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# Garlic Bulb Essential Oil (GBEO) as a Potential Mitigator of Lead Nitrate-Induced Oxidative Stress in Swiss Albino Male Mice

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## Abstract

This research examined the effectiveness of essential oil derived from garlic bulb in alleviating the toxic effects of lead nitrate in animal model Swiss albino mice. Six groups were considered for the study, Group I was untreated and Group II to Group VI animals were given lead nitrate at the dose of 50 mg/kg orally for 30 days. Group III and Group IV received low dose (50 mg/kg), high dose (80 mg/kg) of GBEO, and Group V was given standard drug (Silymarin) respectively, lastly Group VI was given olive oil to see its effect alone, from the 12$^{th}$ day until the experiment's conclusion. To assess GBEO protective effects, various antioxidant parameters, including Superoxide Dismutase (SOD) activity, Lipid Peroxidation (LPO) activity, Glutathione (GSH) levels, Activity of Glutathione Peroxidase (GPx), Catalase (CAT) activity, and levels of Glutathione S-Transferase (GST) were evaluated in Swiss albino mice testicular homogenate. The results revealed that GBEO, particularly the high dose, significantly mitigated lead-induced oxidative stress. This was evidenced by improvements in antioxidant enzyme potencies and preserved GSH levels. These findings indicate that GBEO holds a promising prophylactic treatment for lead poisoning. However, further studies are warranted to optimize the dosing regimen for maximizing its protective efficacy against lead toxicity.

**Keywords:** Garlic Bulb Essential Oil (GBEO), Lead, Oxidative Stress, Swiss Albino Mice, Testes

## 1. Introduction

Lead, a widespread environmental contaminant, presents a major risk to human health by accumulating in multiple organs and disrupting normal physiological functions[1]. Male reproductive health is particularly susceptible to lead-induced damage. Lead exposure promotes the ROS (Reactive Oxygen Species) generation and depletes the antioxidant stores[2]. ROS production results in oxidative stress can induce harmful consequences[3]. This oxidative stress can compromise sperm quality, leading to morphological abnormalities, reduced motility, and impaired fertilization potential[4]. Previous studies also reported decreased sperm quality, impaired testicular function, and hormonal imbalances[5]. This vulnerability stems from the rapid cell division and high metabolic activity of spermatogenesis, which make these cells more susceptible to the oxidative stress.

To overpower harmful consequences of lead exposure, garlic the table food may play effective role in detoxification. Garlic (*Allium sativum*) has a long history of remedial applications and is known for its

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various health benefits. Garlic Bulb Essential Oil (GBEO) is a concentrated extract rich in bioactive compounds with potent free radical scavenging and inflammation-modulating properties[4,5]. Previous research suggests that GBEO may offer protection against lead-induced toxicity in various organ systems[6,7].

So, keeping in mind the health promoting benefits of garlic, the current investigation was designed to explores the remedial effects of Garlic Bulb Essential Oil (GBEO) towards lead nitrate-induced oxidative harm in the male reproductive tissues of mice. To evaluate the impact of GBEO on male fertility, a well-established panel of oxidative stress markers, including measurements of Glutathione-S-Transferase (GST), reduced glutathione (GSH), Glutathione Peroxidase (GPx), Catalase (CAT) Malondialdehyde (MDA) levels (biomarker for lipid oxidative stress), and Superoxide Dismutase (SOD) activity, were measured.

# 2. Experimental Methods

## 2.1 Chemical Acquisition

All chemicals utilized in this research were procured from Thermo Fisher. The chemicals were specifically chosen based on their properties and the study's goals. To guarantee purity and quality, we have meticulously followed the supplier's quality control procedures and verified the certificates of analysis for each compound. We also diligently consulted and adhered to the Safety Data Sheets (SDS) provided by the suppliers, ensuring implemented established laboratory safety protocols. In short, the selection and acquisition of chemicals is directly aligned with our research objectives, ultimately playing a significant role in the study's success.

## 2.2 Plant Material

This study utilized fresh garlic bulbs (*Allium sativum*) sourced from Banasthali Vidyapith, Rajasthan, as the primary plant material. These bulbs were meticulously chosen for the extraction of essential oil through the hydro distillation technique. In this technique water-plant material is carefully heated and then it is condensed. This procedure enables the precise extraction of valuable essential oil from the garlic bulbs.

