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Alleviation of Lead Nitrate-Induced Nephrotoxicity by Garlic Essential Oil in Albino Mice: A Comprehensive Study on Inflammation and Genotoxicity

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Abstract

Lead (Pb) is a hazardous environmental contaminant that has been linked to renal impairments when consumed. As a part of this study, we explored garlic essential oil's potential therapeutic uses against Lead nitrate $(PbNo_3)_2$-mediated nephrotoxicity. Six experimental groups of six mice each were employed and groupings were further formed as - Control, $(PbNo_3)_2$, $(PbNo_3)_2$ + low dosage of garlic essential oil, $(PbNo_3)_2$ + high dosage of garlic essential oil, $(PbNo_3)_2$ + Silymarin and $(PbNo_3)_2$ + olive oil groups. $(PbNo_3)_2$ treatment enhanced the progression of renal inflammation by activating the NF-κB signaling pathway which in turn increased the levels of pro-inflammatory mediators like TNF-α, IFN-γ and IL-6. With that, the reduction of IL-10 cytokine level was also noticed. Interestingly, oral administration of garlic essential oil to some extent improved biological functioning since it was able to substantially decreased the inflammatory processes in the renal tissue. The Lead nitrate intoxicated group also showed substantially higher parameters of the comet assay, including the percentage of DNA in the tail, tail length and the moment of the tail, demonstrating the degree of genetic instability caused by heavy metal exposure. However, garlic essential oil to some extent was able to recover these parameters and ensure molecular repair.

Keywords: Comet Assay, Garlic Essential Oil, Interleukins, Lead Nitrate, Nephrotoxicity

List of Abbreviations: GEO: Garlic Essential Oil, $(PbNo_3)_2$: Lead (II) nitrate, Pb^{2+}: Lead

1. Introduction

Expanding population, industrialisation as well as technological improvements all contribute to an increase in environmental pollution. Heavy metals are among the most dangerous types of contaminants. They possess prolonged biological half-lives. Their persistence, non-biodegradability and the potential for bioaccumulation in living creature tissues can cause serious health consequences and financial losses[1]. Moreover, these noxious metals are known to have the ability to harm the primary target organs such as the endocrine, digestive, cardiovascular, skeletal, pulmonary, hepatic and kidney systems[2]. The focus of this study is on Lead (Pb) toxicity

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in particular because it has been widely used in industrial sectors including those that manufacture paint, ceramics, plastics and autos. Also, it has been confirmed by various studies that Lead accumulation in the body could be linked to a variety of health issues, including nephrotoxicity, neurotoxicity, immunological issues, gastrointestinal diseases, haematological diseases and reproductive issues[3]. One of the organs most susceptible to Lead poisoning is the kidneys and the inhibition of Cytosolic Calcium Ion (Ca^{2+}) influx confirms this vulnerability[4]. A high Lead concentration sets off several physiological reactions that produce pro-inflammatory cytokines and potent inflammatory mediators like Tumour Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6) and Nitric Oxide (NO). Excessive emergence of these mediators may result in oxidative stress and may lead to several harmful immunological health effects. Chelating therapy is used to speed up the removal of Lead deposits in cases of extreme Lead poisoning. The effectiveness of chelating therapy is however constrained by several side effects including transitory leucopenia, hypotension, transient thrombocytopenia, rash, enuresis, Zinc insufficiency and cellular redistribution of lead[5]. Lead additionally causes the release of pro-inflammatory indicators which in turn stimulates apoptotic signalling and promotes the inflammatory response (Figure 1)[6]. Thus, the need for therapies from multiple natural sources to deal with Lead-associated health complications could be met. Since ancient times, all civilisations have employed medicinal plants as a source of remedies. Due to their organic origins, affordability and lack of adverse outcomes, there has been a recent surge of interest in using the biological activity of various ayurvedic medicinal herbs[7]. The bio-prospecting of new plant-derived therapeutics and the rising cost of prescription medications that are needed to maintain individual health and well-being have both increased interest in medicinal plants as a reemerging form of healthcare[8]. Garlic (*Allium sativum*) a common perennial aromatic plant having tremendous meditative properties plays a role in attenuating xenobiotic compounds like toxic metal[9]. According to the accumulated evidence, garlic essential oil also comprises an extensive spectrum of pharmacological properties for example, anti-mutagenic, antioxidant, anti-inflammatory, antimicrobial, neuro-protective, anti-proliferative, hepato-protective, reno-protective and anti-diabetic activities[10]. In accordance with previously conducted research, garlic essential oil was proved to be successful in lowering the toxicity of pesticides, arsenic and strychnine[11]. Moreover, a previous study unveiled that garlic is effective against the cognitive dysfunction of mice when exposed to Lead nitrate[12]. Based on Unaegbu *et al*., research, the consumption of additional vegetables and plants in the diet has been linked fairly to decrease the harmful effects of several pollutants including heavy metals[13]. To the best extent of the literature survey, no

