholesterol transport into mitochondria, a rate-limiting step in testosterone synthesis. However, this increase in intratesticular testosterone is transient and ultimately maladaptive, leading to dysregulated hormone feedback mechanisms, further contributing to spermatogenic disruption[12,31]. This paradoxical rise suggests that Lead exposure initially upregulates testosterone biosynthesis in response to cellular stress, but prolonged oxidative damage and Leydig cell dysfunction eventually impair hormone production. Histological analysis of testicular tissue reveals an important benefit of GBEO treatment: the restoration of normal testicular structure. The high-dose group shows nearly normal-sized tubules with complete sperm production, indicating a stronger effect compared to the low-dose group. This finding is consistent with finding where researcher observed similar improvements in testicular structure with garlic extract in Lead-exposed rats. Restoring normal testicular structure is crucial for healthy sperm production,

suggesting that GBEO has the potential to improve fertility outcomes [2,31]. The molecular mechanisms by which GBEO, particularly its active component allicin, ameliorates Lead-induced damage are multifaceted. Allicin's thiol reactivity allows it to chelate Lead ions (Pb^{2+}), reducing their intracellular concentration and preventing their interference with calcium-binding proteins like calmodulin and voltage-gated calcium channels. This preserves calcium homeostasis, which is essential for both sperm motility and the acrosome reaction, processes vital for fertilization. Additionally, allicin activates nuclear factor erythroid 2–related factor 2 (Nrf2), a transcription factor that upregulates antioxidant genes such as glutathione peroxidase (GPX) and catalase (CAT)[25,26,28]. These enzymes scavenge ROS, protecting the integrity of the spermatogenic cell line and Leydig cells, thereby preventing excessive oxidative stress-induced apoptosis[29,31,32].

The efficacy of high-dose GBEO (Group IV) in reversing Lead-induced damage is evident in the recovery of testes weight, epididymis weight, and sperm concentration, underscores its dose-dependent action. Allicin not only scavenges ROS but also inhibits the NF-κb signaling pathway, reducing pro-inflammatory cytokines like TNF-α and IL-6, which are elevated in response to Lead-induced cellular stress[11,34,35,36]. By mitigating inflammation, GBEO preserves the structural integrity of the testicular tissue, promoting the recovery of seminiferous tubules and maintaining sperm quality. The findings that both high-dose GBEO and silymarin (Group V) restore sperm motility and morphology, with comparable efficacy, suggest that these treatments share a common molecular mechanism, centered on the reduction of oxidative stress and the preservation of mitochondrial function in spermatozoa[8,37,38,39]. Silymarin, rich in flavonolignans such as silibinin, exerts its therapeutic effects through modulation of the same oxidative stress pathways, but its action extends to hepatoprotection and detoxification, critical for mitigating systemic Lead toxicity. Silymarin enhances phase II detoxification enzymes, including glutathione-S-transferase (GST), which conjugates Lead with reduced glutathione (GSH) for excretion[12,30,39,40]. This not only reduces Lead bioavailability in the testes but also supports systemic antioxidant capacity. The restoration of sperm counts and motility in Group V reflects silymarin's ability to preserve mitochondrial membrane potential in sperm cells, thus maintaining ATP production necessary for motility[27,41-43].

The absence of significant improvements in reproductive parameters in the olive oil treatment group (Group VI) emphasizes the specificity of GBEO and silymarin's biochemical actions. While olive oil contains phenolic compounds like hydroxytyrosol, which have mild antioxidant effects, these are insufficient to counteract the extensive ROS-mediated damage induced by Lead. The vacuolation and disorganized structure of the seminiferous tubules in this group underscore the minimal impact of olive oil on reversing testicular damage, as its primary role is as a carrier rather than an active therapeutic agent[24,31]. While the exact mechanisms of GBEO's effects are still being studied, its antioxidant and stimulatory properties are likely significant factors. Lead exposure induces oxidative stress, which can harm sperm and hinder testosterone production. GBEO's antioxidant properties may counteract this damage, protecting sperm and supporting healthy sperm production. Additionally, GBEO's potential stimulatory effects could help to normalize testosterone production, further supporting sperm production and maturation[29,43]. This is supported by the observed restoration of mitochondrial function in sperm cells, as GBEO prevents the opening of mitochondrial permeability transition pores (mptp), preserving ATP production and facilitating the reactivation of dynein atpase, which is essential for sperm motility[19,39]. It has previously shown that the Silymarin, a well-researched antioxidant extracted from milk thistle, proven to be effective in enhancing sperm parameters like motility and morphology in infertile men, and this was the basis of choosing it as a standard drug[32,42]. Silymarin's mechanism involves the inhibition of pro-inflammatory pathways, such as NF-κb, which is often upregulated by Lead-induced oxidative stress[37]. By reducing inflammation and promoting lipid metabolism through pparγ activation, Silymarin supports the recovery of Leydig cell function and testosterone synthesis, further contributing to the normalization of sperm quality[33,34,38].