## 2.3 Hydrodistillation Technique

Fresh garlic cloves (*A. sativum*) underwent hydro distillation, a well-established technique, to yield the essential oil. Locally sourced garlic bulbs from Banasthali Vidyapith were meticulously prepared to optimize the extraction process. The garlic bulbs were finely chopped and homogenized with distilled water using a standard blender for one minute at medium speed. This homogenate was then allowed to soak (macerate) for one hour to improve the extraction efficiency. The hydro distillation process itself employed a vertical distillation apparatus. The garlic homogenate underwent a three-hour heating period with a controlled temperature. The generated vapor was then condensed and separated using an automatic oil or water separator. A pipe water system with low pressure helps to maintain optimal cooling throughout the process. To ensure the accuracy of our results, each extraction was repeated three times (triplicate)[7].

After extraction, the essential oil was carefully dried with sodium sulfate (anhydrous) to eliminate any leftover moisture. The dehydrated oil was then housed within sealed tubes at 4°C, protected from light, to ensure its preservation and stability for future research and applications.

This comprehensive methodology guarantees the reliable extraction and preservation of essential oils from fresh garlic bulbs, contributes the valuable knowledge to the field of natural product chemistry.

## 2.4 Experimental Animals

For experimental consistency, a group of 2–3-month-old male Swiss albino mice (*Mus musculus*) of body weights ranging between 20-30 grams were acquired from Lala Lajpat Rai University, college of Veterinary and Animal Science, Hissar, India. The mice were maintained in a pathogen-free environment with a 50-55% humidity and temperature of 23-25°C. Cages with solid floors and suitable bedding provided hygienic and comfortable conditions. Standard rodent chow and distilled water were available ad libitum throughout the experiment. Prior to study initiation, the mice underwent a one-week acclimatization period, to ensure their physiological stability. We received animal ethical approval from the IAEC (Institutional Animal Ethics Committee) under reference number BV/IAEC/January/2020/10.

## 2.5 Study Design

This meticulously designed experiment investigated the potential protective effects of essential oil derived from

*A. sativum* (garlic) bulbs against lead nitrate intoxication in animal model. Lead nitrate, a known environmental toxin, was administered orally at a dosage of 50 milligrams per kilogram of body weight[8] using distilled water as the solvent. Based on prior investigation, two garlic essential oil doses were selected: 50 mg/kg (designated as the low dose) and 80 mg/kg (designated as the high dose)[9]. The oral administration of garlic oil doses was performed with olive oil used as the carrier. The animals were weighed before the study began.

The healthy subjects of Group I did not receive any treatment. Group II served as the lead nitrate-exposed group. Group III received lead nitrate along with the low dose of garlic essential 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 period lasted 30 days. Lead nitrate administration continued for 30 days in Groups II to VI. Animals received garlic oil (both doses), the standard drug, and the carrier oil from the 12th day along with lead nitrate exposure and continued until the experiment's conclusion as depicted in Figure 1 and Table 1. After experiment completion, prior to sacrifice, the animals were fasted overnight. Euthanasia was then performed via cervical dislocation. Following euthanasia, the testes were carefully extracted, weighed, and if any remaining blood present was removed by using saline solution of 0.9%. Finally, the testes were preserved in an isotonic Phosphate-Buffered Saline (PBS) solution, adjusted to a pH of 7.4.

## 2.6 Tissue Homogenate Preparation

After sacrifice, the testes tissues collected from the Swiss albino mice were cleaned thoroughly using pre-chilled PBS solution on ice[10]. The weight of tissue was then precisely determined. Next, 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 employed for the homogenization process. Sequential homogenization was performed using both large and small-clearance pestles. The tissue was homogenized in an ice bath until the desired level of smoothness was attained.

Homogenization was followed by isolation of the cellular components through centrifugation. The samples were spun at 13,000 x g for a duration two minutes. After centrifugation the supernatant, the liquid layer above the cell pellet, was meticulously aspirated by a pipette.