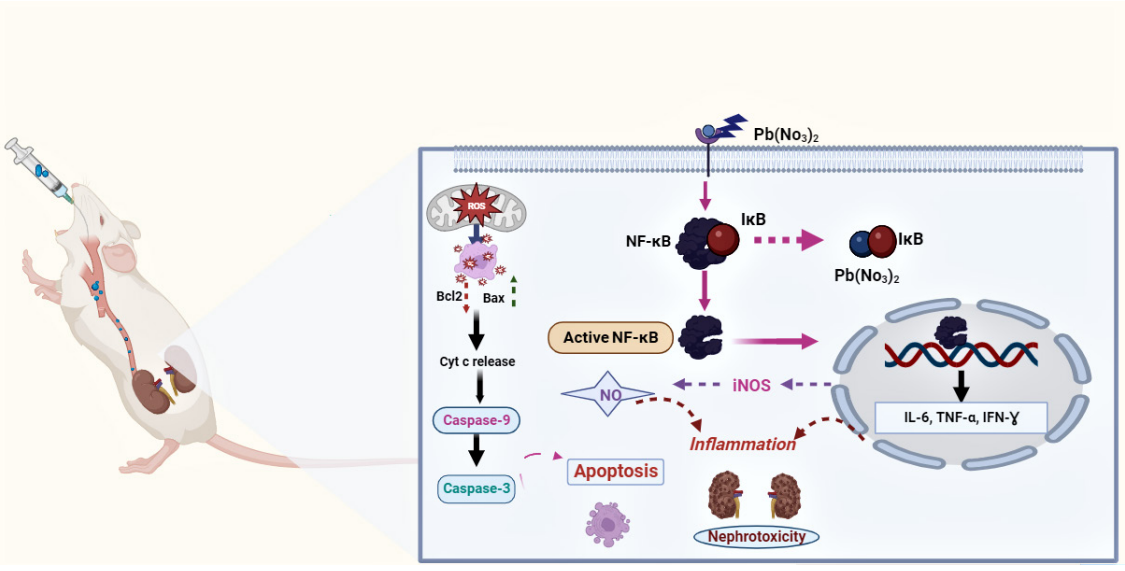


Figure 1. A hypothetical mechanism of Lead nitrate ($Pb(NO_3)_2$ induction nephrotoxicity involving inflammation, apoptosis and genetic instability.

studies or discoveries have been noticed regarding the mechanisms behind the action of garlic essential oil against Lead nitrate-induced renal impairment in mice. The current investigation aims to ascertain how the administration of garlic essential oil influences Lead nitrate-induced genotoxicity and inflammation in mice renal tissues. To achieve this, an evaluation of different inflammatory parameters were performed and along with this, an alkaline comet assay was also studied to figure out whether or not Lead nitrate causes DNA breakage in the renal tissue of mice. Hence, this study was designed to determine whether garlic essential oil might have any preventive benefits on immunological and molecular DNA damage in Lead-nitrate-intoxicated mice.

2. Materials and Methods

2.1 Chemicals

All implemented reagents in the current work were of laboratory benchmark and purchased from putative research facilities and corporations, Sisco Research Laboratories Pvt. Ltd. (SRL) India, Merck, Ranbaxy, HiMedia and SIGMA. The ELISA kits of nuclear factor Kappa B subunit p105 (NF-κB p105), mouse tumour necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), interleukin-6 (IL-6), interleukin-10 (IL-10), Nitric oxide (NO) were purchased from Invitrogen Thermo Fisher.

2.2 Experimental Plant

The raw fresh garlic bulbs were selected arbitrarily from the Banasthali Vidyapith, Rajasthan's native lands and were further utilised in extracting the essential oil.