A more precise future study directly comparing GBEO and silymarin in a Lead-induced infertility model could be valuable to determine their relative efficacy and potential synergistic effects. Interestingly,

the histological analysis provided key insights into the comparative efficacy of these treatments. The high-dose GBEO group displayed almost complete recovery of seminiferous tubule structure and sperm production, while Silymarin showed similar effects in terms of sperm morphology and Leydig cell density. Olive oil, on the other hand, showed minimal independent curative effects, serving primarily as a carrier for the active compounds but failing to significantly restore testicular structure or function. The observed improvements in sperm morphology and motility in the high-dose GBEO and silymarin groups further highlight their role in maintaining the structural integrity of spermatozoa. ROS-mediated damage to the sperm membrane, particularly in the midpiece, impairs mitochondrial function, leading to reduced motility [34]. By scavenging ROS and stabilizing the lipid bilayer of sperm cells, both treatments ensure that ATP production remains intact, supporting sperm movement[28,32,35]. Additionally, the protective effects of GBEO on acrosomal integrity, necessary for sperm-egg fusion, suggest that allicin may also act directly on membrane lipids and proteins, preserving their function in the reproductive tract[35]. Future research could explore optimizing GBEO dosage and investigating the precise molecular pathways through which GBEO exerts its benefits, as well as conducting more targeted clinical trials. In conclusion, this study provides strong evidence for the potential of GBEO as a treatment for male infertility, especially in cases related to Lead exposure. The dose-dependent improvements observed in various reproductive parameters highlight the need for further research to establish GBEO as a safe and effective natural remedy for male reproductive health.

5. Conclusion

This study provides compelling evidence that GBEO offers significant protection against Lead nitrate-induced reproductive toxicity in Swiss albino male mice. Lead exposure led to reduction in testes and epididymis weights, decreased sperm count and motility, altered testosterone level, increased abnormal sperm, and disrupted testicular structure. Additionally, Lead exposure triggered oxidative stress, which severely compromised the testicular microarchitecture, including seminiferous tubule shrinkage and Leydig cell depletion. The supplementation of GBEO, especially at higher doses, effectively counteracted these detrimental effects by reducing oxidative stress, chelating lead ions, and restoring calcium homeostasis, thereby reversing changes in testes weight, epididymis weight, and sperm parameters. GBEO also restored sperm motility and morphology by preserving mitochondrial function and reducing lipid peroxidation in sperm cells, crucial for maintaining ATP production and sperm vitality. Notably, the increase in testosterone concentration observed in the testis homogenate in Lead-exposed animals was normalized with GBEO treatment, suggesting a regulatory effect on steroidogenesis. High-dose GBEO was particularly effective in restoring testicular structure, as evidenced by histological analysis, which revealed the recovery of seminiferous tubule size and an intact spermatogenic cell series. These findings highlight GBEO's potent antioxidant, anti-inflammatory, and metal-chelating properties, positioning it as a promising therapeutic agent for Lead-induced male infertility. The comparable efficacy of high-dose GBEO and silymarin, a standard antioxidant treatment, further underscores its therapeutic potential. Future research should focus on optimizing the dosage and administration protocols of GBEO, and exploring its detailed molecular mechanisms, to validate its efficacy and safety for clinical use in humans.

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7. Author Contribution

T. Aggarwal, and V. Sharma conceptualized the entire experimental work. T. Aggarwal, the primary author of the manuscript, contributed most of the writing,

with additional support from V. Sharma. V. Sharma crafted the study design. T. Aggarwal conducted the experimentation, data analysis, statistical analysis, and data interpretation. All authors have read and agreed to the published version of the manuscript.