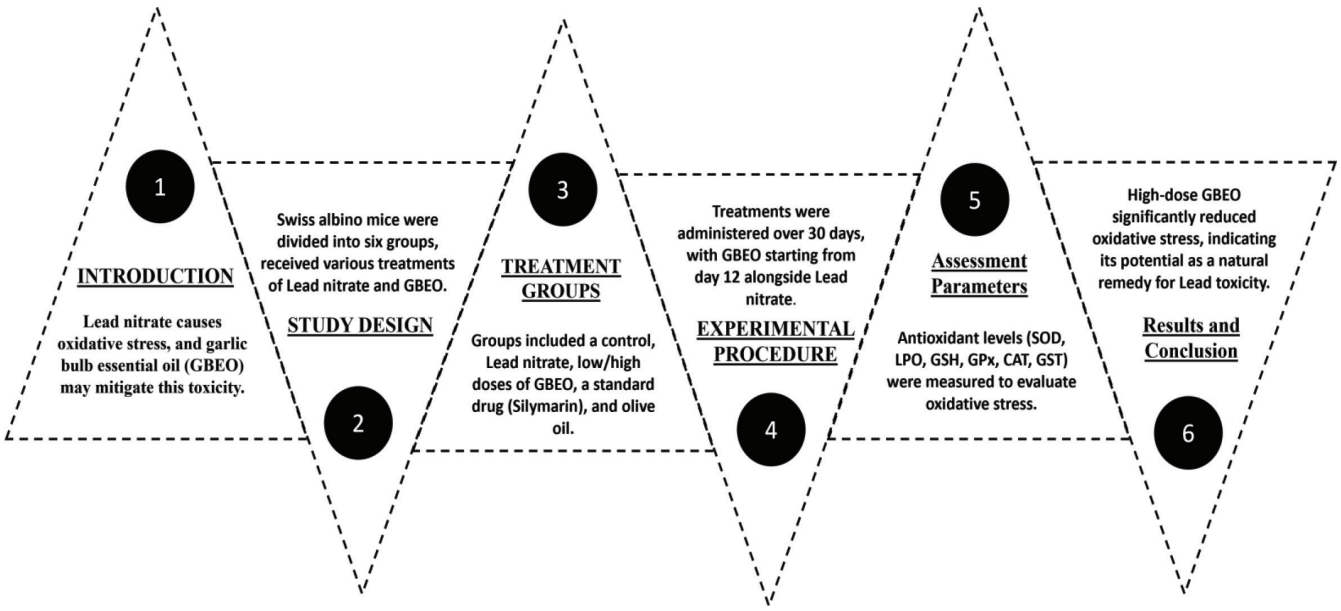



**Figure 1.** Graphical representation of the study on Garlic Bulb Essential Oil (GBEO). Shows treatment groups, 30-day experiment, and antioxidant assessment (SOD, LPO, GSH, GPx, CAT, GST). GBEO reduces oxidative stress, suggesting a natural remedy for lead toxicity.

**Table 1.** Depicts the experimental regime

| 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 12$^{th}$ day) | Olive oil | | |
| IV | LN + GBEO High dose | 50 mg/kg (30 days) | 80 mg/kg (from 12$^{th}$ day) | Olive oil | | |
| V | LN + Silymarin (started from 12$^{th}$ day) | 50 mg/kg (30 days) | NA | Double distilled water | | |
| VI | LN + Olive oil (started from 12$^{th}$ day) | 50 mg/kg (30 days) | NA | Olive oil | | |

This ensured that the pellet, containing the cellular components of interest, remained undisturbed[11].

## 2.7 Oxidative Stress Parameters

### *2.7.1 Superoxide Dismutase Activity*

SOD activity was evaluated through the NBT-mediated superoxide generation and reduction inhibition assay. This method utilizes the competitive reduction of NBT by superoxide anions (O2•-) and superoxide dismutase. Higher SOD activity results in a enhanced inhibition of NBT reduction. Tissue homogenates were incubated using a reaction mixture buffered with phosphate at physiological pH of 7.4, NBT, $Na_2CO_3$, methionine, EDTA, riboflavin, and distilled water. The reaction mixture was incubated under light conditions for one hour under ambient condition. The extent of Nitroblue Tetrazolium (NBT) reduction was quantified by measuring absorbance at 560 nanometers. Enzyme activity was quantified as units formed per minute per milligram of protein (U/min/mg protein) using a standardized formula[12].