2.3 Preparation of Essential Oil

Hydrodistillation was the method used for obtaining the fresh garlic bulb's essential oil. The 250-300 g fresh garlic bulbs were thoroughly scrubbed and scraped before hydrodistillation was performed. After chopping the bulbs, distilled water was stirred in to make a fine, homogenous paste which was subsequently mixed with boiling water in a flask with a flat bottom. A "Clevenger apparatus" (ASGI) connected to a flask containing raw garlic extract was used to obtain the essential oil. Further, the flask was heated for 4-5 hours at 60-70 °C until every last drop of the essential oil was retrieved. An oil-water separator was used to separate the condensed vapour. Then, freshly collected essential oil was used for the subsequent experiments.

2.4 Animal Acquisition

All parts of animal procedures were conducted in accordance with the strict rules established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Two to three-month-old male Swiss albino mice (*Mus musculus*), were procured from Lala Lajpat Rai University of Veterinary and Animal Science, located in Hisar, Haryana, India.

2.5 Animal Preservation

Animals were kept in an enclosure having a pathogen-free area with well-maintained air conditioning, temperature of 23-25 °C, humidity of 50 ± 15 % and housed with an adequate pellet unrestricted water and food consumption. The mice were acclimated for a set amount of time-roughly one week before the studies.

2.6 Dosage Approach

All the doses in the experimental duration were administered via oral gavage needle.

2.7 Experimental Strategy

A total of thirty-six male Swiss albino mice were selected for the current investigation and divided into six groups of six mice each. The animals in Group I served as the control group (untreated) and were given double-distilled water. Group II mice were treated with Lead nitrate. This group was further divided into five subgroups. All the mice of the subgroups received 50 mg/kg of Lead nitrate for 30 days. Moreover, the low and high doses of the garlic essential oil, the standard dose of silymarin and the dose of olive oil were also given to the animals of Groups II b, II c, II d and II e except for Group II a from 12th day to the end of the experiment. A detailed description of these experimental groups is provided in Figure 2. The dose of Lead nitrate was prepared in double distilled water while the Garlic essential oil doses were prepared in olive oil. The study took 30 days to complete. The selection of the Lead nitrate dose was based on previous findings from a study by our lab[12]. The determinations of dosages for garlic essential oil were decided according to the previously published reports[11,14].

2.8 Collection of the Renal Tissues

After completion of the study, the mice of various groups were sacrificed through cervical dislocation therapy. Their kidneys were taken out and washed in a cold saline solution (0.9%) to eliminate any remaining blood. The renal tissues were then further blotted off and weighed.

2.9 Homogenate Preparation

Using a homogeniser (YS1-471 York Tissue Homogeniser), renal tissues were homogenised (10% w/v) in 0.1M sodium phosphate buffer (pH=7.4). The mixture was subsequently spun at 10,000 rpm for 10-15 min at 4°C.

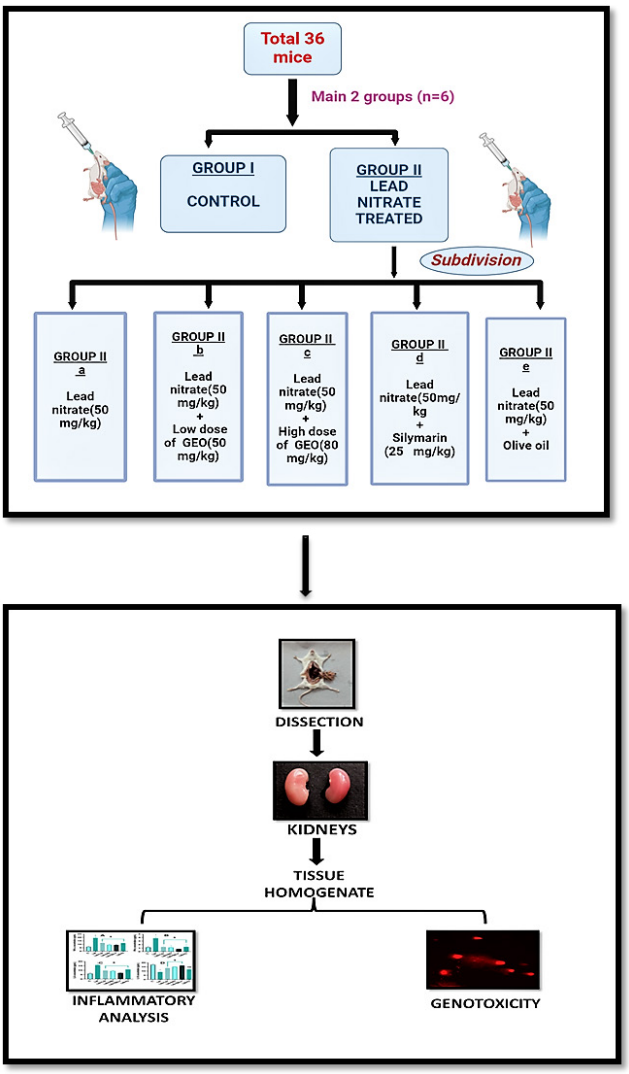


Figure 2. Schematic representation of complete study design.

