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Protective Efficacy of Garlic Essential Oil on Testicular Biochemical Markers in Lead Nitrate-Treated Swiss Albino Male Mice

Tanya Aggarwal and Veena Sharma*

Department of Bioscience and Biotechnology, Banasthali Vidyapith - 304022, Rajasthan, India; veena.sharma61@gmail.com

Abstract

This research examined the potential of garlic bulb essential oil to safeguard male Swiss albino mice from the adverse effects of lead nitrate on their testicular health. Lead nitrate, an environmental toxin, was given via oral route at a dose of 50 mg/kg body weight, with distilled water as the solvent. Based on previous research, two doses of garlic essential oil (GBEO) were selected: 50 and 80 milligrams per kilogram body weight of the mice were administered orally along with carrier olive oil as low and high-dose treatment groups. The treatment period lasted for 30 days, with GBEO, silymarin, and olive oil administered from day 12 alongside continued lead nitrate exposure. The results demonstrated that the high dose of GBEO effectively mitigated lead-induced alterations in total protein content, and reversed elevated levels of AST, ALT, total cholesterol, and ALP, showing comparable efficacy with silymarin. The low dose of GBEO provided partial protection, while olive oil alone was found to be ineffective. The antioxidative, anti-inflammatory, and metal-chelating properties of GBEO, particularly in synergy with olive oil, offer a natural and potent treatment strategy for heavy metal-induced testicular damage. These results could help for developing the way for developing new, natural therapies to combat lead toxicity and other forms of heavy metal poisoning, thus enhancing the clinical relevance and effectiveness of GBEO in protecting reproductive health against environmental toxins. In conclusion, garlic essential oil demonstrates protective efficacy in mitigating lead nitrate-induced testicular toxicity in Swiss albino male mice, likely due to its potent antioxidant properties.

Major Findings: Our study demonstrates that high-dose garlic essential oil (GBEO) is as effective as silymarin in counteracting Lead-induced testicular toxicity, significantly restoring total protein levels and normalizing AST, ALT, total cholesterol, and ALP levels. While the low dose provided partial protection, olive oil alone was ineffective. These findings highlight the therapeutic potential of high-dose GBEO as a promising agent against Lead toxicity, underscoring the need for dose optimization to ensure safe and effective clinical applications. Further research into GBEO's antioxidative, anti-inflammatory, and metal-chelating effects, particularly its synergy with olive oil, could lead to potent natural therapies for heavy metal-induced reproductive toxicity, advancing GBEO as a clinically relevant treatment for safeguarding reproductive health from environmental toxins.

Keywords: AST, ALT, ALP, TPC, GBEO

1. Introduction

Medicinal plants have been integral to human health practices for millennia, and serve as a cornerstone for both traditional and modern medicine. Historically, these plants have been used due to their therapeutic properties, since ancient civilizations. Their significance is underscored by the fact that many contemporary pharmaceuticals are derived from plant-based compounds, highlighting the enduring relationship between humans and nature in the quest for health solutions. Among these botanical options, garlic (*Allium sativum*) is distinguished by its

*Author for correspondence

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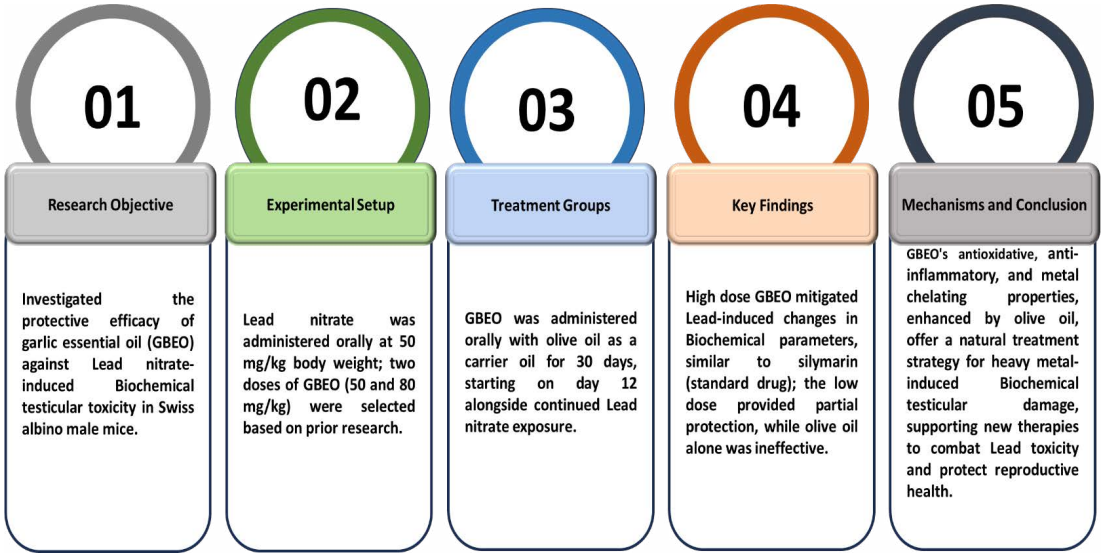


Figure 1. Illustration of graphical abstract.

broad spectrum of therapeutic benefits, including its roles in managing cardiovascular diseases, enhancing immune functions, and exhibiting antimicrobial properties[1]. The bioactive compounds in garlic, particularly sulfur-containing compounds like allicin, are credited for these advantages for well-being, becoming a focal point of attention in both folk and scientific medicine[2].