8. References

1. Oehninger S, Franken DR, Ombelet W. Sperm functional tests. Fertil Steril. 2014; 102(6):1528-33. https://doi.org/10.1016/j.fertnstert.2014.09.044
2. Benoff S. Male infertility and environmental exposure to lead and cadmium. Hum Reprod Update. 2000; 6(2):107-21. https://doi.org/10.1093/humupd/6.2.107
3. Iavicoli I, Fontana L, Bergamaschi A. The effects of metals as endocrine disruptors. J Toxicol Environ Health B Crit Rev. 2009; 12(3):206-23. https://doi.org/10.1080/10937400902902062
4. Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, *et al*. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect. 2005; 113(7):894-9. https://doi.org/10.1289/ehp.7688
5. Zaki A, Aldabah F, Mohammad M, Emran T, Amer A. Effects of environmental exposure to lead and cadmium on male fertility. Mansoura J Forensic Med Clin Toxicol. 2018; 262):179-91. https://doi.org/10.21608/mjfmct.2018.47202
6. Bonde JP. Male reproductive organs are at risk from environmental hazards. Asian J Androl. 2009; 12(2):152-6. https://doi.org/10.1038/aja.2009.83
7. De Queiroz EKR, Waissmann W. Occupational exposure and effects on the male reproductive system. Cad Saude Publica. 2006; 22(3):485-93. https://doi.org/10.1590/S0102-311X2006000300003
8. López-Botella A, Velasco I, Acién M, Sáez-Espinosa P, Todolí-Torró JL, Sánchez-Romero R, *et al*. Impact of heavy metals on human male fertility - an overview. Antioxidants (Basel). 2021; 10(9):1473. https://doi.org/10.3390/antiox10091473
9. Heidari AH, Zamiri MJ, Nazem MN, Shirazi MRJ, Akhlaghi A, Pirsaraei ZA. Detrimental effects of long-term exposure to heavy metals on histology, size, and trace elements of testes and sperm parameters in Kermani sheep. Ecotoxicol Environ Saf. 2021; 207:111563. https://doi.org/10.1016/j.ecoenv.2020.111563
10. Manouchehri A, Shokri S, Pirhadi M, Karimi M, Abbaszadeh S, Mirzaei G, *et al*. The effects of toxic heavy metals lead, cadmium, and copper on the epidemiology of male and female infertility. JBRA Assist Reprod. 2022. https://doi.org/10.5935/1518-0557.20220013
11. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr. 2001; 131(3):955S-962S. https://doi.org/10.1093/jn/131.3.955S
12. Pande M, Mehta A, Pant BP, Flora SJS. Combined administration of a chelating agent and an antioxidant in the prevention and treatment of acute lead intoxication in rats. Environ Toxicol Pharmacol. 2001; 9(4):173-84. https://doi.org/10.1016/S1382-6689(01)00064-3
13. Zheng L, Guo H, Zhu M, Xie L, Jin J, Korma SA, *et al*. Intrinsic properties and extrinsic factors of food matrix system affecting the effectiveness of essential oils in foods: a comprehensive review. Crit Rev Food Sci Nutr. 2023; p. 1-34. https://doi.org/10.1080/10408398.2023.2184767
14. Sharma K, Sharma V. Assessment of chemical constituents of *A. sativum* essential oil extracted using hydro-distillation technique and their pharmacological potential. J Nat Remedies. 2023; p. 977-92. https://doi.org/10.18311/jnr/2023/32468
15. Sharma V, Sharma A, Kansal L. The effect of oral administration of *A. sativum* extracts on lead nitrate-induced toxicity in male mice. Food Chem Toxicol. 2010; 48(3):928-36. https://doi.org/10.1016/j.fct.2010.01.002
16. Doreswamy K, Shrilatha B, Rajeshkumar T, Muralidhara N. Nickel-induced oxidative stress in testis of mice: evidence of DNA damage and genotoxic effects. Andrologia. 2013; 25(6):996-1003. https://doi.org/10.1002/j.1939-4640.2004.tb03173.x
17. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res. 2003; 93(1-3):141-54. https://doi.org/10.1385/BTER:93:1-3:141
18. Hammami I, May MVE. Impact of garlic feeding (*A. sativum*) on male fertility. Andrologia. 2012; 45(4):217-24. https://doi.org/10.1111/and.12009
19. Sudjarwo SA, Sudjarwo GW, Koerniasari. Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. Res Pharm Sci. 2017; 12(5):381. https://doi.org/10.4103/1735-5362.213983
20. Ola-Mudathir KF, Suru SM, Fafunso MA, Obioha UE, Faremi TY. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol. 2008; 46(12):3604-11. https://doi.org/10.1016/j.fct.2008.09.004
21. Kempinas WG, Lamano-Carvalho TL. A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. Lab Anim. 1988; 22(2):154-6. https://doi.org/10.1258/002367788780864547
22. Helke KL. Book review: Comparative anatomy and histology: A mouse, rat, and human atlas. Vet Pathol. 2018; 55(6):918. https://doi.org/10.1177/0300985818795862