After that, the supernatant was used to study various parameters related to inflammation.

2.10 Renal Inflammatory Biomarkers

The ELISA kits were procured from Invitrogen by Life Technologies (Thermo Fisher) and were used as per the directions given by the manufacturer for estimating the inflammatory parameters in the renal tissue of mice. NF-κBp65 (Total) ELISA Kit (Catalogue Number: 85-86081); TNF-α ELISA Kit (Catalogue Number: KMC3011); IFN-γ ELISA Kit (Catalogue Number: BMS228); IL-6 ELISA Kit (Catalogue Number: A35573); IL-10 ELISA Kit (Catalogue Number: BMS614INST); Nitric oxide (NO) levels were determined with the calorimetric assay kit (Elabscience, Catalogue Number: E-BC-K035-M).

2.11 DNA Damage (Comet Assay)

To determine the level of DNA damage in the renal tissue by Lead nitrate intoxication and to check the anti-genotoxic potential of the garlic essential oil, the DNA Comet Assay (alkaline variation) was performed in accordance with Recio *et al.*, approach with certain modifications[15]. The tissues were taken out of the animal right after sacrifice. Following the McKelvey-Martin *et al.*, suspensions of single cells were obtained[16]. Following that, renal tissues were rinsed in a cold medium (RPMI medium 1640, 15% DMSO, 1.8% NaCl). They were washed and then sliced into little pieces before being put in a petri dish with 1.5 mL medium. After transferring the sample to a microfuge tube, the larger tissue fragments were allowed to settle and the supernatant containing the cell suspension was subsequently centrifuged at 1,000 rpm for 5 minutes at 4°C and collected. The pellet was then re-suspended in 500 µl of the medium after the supernatant had been discarded. Further, on the slides that had already been covered with 1.5% normal melting agarose, the cell suspension was then blended with 0.65% low melting agarose solution. To remove the cell membranes, slides were treated at 4°C for 50 min in cold lysis buffer (1% Triton X-100, 2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 10% DMSO, pH 10). The slides were then put in a horizontal electrophoresis device and treated for 25 minutes in electrophoresis buffer (5 M NaCl, 0.2 M EDTA, pH 10) to enable the DNA to relax and reveal alkali-sensitive sites. Then, electrophoresis was performed at 25 V and 300 mA for about 25 minutes. Coated slides were then rinsed with

methanol and neutralised using Tris buffer (0.4 M Trizma base, pH 7.5). Ethidium bromide (EtBr) was used to stain the slides. All of the aforementioned measures were taken to complete darkness to minimise DNA deterioration. Additionally, the analysis was performed on 50 cells for each group. A fluorescence microscope (Leica, Germany) fitted with the proper filters (N2.1, excitation wavelengths of λ510–λ560 nm and wavelengths of 590 nm were emitted) was used for taking cell images. For that, at least 100 photos of DNA comets were taken from each preparation. The following parameters were then calculated: "the percentage of DNA in the tail" (%DNA in tail), tail length and the "Olive Tail Moment" (OTM). The parameter "percentage of DNA in the tail of a comet" is the number of single-stranded fragments that were formed as a result of breaks and the realisation of alkaline-DNA segments and migrated towards the anode during electrophoresis. Tail length is the length of DNA migration. "Olive tail moment" is the distance from the centre of the nucleus to the centre of the comet's tail density multiplied by "% DNA in the comet's tail"[17]. Then the images from the microscope were sent to the COMETSCORE analyser software 2.0 via a computer connection.