### *2.7.2 Lipid Peroxidation Assay*

Tissue LPO was assessed via the TBARS method, which quantifies the formation of Malondialdehyde (MDA), a byproduct of lipid peroxidation, by its reaction with TBA to form a pink chromogen. Briefly, tissue homogenates were incubated with a solution containing TBA, acetic acid, and a detergent (SDS) at 95°C for 1 hour. Following incubation, the reaction components were extracted with n-butanol : Pyridine to isolate the MDA-TBA chromogen. Light absorption of the chromogen was quantified at its peak wavelength of 532 nanometers (532 nm). The LPO level was determined by calculating the tissue concentration of MDA, expressed as nanomoles MDA formed per milligram of tissue (nmol MDA/mg tissue) using a standardized molar extinction coefficient[13].

### *2.7.3 Reduced Glutathione Assay*

The level of GSH was measured and analyzed using the Ellman's method. The method utilizes the reaction between GSH and Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid), DTNB) to generate a measurable yellow chromophore. Briefly, tissue homogenates were incubated in a reaction mixture with phosphate buffer (pH 7.0), DTNB, and EDTA. Light absorption by the formed DTNB-GSH adduct was quantified at its optimal wavelength of 412 nm. The GSH concentration in the samples was measured and reported as micromolar GSH per milligram of protein (µmol GSH/mg protein) using a standard formula and the molar extinction coefficient of the DTNB-GSH complex[14].

### *2.7.4 Glutathione Peroxidase Activity*

GPx activity was evaluated indirectly by measuring the rate of GSSG (NADPH-dependent conversion of oxidized glutathione) formation back to GSH by GR (Glutathione Reductase). The downregulation of reading at 340 nm, corresponding to NADPH oxidation, reflects GPx activity. Briefly, tissue homogenates were incubated with a assay mixture comprised Tris-HCl buffer (pH 7.4), GSH, sodium azide (to inhibit catalase activity), Hydrogen Peroxide ($H_2O_2$), and distilled water. After incubation, the enzymatic reaction was terminated by addition of TCA (Trichloroacetic Acid), and the proteins were precipitated

by centrifugation. The remaining GSH in the supernatant was then condensed with Ellman's reagent DTNB to yield a yellow-coloured compound with a maximal absorbance at 420 nm, was quantified spectrophotometrically. The absorbance data was then used to calculate GPx activity, expressed as µg GSH consumed per minute per milligram of protein (µg GSH utilized/min/mg protein) using a standard formula[15].

### *2.7.5 Catalase Activity Assay*

CAT activity was determined by monitoring the kinetic disappearance of $H_2O_2$ at its characteristic absorbance peak of 240 nm. Tissue homogenates were incubated with a $H_2O_2$ solution buffered with phosphate buffer (pH 7.4). The decrease in absorbance at 240 nm over time, which correlates directly to the $H_2O_2$ consumption by CAT activity, was employed to quantify enzyme activity. The molar extinction coefficient of $H_2O_2$ (4.6 $M^{-1}cm^{-1}$) and a standard equation were used to calculate the activity, expressed as micromoles of H2O2 consumed per minute per milligram of protein (µmol $H_2O_2$/min/mg protein)[16].

### *2.7.6 Glutathione-S-transferase Assay*

GST activity was quantified spectrophotometrically by monitoring the formation of a GSH-CDNB conjugate, a reaction catalyzed by GST enzymes. Briefly, the reaction system was comprised of GSH, CDNB and phosphate buffer (pH 7.4) to maintain a suitable physiological environment for the reaction. Following the addition of tissue homogenate, the reaction was initiated, and the resulting increase in absorbance at 340 nm was continuously measured over time. The rate of absorbance increase reflects GST activity, as it catalyses the formation of a coloured product (GSH-CDNB conjugate). GST activity was calculated based on the rate of CDNB-GSH adduct formation, quantified spectrophotometrically using the adduct's molar extinction coefficient (ε) and a standard formula. The activity is expressed in millimoles of CDNB-GSH conjugate formed per minute per milligram of protein (mmol CDNB-GSH conjugate/min/mg protein)[17].