Despite the well-documented benefits of garlic, the increasing prevalence of environmental toxins, particularly lead, poses significant health risks. Lead toxicity remains a critical public health concern, affecting various systems within the body, with particularly deleterious effects on the nervous system, cardiovascular health, and renal function[3]. Notably, the reproductive system, particularly the testes, is highly susceptible to lead exposure. Sustained lead intake has been proven to negatively influence spermatogenesis, and diminish testosterone production and morphological changes in testicular tissue, leading to reduced fertility and other reproductive dysfunctions[4,5]. These effects are attributed to lead's interference with the hypothalamic-pituitary-testicular axis, which is of the utmost importance for regulating male reproductive hormones and spermatogenesis. Given the serious implications of lead exposure on male reproductive health, there is a pressing need to explore potential protective agents that can mitigate its harmful effects[6].

A previous study suggests garlic as a potential natural remedy against lead and various other ranges of toxins[7]. So, keeping in mind the earlier research performed on garlic, this research aimed to assess the ability of GEO to protect against deleterious effects of LN in a decided male animal model, focusing on the testes as a target organ. The findings of this study hold potential implications for developing therapeutic strategies utilizing natural products, such as garlic, to combat heavy metal toxicity and improve male reproductive health outcomes. It is important to note that males are disproportionately exposed to lead contamination due to occupational hazards in industrial settings and other factors. Studies have consistently demonstrated higher lead levels in male populations compared to females[8,9]. This gender imbalance highlights the critical need for specialized approaches to male health issues.

2. Experimental Methods

2.1 Chemical Acquisition

Thermo Fisher was the exclusive supplier of all chemicals utilized for this study. The selection was based on the specific properties needed for our research objectives. We ensured purity and quality by adhering to the supplier's quality control procedures and verifying each compound's certificates of analysis. Safety Data Sheets (SDS) were thoroughly consulted and followed, ensuring strict compliance with laboratory safety protocols. Thus, our chemical acquisition process aligns closely with our research goals, significantly contributing to the study's success.

2.2 Plant Material

Fresh garlic bulbs (*A. sativum*) harvested from the vicinity of Banasthali Vidhyapith, Rajasthan, were used as the primary plant material in this study. These bulbs were carefully selected to extract essential oil through a hydro distillation procedure. In this method, heating of the water-plant mixture and condensation of the vapours enable the precise extraction of essential oils from garlic bulbs.

2.3 Hydrodistillation Technique

Fresh garlic cloves (*A. sativum*) were subjected to hydrodistillation, a well-established method for essential oil extraction. Garlic bulbs sourced locally from Banasthali Vidhyapith were finely chopped and homogenized with distilled water using a standard blender for one minute at medium speed. The homogenate was soaked for one hour to improve extraction efficiency. Using a vertical distillation apparatus, the homogenate was heated for three hours at a controlled temperature. The vapours produced were condensed and separated with an automatic oil/water separator. A low-pressure pipe water system maintained optimal cooling. To ensure accuracy, each extraction was repeated three times[10]. Residual moisture was removed from the essential oil by drying it with anhydrous Sodium Sulfate, then stored in sealed tubes at 4°C, protected from light, preserving its stability for future research. This thorough methodology ensures reliable extraction and preservation of essential oils from garlic bulbs, thus contributing valuable knowledge to natural product chemistry.

2.4 Experimental Animals

For experimental consistency, adult male *Swiss albino* mice (*Mus musculus*), aged 2-3 months and weighing 20-30 grams, were acquired from the College of Veterinary and Animal Science, Lala Lajpat Rai University, Hissar, India. To minimize the influence of external pathogens, the mice were housed under specific pathogen-free conditions with 50-55% humidity and a temperature of 23-25°C. They were kept in cages with solid floors and suitable bedding provided hygienic and comfortable conditions. Standard rodent chow and distilled water were available unrestricted. The mice underwent a one-week acclimatization period before the study to establish physiological stability. Ethical approval for this animal study was granted by the IAEC (Institutional Animal Ethics Committee) under reference number BV/IAEC/January/2020/10.

2.5 Study Design

This research intended to evaluate the potential preventive capabilities of essential oil derived from *A. sativum* (garlic) bulbs against lead nitrate intoxication in an animal model. Lead nitrate, a known environmental toxin was given orally at a dosage of 50 milligrams per kilogram body weight, using distilled water as the solvent[11]. Based on previous research, garlic essential oil was given at low and high doses of fifty and eighty milligrams per kilogram body weight, respectively[12]. Concentrated garlic essential oil, suspended in olive oil, was also administrated through an oral route. The animals were weighed before the study began. Thirty-six mice were used in the study and they were segregated into 6 groups with 6 mice in each group.