23. Kopera IA, Bilinska B, Cheng CY, Mruk DD. Sertoli-germ cell junctions in the testis: a review of recent data. Philos Trans R Soc Lond B Biol Sci. 2010; 365(1546):1593-605. https://doi.org/10.1098/rstb.2009.0251
24. Buranaamnuay K. The MTT assay application to measure the viability of spermatozoa: a variety of the assay protocols. Open Vet J. 2021; 11(2):251. https://doi.org/10.5455/OVJ.2021.v11.i2.9
25. Rezaei M, Nikkhoo B, Moradveisi B, Allahveisi A. Effect of sperm selection methods on ICSI outcomes in patients with oligoteratozoospermia. BMC Urol. 2021.
26. Hammami I, May MVE. Impact of garlic feeding (*A. sativum*) on male fertility. Andrologia. 2012; 45(4):217-24. https://doi.org/10.1111/and.12009
27. Sudjarwo SA, Sudjarwo GW, Koerniasari. Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. Res Pharm Sci. 2017; 12(5):381. https://doi.org/10.4103/1735-5362.213983
28. Ola-Mudathir KF, Suru SM, Fafunso MA, Obioha UE, Faremi TY. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol. 2008; 46(12):3604-11. https://doi.org/10.1016/j.fct.2008.09.004
29. Ryan KJ. Biochemistry of aromatase: significance to female reproductive physiology. Endocr Rev. 1982; 42(8):3342-3344.
30. Zhai Q, Narbad A, Chen W. Dietary strategies for the treatment of cadmium and lead toxicity. Nutrients. 2015; 7(1):552-71. https://doi.org/10.3390/nu7010552
31. Faraji T, Momeni HR, Malmir M. Protective effects of silymarin on testis histopathology, oxidative stress indicators, antioxidant defence enzymes and serum testosterone in cadmium-treated mice. Andrologia. 2019; 51(5):13242. https://doi.org/10.1111/and.13242
32. Suri S, Khan SS, Naeem S, Nisa ZU, Alam N, Majeed S, *et al*. The beneficial effect of *Allium cepa* bulb extract on reproduction of rats: A two-generation study on fecundity and sex hormones. Plos One. 2024; 19(3):0294999. https://doi.org/10.1371/journal.pone.0294999
33. El-Saadony MT, Saad AM, Korma SA, Salem HM, El-Mageed TA, Alkafaas SS, *et al*. Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. Front Immunol [Internet]. 2024; 15. https://doi.org/10.3389/fimmu.2024.1277074
34. El-Aziz GSA, Hindi EA, Aggad WS, Alturkistani HA, Halawani MM, Alyazidi AS. Evaluation of the potential protectivity of both *A. sativum* and *Zingiber officinale* on cadmium-induced testicular damage in rats. J Microsc Ultrastruct. 2022; 12(2):62-70. https://doi.org/10.4103/jmau.jmau_81_21
35. Aggarwal T, Rajpoot A, Sharma V. GBEO as a potential mitigator of lead nitrate-induced oxidative stress in Swiss albino male mice. Toxicol Int. 2024; 31(4):677-86. https://doi.org/10.18311/ti/2024/v31i4/45001
36. Aggarwal T, Sharma V. Pathways and interventions in lead-induced male reproductive toxicity and testosterone dysregulation. Biochem Cell Arch. 2024; 24(2). https://doi.org/10.51470/bca.2024.24.2.1393
37. Aggarwal T, Sharma V. Protective efficacy of garlic essential oil on testicular biochemical markers in lead nitrate-treated Swiss albino male mice. J Ecophysiol Occup Health. 2025; 25(1):1-12. https://doi.org/10.18311/jeoh/2025/45791
38. Gupta S, Aggarwal T, Sharma V. Unravelling protective efficacy of *A. sativum* essential oil against lead nitrate-induced toxicity in Swiss albino male mice lungs: a study on biochemical parameters. Biochem Cell Arch. 2024; 24(2). https://doi.org/10.51470/bca.2024.24.2.1587
39. Rajpoot A, Aggarwal T, Sharma V. Unraveling the enigma of cardiac damage caused by lead: understanding the intricate relationship between oxidative stress and other multifactorial mechanisms. Toxicology. 2024; p. 153984. https://doi.org/10.1016/j.tox.2024.153984
40. Sharma S, Sharma V. Alleviation of lead nitrate-induced nephrotoxicity by garlic essential oil in albino mice: A comprehensive study on inflammation and genotoxicity. Toxicol Int. 2025; 31(2):283-9. https://doi.org/10.18311/ti/2024/v31i2/35881
41. Gupta S, Sharma V. Administration of garlic essential oil restored the altered enzymatic and non-enzymatic parameters and pulmonary histoarchitecture in mice subjected to lead nitrate [Internet]. J Nat Remedies. 2024; 24(8):1695-707. Available from: https://informaticsjournals.co.in/index.php/jnr/article/view/35969 https://doi.org/10.18311/jnr/2024/35969
42. Rajpoot A, Sharma V. Protective effects of *A. sativum* essential oil against lead nitrate-induced cardiotoxicity: Modulation of lipid metabolism, nitric oxide dynamics, inflammatory mediators, and histological profiles in Swiss albino mice. Toxicol Rep. 2025; p. 101950. https://doi.org/10.1016/j.toxrep.2025.101950