## 3. Results

This study explored the consequences of lead nitrate exposure and various treatment interventions on male reproductive health in different experimental groups. Authors noticed the effectiveness of Garlic Bulb Essential Oil (GBEO) at specific doses to counteract lead nitrate's toxic effects, on a specific set of oxidative stress markers, including SOD, GSH, GPx, GST, LPO, and CAT. One way analysis of variance (ANOVA) followed by Tukey's post-hoc test was employed to determine statistically significant differences between groups. A significance level of $p < 0.05$, $< 0.01$, and $< 0.001$ was employed to assess statistical significance.

### 3.1 SOD Activity

Our findings underscore the connection between oxidative stress and Superoxide Dismutase (SOD) activity, particularly for testicular tissues. Lead exposure alone (Group II) caused a marked reduction in SOD concentration relative to the control group ($p < 0.01$), indicating disruption of natural antioxidant defences within testes. The high dose of garlic oil (GBEO) in Group IV demonstrated a upregulation of SOD enzymatic activity ($p < 0.01$) compared to lead exposure subjects. This suggests its effectiveness in countering lead-induced SOD reduction. Notably, the standard drug Silymarin (Group V) and olive oil treated animals (Group VI) showed no statistically significant changes when contrasted to lead administrated group ($p > 0.05$). Interestingly, a dose-dependent response of GBEO on SOD activity was observed. The high dose group (Group IV) exhibited a significantly greater effect ($p < 0.05$) than the group treated with low dose (Group III) and the olive oil group VI. Comparisons between the low dose GBEO group, Silymarin, and olive oil (Groups III, V, and VI). As shown in Figure 2A: graph A, the differences between the groups were not statistically significant ($p > 0.05$).

### 3.2 Lipid Peroxidation (LPO)

Results show significant alterations in LPO activity across treatment groups. In group II mice lead exposure caused a dramatic increase in LPO activity against the control group ($p < 0.001$), signifying substantial oxidative stress. GBEO in both low and high dosages (Groups III and IV) offered protection against lead-induced LPO increase, demonstrates a statistically significant difference from the lead subjected Group II animals ($p < 0.05$ and $p < 0.001$, respectively). This indicates a dose-dependent response of GBEO. On comparison with lead nitrate treated animals ($p < 0.01$), the standard drug Silymarin (Group V) also displayed effectiveness in reducing LPO activity.