Group I served as the healthy subjects or control group, received no treatment. Group II was subjected to

Table 1. Detailed administration schedule and treatment overview

Day	**Group I** (Control)	**Group II** (Lead Nitrate)	**Group III** (Lead Nitrate + Low Dose GBEO)	**Group IV** (Lead Nitrate + High Dose GBEO)	**Group V** (Lead Nitrate + Silymarin)	**Group VI** (Lead Nitrate + Olive Oil)
1-11	No treatment (Distilled water)	Lead Nitrate (50 mg/kg)	Lead Nitrate (50 mg/kg)	Lead Nitrate (50 mg/kg)	Lead Nitrate (50 mg/kg)	Lead Nitrate (50 mg/kg)
12-30	No treatment (Distilled water)	Lead Nitrate (50 mg/kg)	Lead Nitrate (50 mg/kg) + GBEO (50 mg/kg)	Lead Nitrate (50 mg/kg) + GBEO (80 mg/kg)	Lead Nitrate (50 mg/kg) + Silymarin (standard dose)	Lead Nitrate (50 mg/kg) + Olive Oil (carrier)
31	Animals were sacrificed by Cervical dislocation					

lead Nitrate. Group III underwent lead nitrate treatment and concurrently administered a low dose of garlic essential oil (GBEO). Group IV received a toxicant and a high dose of GBEO. Group V was treated with lead nitrate and the standard drug, Silymarin. Lead nitrate and olive oil were administered to Group VI. The total treatment period lasted 30 days. Lead nitrate administration was continuous from the first day up to the end of the experiment in Groups II to VI. From day 12, animals also received garlic oil (both doses), the standard drug, and the carrier oil along with lead nitrate until the experiment's conclusion. Before sacrifice, the animals were fasted overnight and euthanized via cervical dislocation. Testes were carefully collected, weighed, and cleaned off any remaining blood using a saline solution of 0.9%. The testes were then preserved in a physiological or isotonic phosphate-buffered saline solution with a pH of 7.4 for the experimental purpose.

2.6 Tissue Homogenate Preparation

For the homogenate preparation, the required tissue weight was precisely determined. A homogenization buffer was prepared using PBS at a ratio of 100 mg tissue per 900 µL of buffer. To achieve proper tissue disruption a Dounce homogenizer maintained at a low sample temperature (2-8°C) was used. The tissue was sequentially homogenized with both large and small-clearance pestles in an ice bath until the desired smoothness was attained. Cellular components were then isolated through centrifugation at a relative centrifugal force of 13,000 times gravity for two minutes. The supernatant was carefully aspirated, ensuring the pellet containing the cellular components remained undisturbed[13].

2.7 Biochemical Parameters

2.7.1 Total Protein Estimation

The overall protein concentration in testes tissue homogenate was estimated using the widely accepted Lowry method, a colourimetric assay. This protocol employed Solution A, comprising 4 mg/mL sodium hydroxide and 20 mg/mL sodium carbonate, Solution B containing 10 mg/mL potassium sodium tartrate and 5 mg/mL copper Sulfate, and Folin-Ciocalteau reagent. A series of bovine serum albumin standards, ranging from 0.5 to 8 micrograms per millilitre, were prepared using distilled water. Protein concentration in the homogenized testes tissue samples was determined via a standard curve. Lowry's assay involved incubation of the test solution with Solution A, followed by the addition of Solution B which was then subsequently followed by incubation. Colour intensity was quantified at a wavelength of 750 nanometres using a microplate spectrophotometer. Using the standard curve as a reference, the protein concentration of the samples was determined. This protocol ensures accurate and reproducible estimation of total protein content in testes tissue homogenate and provides valuable data for analysis[14,15].

2.7.2 Glutamic Oxaloacetic Transaminase (AST) Assay

According to the procedure proposed by Reitman and Frankel, 1957 the enzymatic assay for Glutamic Oxaloacetic Transaminase (GOT), Alternatively termed Aspartate Aminotransferase (AST), was carried out. One ml of the AST substrate (containing 2 mM α-ketoglutarate and 200 mM dl-aspartate) was transferred via pipette into a test tube and subjected to incubation at 40°C for a ten-minute duration. Subsequently, 0.2 millilitres of test serum was introduced and thoroughly mixed, and the reaction was allowed to proceed for precisely one hour. The reaction was ceased immediately by introducing 1 ml of 2,4-dinitrophenylhydrazine reagent (1 mM per litre). Then 10 millilitres of 0.4 normality sodium hydroxide were added to the solution after it had stabilized at room temperature for at least twenty minutes. The optical density of the solution was subsequently determined at 505 nm exactly 60 minutes later, using water as a blank. A serum reference was prepared by mixing 1 ml substrate, 0.2 ml serum, and 1 ml hydrazine reagent. After at least 20 minutes, 10 ml alkali was added and the OD was measured accordingly as above.

A proportional relationship exists between the variation in optical density and the AST enzymatic activity present in the serum sample. A standard curve is used to convert ΔOD to AST units, which is defined as micromoles of oxaloacetate plus pyruvate produced per minute at 25°C[16].

2.7.3 Glutamic Pyruvic Transaminase (ALT) Assay

The determination of Glutamic Pyruvic Transaminase (GPT), synonymous with Alanine Aminotransferase (ALT), was performed according to the Reitman and

Frankel method (1957). Here 1 ml of the ALT substrate (containing 2 mM α-ketoglutarate and 200 mM dl-alanine) was preincubated at 40°C for 10 minutes then the 0.2 ml of test serum was added to it and blended. The reaction was initiated for exactly 30 minutes before being stopped with 1 ml hydrazine reagent. The rest of the procedure for the ALT was the same as that of AST. The absorbance was determined at 505 nm 30 minutes after adding 10 ml 0.4 N NaOH. A serum control was also prepared parallelly. The change in absorbance correlates directly with the ALT enzyme activity in the sample. A pyruvate standard curve is used to convert ΔOD to ALT units, defined as micromoles of pyruvate produced per minute at 25°C[16].