2.12 Statistical Analysis

Data were subjected to statistical investigation to test the significance between means of different groups. For every group, the data is shown as the Mean ± SEM. Using the Statistical Package for the Social Sciences (SPSS) software, version 20.0, one-way Analysis of Variance (ANOVA) test and Tukey's post hoc test to compare the control and treatment groups statistically. To identify statistically significant variations between the means under comparison, a significance threshold of $p<0.05$ was employed. All calculations were carried out using Prism software, version 4.02, developed by Graph Pad Software in San Diego, CA, USA.

3. Results

3.1 Mitigating Lead Nitrate-Induced Inflammation with Garlic Essential Oil

3.1.1 NF-κB (Nuclear Factor Kappa-B)

Lead nitrate caused a substantial increase ($P<0.05$) in activation of NF-κB from 3.246 to 13.28 at 450nm as compared to control group I of mice. The groups of mice that received garlic essential oil's low dose, high dose, standard dose (silymarin) and vehicle olive oil attenuated the NF-κB levels significantly to the 4.56, 4.333, 3.893 and 5.73 extents at 450 nm when compared to the Lead nitrate intoxicated group (13.28) (Figure 3).

Abbreviations: C stands for Control group, LN stands for Lead nitrate group, LN+LD stands for Lead Nitrate + Low Dose of garlic essential oil, LN+HD stands for Lead Nitrate + High Dose of garlic essential oil, LN+SILY stands for the Lead Nitrate + Standard Dose Silymarin, LN+O stands for Lead Nitrate + vehicle olive oil.

3.1.2 Estimation of the Cytokine Levels in the Renal Tissue

3.1.3 TNF-alpha (Tumor Necrosis Factor- alpha)

In the present study, TNF-alpha levels were significantly elevated in the Lead nitrate-treated group (19.29pg/mL) when compared to the control group (6.48 pg/mL). In the case of garlic essential oil low dose, high dose, standard

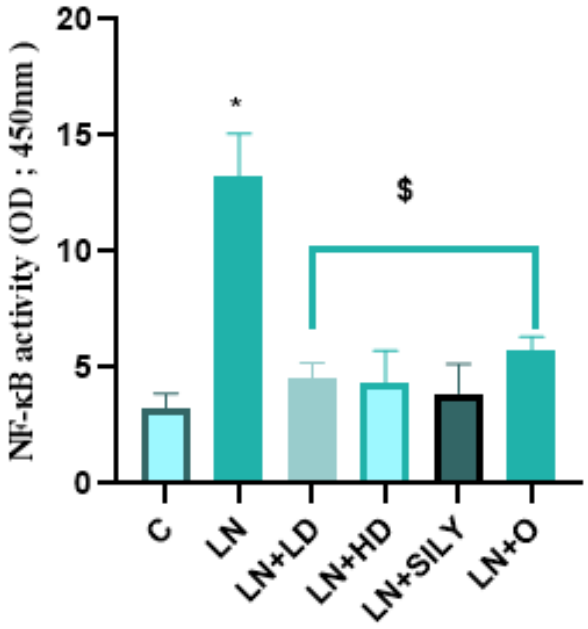


Figure 3. Histogram depicting the changes in the levels of NF-κB (transcription factor) after the various treatments. The values were illustrated as mean ± SEM (n = 6), which are statistically favourable at $p<0.05$.*$p<0.05$ depicts the LN (II a) relative to the group under C (I). $ $p<0.05$ depicts the LN (II a) in comparison with LN + LD (II b), LN+HD (II c), LN + Sily (II d) and with LN + O (II e) respectively.

compound dose and vehicle olive oil groups the TNF-alpha levels got normalised significantly up to 12.11 pg/mL, 9.25 pg/mL, 9.14 pg/mL and 11.82 pg/mL when compared to the toxicated group 19.29 pg/mL (Figure 4).

3.1.4 IFN-γ (Interferon-Gamma)

In the present study, IFN-γ levels were significantly elevated in the Lead nitrate-treated group (3.81 pg/mL) when compared to the control group (1.22 pg/mL). In the case of garlic essential oil low dose, high dose, standard compound dose and vehicle olive oil groups the IFN-γ levels got normalised significantly up to 1.33 pg/mL, 1.18 pg/mL, 0.77 pg/mL and 1.35 pg/mL when compared to the toxicated group 3.81 pg/mL (Figure 4).

3.1.5 IL-6 (Interleukin-6)

In the present study, IL-6 level got significantly elevated in the Lead nitrate-treated group (15.91 pg/mL) when compared to the control group (6.28 pg/mL). In the case of garlic essential oil low dose, high dose, standard dose and vehicle olive oil groups, the IL-6 levels got normalised significantly up to 9.73 pg/mL, 9.23 pg/mL, 7.03 pg/mL and 10.9 pg/mL when compared to the toxicated group 15.91 pg/mL.