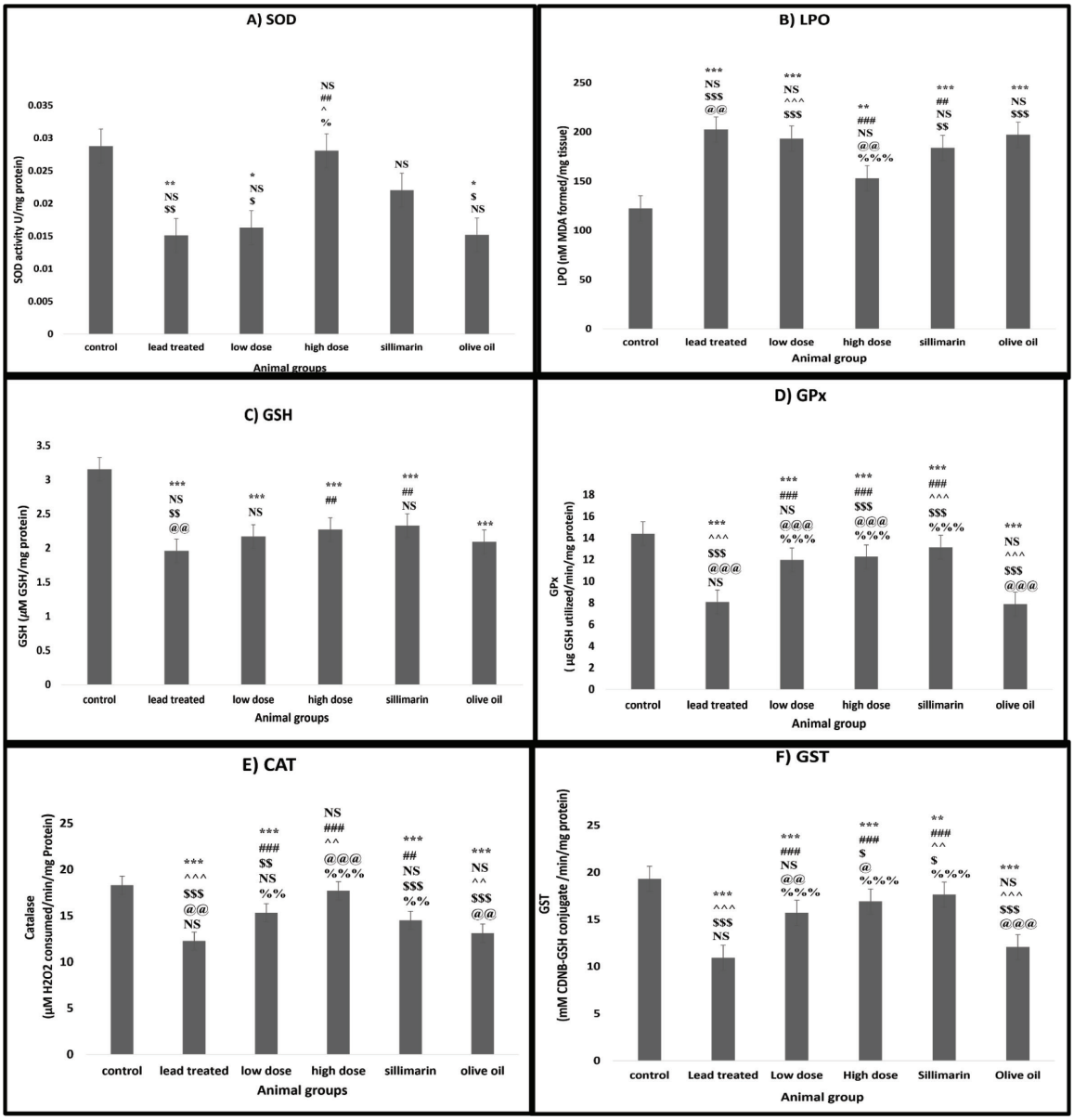



**Figure 2.** Graph A-F show the testicular redox and biochemical parameters between the different treatment groups. Data are represented as mean ± SEM with a sample size of n=6 per group. Statistical significance is indicated at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels. NS stands for non-significant. 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), ($p < 0.05$, $$p < 0.01$, $$p<0.001compared to GroupIV), (@$p < 0.05$, @@$p < 0.01$, @@@$p < 0.001$ compared to Group V), and (%$p < 0.05$, %%$p < 0.01$, %%%$p < 0.001$ compared to Group VI).

However, with olive oil alone (Group VI) no significant effect was detected ($p > 0.05$). The group treated with high dose of GBEO exhibited a statistically relevant recovery in LPO ($p<0.001$) over both the low dose group (Group III) and Silymarin Group V. No substantial recovery in LPO was seen when Group III, V, and VI were compared with each other, as shown in Figure 2, Graph B.

## 3.3 GSH Levels

Toxicant treated Group II showed a marked decrease in GSH level in comparison to group with no treatment ($p < 0.001$), indicating disruptive effect on GSH homeostasis, a key component of the antioxidant defence system. Conversely, in all treatment groups which received lead nitrate alongside protective agents (from Groups III to

Group V) GSH content was found to be significantly elevated p<0.05 when contrasted to the lead alone subjected group, suggesting protection against lead-induced GSH depletion. Notably, olive oil alone (Group VI) did not exert any significant protective effect (p > 0.05), proving it a better carrier oil for studying effect of GBEO. Further analysis showed no notable variations in GSH levels were observed among the other groups that received GBEO, Silymarin, or olive oil (Groups III, IV, V, and VI). However, the high dose GBEO group (Group IV) demonstrated a potential advantage p < 0.05 over olive oil alone Group VI. These findings suggest that both GBEO and Silymarin offer protection against lead-induced GSH depletion, Silymarin may be more effective (Figure 2; Graph C).