2.7.4 ALP Activity

The methodology of Tietz *et al.* (1983) was employed to determine Alkaline Phosphatase (ALP) activity. ALP catalyzes the hydrolysis of colourless p-nitrophenyl phosphate (pNPP) to yield yellow-coloured p-nitrophenol and phosphate at pH 10.3. The resulting absorbance change at 405 nanometers is directly proportional to ALP enzyme activity. The reagents include AMP buffer (300mM AMP, 2mM magnesium acetate, 0.8mM zinc Sulfate) and 10mM pNPP substrate. The procedure involves mixing the sample with a working ALP reagent, aspirating immediately for measurement, and noting the absorbance at 30-second intervals up to 120 seconds. ALP activity is calculated using the kinetic factor based on the change in absorbance per minute[17].

2.7.5 Cholesterol Estimation

The colourimetric method proposed by Zak in 1981 was employed to determine the cholesterol concentration in the samples. To initiate the reaction, 0.2 mL of the sample or standard solution was transferred to a test tube, followed by the addition of 0.2 mL of ferric chloride solution and 6 mL of concentrated sulfuric acid. The tube was then gently inverted to ensure thorough mixing. The reaction mixture was subsequently incubated in a water bath at 37°C for 10-15 minutes, allowing for the development of a characteristic colour.

The colored reaction mixture was then placed into a cuvette and the light absorption was measured at a wavelength of 560 nanometres using a spectrophotometer. A blank solution containing all the reagents except the cholesterol was used as a reference. A calibration curve was constructed by graphing the absorbance of the standard solutions against their respective concentrations. The concentration of cholesterol in the unknown samples was estimated by interpolating their absorbance values onto the calibration graph[18].

3. Statistical Analysis

Results are presented as mean ± Standard Error of the Mean (SEM) to represent both the central tendency and variability of the findings. Statistical significance among multiple groups was assessed using one-way ANOVA, followed by Tukey's post-hoc test. SPSS 20.0 software was used for all statistical analyses. Statistical significance was considered at p-values less than 0.05, 0.01, and 0.001, respectively.

4. Result

This study sought to explore the potential protective benefits of Garlic Essential Oil (GEO) on testicular biochemical markers in adult male *Swiss albino* mice exposed to lead nitrate. To evaluate this, key testicular biochemical parameters were assessed across various experimental groups. The findings are graphically represented for comprehensive analysis.

4.1 Protein Content

As illustrated in Figure 2A and further supported by Table 2, in healthy subjects, the TPC was considerably higher than the Group that was treated with lead nitrate alone with a p-value of < 0.001, indicating a notable decline in TPC due to toxicant exposure. Group III with healthy subjects also showed a notable reduction in TPC ($p < 0.001$), suggesting that the low dose of GBEO did not fully mitigate the lead toxicity. However, when Group IV animals were compared with normal mice, there was no statistically significant variation ($p > 0.05$). This demonstrates that the high dose of GBEO effectively preserved TPC. None of the other treated groups showed significantly higher TPC than the Group treated with lead nitrate + Silymarin, with a p-value of <0.01, indicating that conventional drug provided partial but not complete protection. Lastly, Group I also showed a significantly higher TPC compared to Group VI (lead nitrate + olive oil) with a p-value of <0.001, underscoring the lack of protective effect from olive oil alone.