3.1.6 IL-10 (Interleukin-10)

In the present study, IL-10 level significantly declined in the Lead nitrate-treated group (8.92 pg/mL) when compared to the control group (17.6 pg/mL). In the case of garlic essential oil low-dose, high-dose and standard compound-dose groups, the IL-10 levels elevated significantly up to 13.6 pg/mL, 15.1 pg/mL and 16.50 pg/mL when compared to the toxicated group 8.92 pg/mL. In the case of the olive oil group, the IL-10 level enhanced non-significantly up to 12.01 pg/mL when compared to the toxicated group at 8.92 pg/mL (Figure 4).

Abbreviations: C stands for Control group, LN stands for Lead Nitrate group, LN+LD stands for Lead Nitrate + Low Dose of garlic essential oil, LN+HD stands for Lead Nitrate + High Dose of garlic essential oil, LN+SILY stands for the Lead Nitrate + standard dose Silymarin, LN+O stands for Lead Nitrate + vehicle olive oil.

3.1.7 NO (Nitric Oxide)

The NO is a major signalling molecule that plays a vital role in oxidative stress and inflammation. During

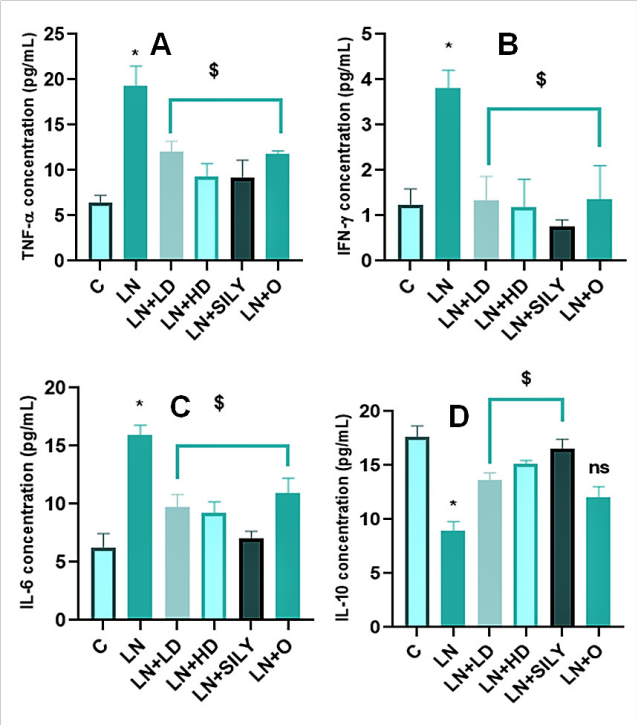


Figure 4. Histogram (A-D) depicting the changes in the levels of cytokines after the various treatments. The values were illustrated as mean ± SEM (n=6), which are statistically favourable at $p<0.05$.*$p<0.05$ depicts the LN (II a) relative to the group under C (I). $ $p<0.05$ depicts the LN (II a) in comparison with LN + LD (II b), LN+HD (11 c), LN + Sily (II d) and with LN + O (II e) respectively.

stressful conditions, overproduction of NO could be seen which ultimately causes inflammation. In the present study, NO levels were found to be significantly elevated in the Lead nitrate-treated group (2.022 µmol/L) when compared to the control group (1.25 µmol/L). In the case of garlic essential oil low dose, high dose and standard compound groups the NO levels significantly declined to 1.1216 µmol/L, 0.7162 µmol/L and 0.6711 µmol/L when compared to the toxicated group 2.022 µmol/L. In the case of the olive oil group, the NO level reduced non-significantly up to 1.43 µmol/L when compared to the toxicated group 2.022 µmol/L (Figure 5).

Abbreviations: C stands for Control group, LN stands for Lead Nitrate group, LN+LD stands for Lead Nitrate+ Low Dose of garlic essential oil, LN+HD stands for Lead Nitrate+ High Dose of garlic essential oil, LN+SILY stands for the Lead nitrate+ standard dose Silymarin, LN+O stands for Lead nitrate + vehicle olive oil, ns stands for non-significant.