### 3.4 GPx Level

Consistent with the observed oxidative stress pattern, toxicated group (Group II) resulted in a remarkably (p < 0.001) suppressed GPX activity compare to the non-treated group. Other groups administered with lead nitrate alongside protective agents (Groups III-V) displayed statistically significant differences against the lead-exposed group (p < 0.05), suggesting protection against lead-induced GPX activity reduction. Notably, olive oil alone (Group VI) did not exert any significant protective effect. Even no significant effect was detected between the groups received GBEO or silymarin (Groups III, IV, and V, p > 0.05). The high dose GBEO group (Group IV) demonstrated a potential advantage (p < 0.05) over olive oil Group VI. These findings suggest that both GBEO and silymarin may offer protection against Lead-induced GPX activity reduction (Figure 2; Graph D).

### 3.5 Catalase Activity

The comparison of catalase activity across the treatment groups demonstrates the protective effects of Garlic Bulb Essential Oil (GBEO) contrary to lead nitrate-caused oxidative stress in Swiss albino mice. Lead nitrate-treated group displayed a notable reduction in catalase activity when compared to the control group, with a statistically significant difference (P < 0.001), confirming oxidative stress. Treatment with GBEO (both doses) and Silymarin significantly increased catalase activity compared to Group II, P < 0.001, while alone olive oil treated animals showed no significant change with p value more than 0.05. Low dose GBEO treated subjects showed a marked decrease in catalase activity compared to the healthy group animals, P < 0.001, though it improved the CAT level relative to lead treated group, P < 0.001 reflecting that low dose may offer some protection. Catalase activity in Group III was significantly lower than in Group IV with P value less than 0.01, suggesting that high dose provided better protection. There was no discernible difference between Group III and Group V (P > 0.05) means silymarin and low dose offers almost same protection. Group treated with high dose of garlic oil showed no notable difference from the control (P > 0.05) suggesting an effective reversal of catalase activity. These finding suggest that high dose garlic oil can significantly mitigate lead nitrate-induced oxidative stress in comparison to the low dose.

### 3.6 GST Level

Lead nitrate exposure resulted in a statistically (p < 0.001) notable decline in GST content against the group with healthy subjects (p < 0.001), suggesting its disruption of the antioxidant defense system. Supplementation with both low and high doses of GBEO alongside lead nitrate (Groups III and IV) led to substantial recovery of GST activity when measured against lead-exposed group (p < 0.001 for both doses). Similarly, treatment with Silymarin (Group V) significantly increased GST activity in contrast to Group II (p < 0.001). Notably, olive oil administration (Group VI) did not show significant protective effect (p > 0.05). Further analysis revealed a dose-dependent response within the GBEO treatment groups. The low dose of GBEO (Group III) exhibited significantly higher GST measurements compared to the group administered with olive oil (Group VI, p < 0.001), but lower level of GST was noticed in contrast to silymarin Group V (p < 0.01). Whereas, the group given with high dose of GBEO (Group IV) demonstrated the highest GST activity, with no significant differences with the silymarin Group V (p > 0.05). These findings suggest that both GBEO doses could be effective in mitigating lead-induced GST activity reduction as silymarin (Figure 2; Graph F).

## 4. Discussion

This study build is upon the well-established connection between lead exposure and oxidative stress. Lead is known to disrupt antioxidant defence mechanisms, leading to the surplus of Reactive Oxygen Species (ROS), which consequently damages cellular structures, including

the male reproductive system. Our findings provide further evidence of this disruption, as seen in significant alterations in oxidative stress markers such as Superoxide Dismutase (SOD), Lipid Peroxidation (LPO), Glutathione (GSH), Catalase (CAT), Glutathione S-Transferase (GST), and Glutathione Peroxidase (GPx) in lead-exposed male mice. These changes are consistent with previous studies that have documented lead's deleterious effects on antioxidant enzymes and its role in inducing oxidative stress, ultimately contributing to reproductive toxicity[18].

Specifically, the marked decrease in SOD activity in the lead-exposed group aligns with existing literature that shows lead's inhibitory effect on this key antioxidant enzyme. SOD plays a crucial role in dismutation of superoxide radicals, and its depletion indicates weakened oxidative defenses[19,20]. However, the observed upregulation of SOD activity in the group treated with high-dose Garlic Bulb Essential Oil (GBEO) supports previous findings on the antioxidant potential of garlic compounds, particularly in restoring SOD activity in oxidative stress conditions[21,22]. The dose-dependent effect of GBEO on SOD activity, with a more pronounced response at higher doses, suggests that GBEO may offer potent protective effects. This warrants further exploration of optimization of its therapeutic dosage for mitigation of oxidative damage.