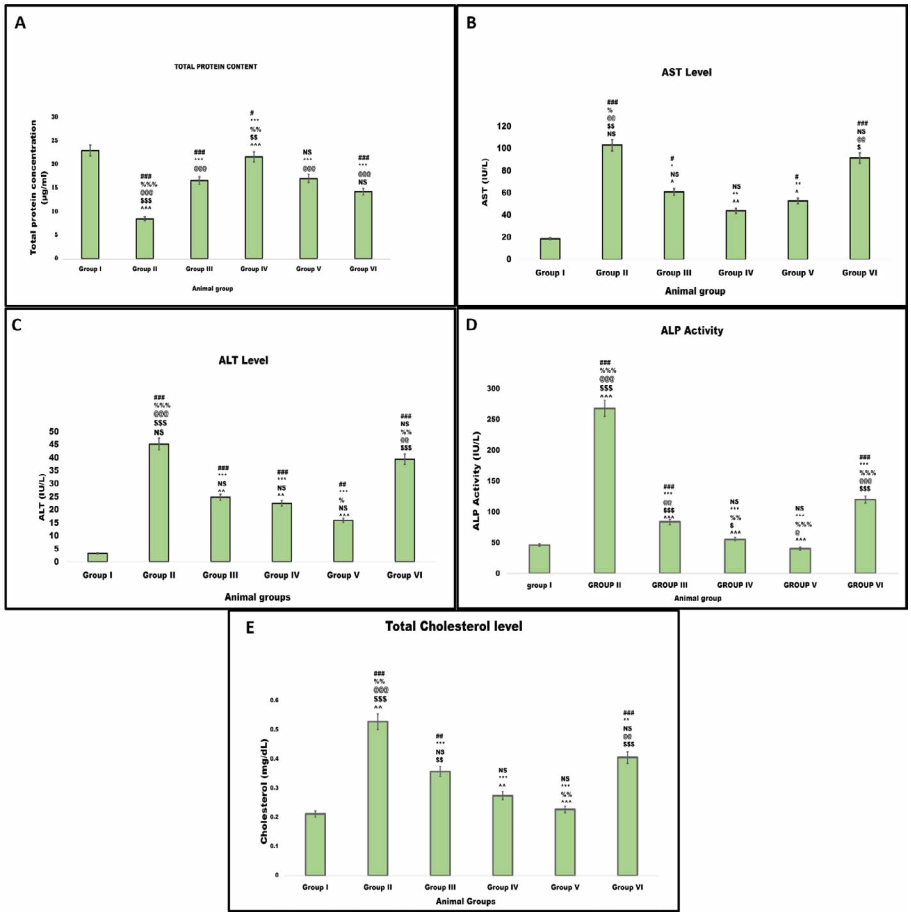


Figure 2. Bar graphs (A–E) show biochemical parameters in the testes of male *Swiss albino* mice. Statistical significance is indicated as follows: # (vs. Group I), * (vs. Group II), % (vs. Group III), @ (vs. Group IV), $ (vs. Group V), ^ (vs. Group VI). Each symbol's significance level is denoted by the number of symbols used (e.g., *p < 0.05, **p < 0.01, ***p < 0.001).

Table 2. The table depicts the biochemical parameters of the testes in different experimental groups. Values are presented as mean ± SEM. Superscripts denote statistically significant differences as follows: a: vs. Group I; b: vs. Group II; c: vs. Group III; d: vs. Group IV; e: vs. Group V; f: vs. Group VI. NS indicates non-significant differences

	TPC (ug/ml)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total cholesterol (mg/ml)
Group I	22.9 ± 0.04	18.8 ± 1.1	3.3 ± 0.41	46.2 ± 2.9	0.211 ± 0.01
Group II	8.46± 0.014 $^{a\,c\,d\,e\,f}$	102 ± 1.2^{a}	45.3 ± 3.1 $^{a\,c\,d\,e}$	269.2 ± 2.4$^{a\,c\,d\,e}$	0.527 ± 0.005$^{a\,d\,e}$
Group III	16.5 ± 0.014 $^{a\,b\,d}$	60 ± 2.1^{NS}	24.86 ± 0.47$^{a\,b}$	83.6 ± 1.3 $^{a\,b}$	0.356 ± 0.01^{b}
Group IV	21.6 ± 0.02 $^{b\,f}$	43 ± 0.94^{NS}	22.56 ± 0.17^{ab}	55.6 ± 0.72$^{a\,b}$	0.274 ± 0.009^{b}
Group V	17.03 ± 0.03 $^{b\,d}$	52 ± 0.86	16.06 ± 0.64^{bf}	40.4 ± 0.82$^{b\,f}$	0.226 ± 0.008$^{b\,f}$
Group VI	14.2 ± 0.05 $^{a\,b\,d}$	91 ± 6.08^{a}	39.57 ± 0.64^{ae}	120.2 ± 1.6$^{a\,e}$	0.404 ± 0.009$^{a\,e}$

When comparing Group II with other groups, it was evident that lead nitrate significantly reduced TPC. Group II had significantly lower TPC compared to Groups III, IV, V, and VI with a statistically significant difference of $p < 0.001$. This indicates that all treatments (GBEO, control drug, and base oil) provided some level of protection against lead-induced TPC reduction. Particularly GBEO high dose and Silymarin provided the most significant results. Assessing the low-dose regimen group to other groups revealed that Group III had significantly reduced TPC than Group given with high garlic oil dose ($p < 0.01$), this highlights the superior protective effect of the high dose GBEO over low dose. Statistical analysis revealed no significant differences in total protein content between

Groups III, V, and VI ($p > 0.05$), suggesting almost the same protective effects offered by the low-dose GBEO, Silymarin, and olive oil.

4.2 AST Level

As demonstrated in Figure 2B and detailed in Table 2, the Benchmark group exhibited substantially reduced AST values in comparison to the lead nitrate Group, with a p-value of <0.001, indicating an ample increase in AST due to lead nitrate ingestion in Group II animals. The baseline group also had a remarkable decline in AST levels compared to the Group that received low-dose GBEO along with lead nitrate, ($p < 0.05$). This suggests that the low dose of GBEO partially counterbalanced the lead-mediated AST increase. No statistically significant variation in AST level was observed when contrasting Group I with the Group exposed to lead nitrate + high-dose GBEO with a p-value of >0.05, demonstrating the high dose of GBEO effectively maintained AST levels close to the non-treated group. Compared to the animal Group administered with lead nitrate and reference drug, Group I animal showed a significant decline in AST level ($p < 0.05$), indicating that the reference drug (Silymarin) provided partial protection. Group I had significantly lower AST levels compared to Group VI (lead nitrate + olive oil) with a p-value of <0.001, highlighting that carrier oil alone did not offer substantial protection.

The lead nitrate treatment group showed significantly higher AST levels relative to the Group with no treatment ($p < 0.001$). Group II exhibited a marked increase in AST level relative to Group III ($p < 0.05$), indicating that the low dose GBEO provided some protection. The intoxicated group also had considerably higher AST levels compared to the conventional drug-treated Group ($p < 0.01$), but no significant difference was observed with the Group given carrier oil alone ($p > 0.05$). This underscores the limited protective effect of olive oil. On comparing Group III to other groups, Group III had significantly lower AST levels compared to Group I ($p < 0.05$) and Group VI ($p < 0.05$). This indicates that low-dose GBEO offers partial protection, but is not as effective as the high-dose and standard drug. Statistically insignificant differences were detected in AST levels between Groups III, IV, and V ($p > 0.05$), suggesting almost equivalent protective capacities of low-dose GBEO, high-dose GBEO, and Silymarin.

4.3 ALT Level

Group I (control) had significantly lower ALT levels compared to Group II (lead nitrate only), with a p-value of <0.001, indicating a marked increase in ALT due to lead nitrate exposure. Similarly, Group I showed significantly lower ALT levels compared to treatment Groups III, IV, and V, consisting of lead nitrate combined with low-dose GBEO, high-dose GBEO, and silymarin, respectively exhibited significantly reduced ALT levels compared to the control group ($p < 0.001$, <0.001, and <0.01, respectively). This suggests that both doses of GBEO and Silymarin provided partial protection. Group I also had a significantly lower ALT level compared to Group VI (lead nitrate + olive oil) with a p-value of <0.001, highlighting that olive oil alone did not offer significant protection. Group II exhibited significantly higher ALT levels compared to the control group ($p < 0.001$). When Group III, IV, and V were compared with the lead-treated group then significant decline in ALT levels was noticed ($p < 0.001$ for each), indicating that both doses of GBEO and Silymarin provided some protection. However, statistically insignificant differences were detected in ALT between Group II and Group VI ($p > 0.05$), underscoring the limited protective effect of olive oil, as observed in Figure 2C, and Table 2.

Group III had significantly higher ALT levels than the healthy Group ($p < 0.001$) and Group animals given with high dose of GBEO ($p < 0.01$), whereas no statistically significant variation was observed in comparison to Group IV ($p > 0.05$). This indicates that the low and high-dose GBEO provided the same protection. When compared to Group V, Group III exhibited significantly elevated levels of ALT ($p < 0.05$), suggesting that Silymarin was more effective than both doses of GBEO.

4.4 ALP Activity

The graph illustrating Alkaline Phosphatase (ALP) activity levels across the different groups highlights significant variations. When comparing Group I (control) with Group II (lead nitrate only), a highly significant elevation in ALP activity was observed ($P < 0.001$) demonstrating the detrimental impact of lead nitrate. A comparison between Group I and Group III (low-dose garlic essential oil) also showed a highly significant increase in enzyme ($P < 0.001$). This indicates that the low dose was insufficient to counteract the lead-induced rise in ALP activity. However, when comparing Group I

with Group IV (high-dose garlic essential oil) and Group V (Silymarin), no significant differences were noticed ($P > 0.05$ for both). This suggests the protective effect of both high-dose garlic oil and Silymarin. Conversely, the comparison between Group I and Group VI (olive oil) revealed a significant increase in ALP ($P < 0.001$), confirming that olive oil alone did not offer any protective benefit. The administration of low-dose GBEO in Group III showed a significant reduction in ALP activity compared to Group II ($P < 0.001$), indicating a protective effect of GBEO. Group IV, which received the high dose of GBEO, demonstrated a remarkable normalization of ALP activity, but there was no significant difference when compared to Group I mice ($P > 0.05$) when compared to Group II mice ($p < 0.001$). Also, mice of Group III showed lower levels of ALP ($P < 0.01$). This suggests that the higher dose of GBEO is more effective in mitigating lead-induced toxicity and, underscoring the dose-dependent efficacy of GBEO. Group V, treated with the standard drug Silymarin, showed no significant difference in ALP activity compared to Group I ($P > 0.05$), but there was a highly significant reduction compared to Group II ($P < 0.001$). This indicates that Silymarin is effective in preventing lead-induced testicular toxicity, similar to the high dose of GBEO.

Lastly, Group VI, treated with olive oil, showed significantly elevated ALP activity when compared to the control Group I ($P < 0.001$), but significantly lower ALP activity was recorded compared to Group II mice ($P < 0.001$). This indicates minimal protection offered by olive oil. But overall comparison showed that olive oil alone does not provide sufficient protection against lead-induced incline in ALP level as evidenced in Figure 2D and Table 2 both low and high doses of GBEO provide significant protection against lead-induced testicular toxicity, as evidenced by the reduction in ALP activity.

4.5 Total Cholesterol Level

The total cholesterol level showed significant variations among the groups. On comparing Group I (control) with Group II (lead nitrate only), a highly significant increase in total cholesterol level was observed ($P < 0.001$), which indicates the detrimental impact of lead nitrate exposure. When comparing Group I with Group III (low-dose garlic essential oil), the increase in total cholesterol was significant ($P < 0.01$), suggesting partial protective effects at the lower dose. Statistical analysis revealed no significant differences when compared to Group I with Group given high-dose garlic essential oil, $P > 0.05$ and Group V administered with Silymarin, $P > 0.05$. This indicates effective protection offered by both high-dose garlic oil and Silymarin. However, a significant increase of cholesterol in Group VI than the group I mice (olive oil, $P < 0.001$), shows the ineffectiveness of olive oil as a protective agent. Group II (lead nitrate only) comparisons revealed significant differences with all other groups including Groups III, IV and V all are $P < 0.01$, as shown in Figure 2E and presented in Table 2. These results confirm the protective effects of garlic essential oil (both doses), Silymarin (standard drug) and olive oil against lead-induced cholesterol elevation.