Lipid Peroxidation (LPO) is another key indicator of oxidative stress, and the significant increase in LPO in the lead-treated group demonstrates the extent of membrane damage due to ROS accumulation[4,16,19]. Our results show that both low and high dose of GBEO reduced LPO levels. This finding corroborates with the previous studies that highlighted garlic's ability to reduce lipid peroxidation in Swiss albino male mice[15]. Notably, the high-dose GBEO group exhibited greater protection against LPO than the standard antioxidant drug, Silymarin, reinforcing the potential of GBEO as a natural therapeutic agent for managing lead-induced toxicity.

Similarly, the observed reduction in GSH levels in lead-exposed mice is consistent with prior research that demonstrates how lead exposure depletes GSH, a critical intracellular antioxidant involved in neutralization of ROS[20,21,23]. The ability of GBEO to significantly restore GSH levels in a dose-dependent manner further supports its role in enhancing cellular antioxidant defenses[24]. While both GBEO and Silymarin offered protection against GSH depletion, the comparable efficacy of high-dose GBEO to Silymarin suggests that GBEO could serve as an alternative or complementary treatment strategy to traditional antioxidants.

Regarding GPx and GST activity, the reduction in these enzymes in the lead-exposed group aligns with the established effects of lead on antioxidant enzyme activity, where lead exposure impairs enzymatic function, leading to oxidative damage[20]. The recovery of GPx and GST activity in groups treated with GBEO, especially at higher doses, suggests that GBEO may help to restore enzymatic balance, as seen in previous studies on garlic's effects in oxidative stress models[19]. The dose-dependent response observed for GST activity, in particular, emphasizes the importance of optimization of GBEO dosage to achieve the best therapeutic outcomes.

Catalase (CAT) activity was similarly reduced in lead-exposed mice, indicating compromised oxidative defense mechanisms[22,23]. The significant restoration of CAT activity in GBEO-treated groups supports the notion that garlic's bioactive components can scavenge ROS and can restore antioxidant enzyme levels[25-27]. The dose-dependent improvement in CAT activity further reinforces the need for dose optimization to maximize the therapeutic benefits of GBEO.

This study also acknowledges several limitations, including the relatively small sample size, which may limit the generalizability of the results, and the fixed dose range, which may not fully capture the optimal dosage for GBEO's protective effects. Future research should investigate a broader range of doses, include larger sample sizes, and employ mechanistic studies, to fully elucidate how GBEO exerts its protective effects, whether through direct ROS scavenging or modulation of antioxidant enzymes. Thus, the findings of this study suggest that possibly GBEO could be a promising therapeutic agent for mitigating the detrimental effects of lead exposure. Further research is warranted to elucidate the underlying mechanisms and to explore the potential clinical applications of GBEO in the management of lead toxicity.

## 5. Conclusion

Rigorous experimentation and analysis revealed that GBEO, especially at higher dose, effectively combat lead-induced oxidative stress by enhancing antioxidant enzyme activity and maintaining glutathione levels. These results illuminate the potential of GBEO as a natural remedy for lead toxicity, offering valuable insights for the development of novel preventive and therapeutic approaches. To fully

understand the underlying molecular pathways through which GBEO mediates its protective actions and assess its potential for clinical application, further research is warranted. However, further studies are warranted to optimize the dosing regimen for maximizing its protective efficacy against lead toxicity.

## 6. Acknowledgements

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## 7. Author Considerations

The entire experimental work was conceptualized by T. Aggarwal, A. Rajpoot, and V. Sharma. T. Aggarwal has contributed most of the manuscript writing, with additional support provided from V. Sharma. The study plan was crafted by V. Sharma. **T**. Aggarwal performed the experiments, T. Aggarwal and A. Rajpoot analysed the data (descriptive and statistical analysis), and interpreted the results (Straightforward and emphasizes specific tasks).

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