Group III, which received the low dose of GBEO, showed a significant reduction in cholesterol compared to Group II ($P < 0.01$) but it remains higher in Group III in comparison to animals of Groups IV and V, reflecting a dose-dependent effect of GBEO. When comparing Group III with Group IV, a significant difference in cholesterol level was observed ($P < 0.05$), emphasising the superior efficacy of the high dose of GBEO.

The analysis of Group VI, treated with olive oil, showed significantly elevated cholesterol levels when compared to Groups I, IV, and V ($P < 0.001$), but no significant difference was observed when compared to Group III subjects ($P > 0.05$), this indicates that olive oil does not provide adequate protection against lead-induced cholesterol elevation. Further, it was noticed that high-dose GBEO was highly effective in normalizing cholesterol levels in lead nitrate-treated mice, comparable to the standard treatment drug Silymarin, while the low dose of GBEO provides moderate protection. These findings underscore the dose-dependent protective efficacy of garlic essential oil in combating lead-induced hypercholesterolemia.

5. Discussion

The present study evaluated the protective potential of garlic essential oil (GBEO) in mitigating lead nitrate-induced toxicity in male *Swiss albino* mice particularly biochemical markers such as Total Protein Content (TPC), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), total cholesterol, and Alkaline Phosphatase (ALP). The results were compared with those

from previous studies on similar models to contextualize our findings.

Lead ingestion has been shown to elicit oxidative stress and inflammation, leading to an overproduction of reactive oxygen metabolites that leads to damaged cellular proteins, through processes like oxidation, nitration, and glycation. Our findings indicate a marked reduction of overall protein content among mice subjected to lead nitrate compared to the non-exposed group, indicating substantial protein degradation due to lead toxicity. These findings align with previous studies reporting lead's disruption of protein synthesis and increased protein catabolism in various tissues by promoting the proteolytic activity of calpains and other proteases[19]. Furthermore, lead exposure can lead to mitochondrial dysfunction, which exacerbates oxidative stress and impairs cellular energy metabolism, contributing to cellular damage and apoptosis[11]. The detrimental impact of lead on DNA and RNA could underlie its ability to inhibit protein synthesis by disrupting transcriptional and translational machinery[20]. Additionally, lead disrupts intracellular calcium homeostasis by altering the function of calcium channels and pumps, leading to cellular dysregulation[21]. It also damages the endoplasmic reticulum, leading to reduced protein synthesis and increased ER stress. Administration of a high dose of GBEO (Group IV) effectively preserved TPC and showed no significant difference from the non-exposed group. These results are consistent with the results of earlier studies that demonstrated the antioxidative and protective properties of garlic compounds against heavy metal toxicity[11]. Conversely, the low dose of GBEO (Group III) and silymarin (Group V) provided partial protection, while olive oil (Group VI) did not offer significant protection, underscoring the superior efficacy of GBEO at higher doses.

AST and ALT levels were markedly elevated in the lead nitrate-exposed group, reflecting testicular tissue damage. Lead induces cell lysis through its interference with potassium and calcium channels, leading to increased cellular permeability and subsequent lysis[22], This lysis also involves alterations in the cytoskeleton, which increases susceptibility to cell damage. These findings corroborate findings from previous studies, which have shown that lead exposure induces liver injury, resulting in the leakage of these enzymes into the bloodstream[11]. The high dose of GBEO markedly reduced AST and ALT levels, similar to silymarin, suggesting its potent testicular protective effect. Previous research has indicated that garlic's sulfur-containing compounds can mitigate oxidative stress and inflammation, thereby protecting testicular function[23]. The mechanism may involve the reduction of lipid peroxidation, as garlic compounds scavenge ROS, preventing membrane damage. The low dose of GBEO showed partial efficacy, while olive oil alone was ineffective, highlighting the dose-dependent protective effect of GBEO.

Lead nitrate exposure significantly increased total cholesterol levels. This finding is consistent with earlier studies, associating lead toxicity with disrupted lipid metabolism and increased cholesterol synthesis[24]. The observed hypercholesterolemia may be attributed to altered expression of genes involved in cholesterol metabolism, particularly those regulating HMG-CoA reductase and Low-Density Lipoprotein (LDL) receptors[11,19]**.** High-dose GBEO and silymarin (standard drug) treatments effectively normalized cholesterol levels, reflecting their lipid-lowering and antioxidative properties. These results are supported by prior studies documenting garlic's ability to modulate lipid profiles and reduce cholesterol levels in toxic conditions[25]. The low dose of GBEO provided limited protection, whereas olive oil showed no significant effect, emphasizing the need for a higher dose of GBEO for effective protection.

The study found that ALP activity was substantially higher in the group treated with lead nitrate, indicative of cholestatic testicular damage. The increase in ALP activity can be linked to bile duct injury and liver dysfunction caused by lead exposure, which leads to impaired biliary excretion. This finding is consistent with previous research linking lead exposure to increased ALP activity[26]. High-dose GBEO and silymarin effectively maintained ALP levels close to those of the healthy subjects, showcasing their protective role against lead-induced cholestasis. Prior studies have also highlighted the protective effects of garlic on improved ALP activities[27]. The low dose of GBEO provided partial protection, and olive oil was ineffective, reaffirming the importance of adequate dosing for GBEO's efficacy.

The mechanisms behind these protective effects are multifaceted. Garlic essential oil is abundant in organosulfur compounds like allicin, diallyl disulfide, and diallyl trisulfide, which possess strong antioxidative properties[28]. These compounds scavenge ROS and potentiate the function of intrinsic antioxidant enzymes including superoxide dismutase, catalase, and glutathione

peroxidase by enhancing their transcriptional activity and stability. By reducing oxidative stress, GEO helps to protect cellular proteins, enzymes, and lipids from oxidative damage, thereby maintaining TPC, ALP, ALT, and TC levels. Additionally, GEO exhibits anti-inflammatory effects, mitigating the inflammatory response induced by lead exposure[29]. The organosulfur compounds in garlic inhibit the generation of pro-inflammatory cytokines, including Tumor necrosis factor-alpha, Interleukin-1 beta, and interleukin-6, through suppression of nuclear factor kappa B activation and related inflammatory pathways. This reduces inflammation-mediated damage to testicular tissues and helps to preserve biochemical parameters[25].

Garlic compounds, particularly allicin, also possess metal-chelating properties, permitting their complexation with lead ions and promoting their elimination from the organism's body. By reducing the bioavailability of lead, GEO decreases the toxic burden on the testes, protecting against lead-induced disruptions in biochemical parameters. This metal-chelating effect may involve the formation of stable thiol-lead complexes, which are more readily excreted. Moreover, GBEO helps to stabilize cell membranes by preventing lipid peroxidation and maintaining membrane integrity, crucial for preserving the function of membrane-bound enzymes like ALP and preventing the leakage of intracellular enzymes like ALT[30]. The stabilization of cellular membranes also helps to maintain normal cholesterol metabolism within the testes.

The protective effects of GEO can be enhanced when combined with olive oil. Olive oil is a source of monounsaturated fatty acids and polyphenols, which have their own antioxidative and anti-inflammatory properties[31]. The synergistic action of olive oil and GEO provides a more robust defence against oxidative stress and inflammation, further preserving testicular biochemical parameters. Olive oil also acts as an excellent carrier, improving the bioavailability and absorption of GEO's active compounds, thereby enhancing their protective efficacy[32]. Additionally, comparing GBEO with other natural remedies, such as curcumin or green tea extract, could provide insights into alternative or complementary therapeutic strategies for mitigating heavy metal toxicity.

Comparing our findings with published literature, it is evident that GBEO, particularly at higher doses, exhibits significant protective effects against lead-induced toxicity. The antioxidative, anti-inflammatory, and hepatoprotective properties of garlic constituents play a central role in mitigating the biochemical alterations induced by lead exposure. The outcomes of this investigation align with the conclusions of prior investigations that have documented similar safeguarding properties of garlic and its components in various animal studies[33,34]. Given the rising levels of environmental lead exposure due to industrial activities, understanding the role of natural antioxidants like GBEO in public health could be crucial for developing community-based interventions Intending to decrease the occurrence of lead-induced health problems.

In conclusion, the high dose of garlic essential oil demonstrated robust protective effects against lead-induced biochemical alterations, comparable to the standard drug silymarin. The findings indicate that GBEO could be a potential medication for managing lead toxicity, warranting further investigation and clinical validation.

6. Conclusion

The high dose of garlic essential oil (GBEO) showed comparable efficacy to silymarin, the chosen standard drug, in counteracting lead-induced testicular toxicity by effectively mitigating alterations in total protein content and reversal of AST, ALT, total cholesterol, and ALP levels. The low dose of GBEO provided partial protection, while olive oil alone was ineffective. These findings underscore the potential of GBEO, especially at higher doses, as a promising therapeutic agent to protect against and reverse lead-induced testicular toxicity. This study highlights the importance of dose optimization in future research to determine the most effective and safe dosage for clinical applications. Additionally, further studies should explore the underlying mechanisms of GBEO's protective effects, as well as its long-term safety profile. The antioxidative, anti-inflammatory, and metal-chelating properties of GBEO, particularly in synergy with olive oil, offer a natural and potent treatment strategy for heavy metal-induced testicular damage. These results pave the way for developing new, natural therapies to combat lead toxicity and potentially other forms of heavy metal poisoning. By advancing our understanding of GBEO's therapeutic benefits and refining its application, we can enhance its clinical relevance and contribute to safer, more effective treatments for protecting reproductive health against environmental toxins.

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8. Author Contributions

T. Aggarwal and V. Sharma conceptualized the entire experimental work. T. Aggarwal, the principal author of the manuscript, contributed the majority of the writing, with additional support from V. Sharma. V. Sharma designed the whole study design. T. Aggarwal carried out the experimentation, data analysis, statistical analysis, and data interpretation.

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