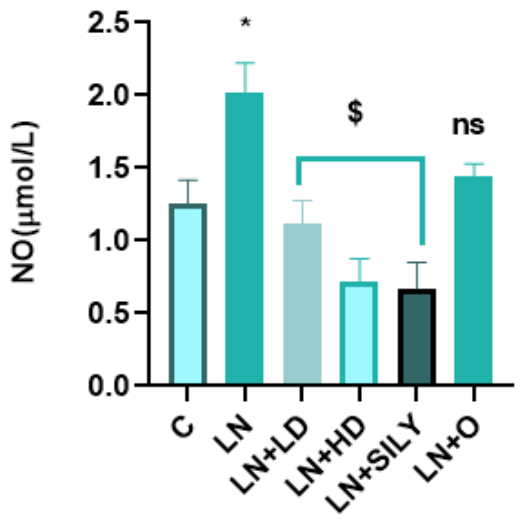


Figure 5. Histogram depicting the changes in the levels of nitric oxide after the various treatments. The values were illustrated as mean ± SEM (n=6), which are statistically favourable at $p<0.05$.* $p<0.05$ depicts the LN (II a) relative to the group under C (I). $ $p<0.05$ depicts the LN (II a) in comparison with LN + LD (II b), LN+HD (II c), LN + Sily (II d) and with LN + O (II e) respectively.

3.2 Mitigating Lead Nitrate-Induced Molecular Damage with Garlic Essential Oil

Comet results from the kidney tissues of mice in our study showed higher comet values, indicating damage from metal toxicity (Table 1). Mice given Lead nitrate showed this harm particularly (Figure 6). From the analysis of the comet assay results depicted well in Table 1, it was noticed that a proportion of the tail length, % DNA in the tail and tail moment were significantly increased ($p<0.05$) in the Lead nitrate treated group (5.72, 22.22, 127.53 respectively) when compared to the control group (3.01, 15.14, 45.61). This shows the toxic impact of Lead nitrate on the renal tissue of mice. However, in the case of garlic essential oil low and high doses, silymarin (standard dose) and olive oil groups the DNA damage parameters reduced significantly ($p<0.05$) when compared to the Lead nitrate intoxicated group. A high dose of garlic, however, has been noticed to be more beneficial than a lower dose in reducing these DNA damage metrics. This eventually brings us to the conclusion that using a high

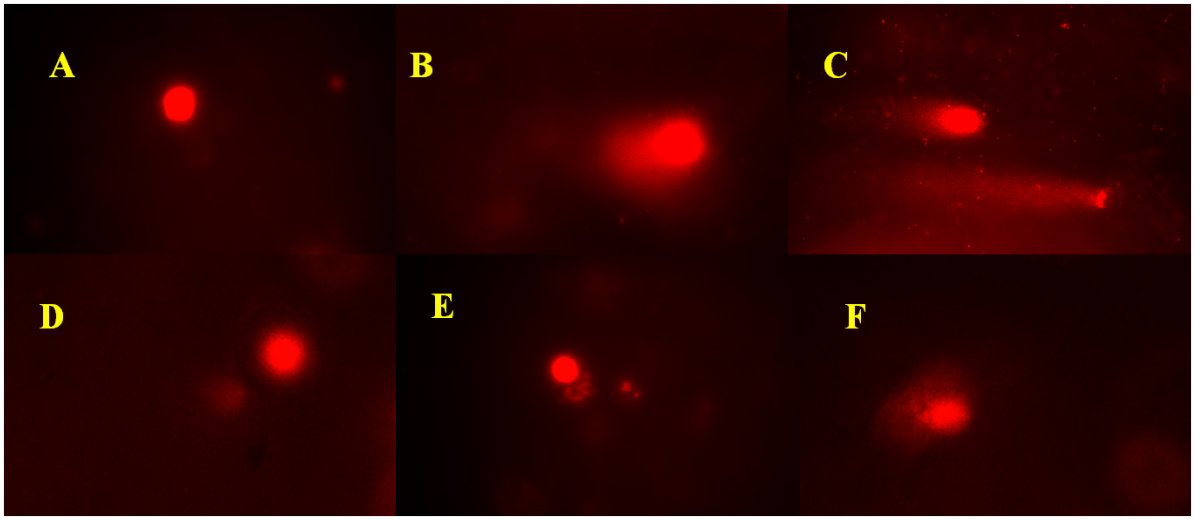


Figure 6. Here are some typical pictures of comet nuclei found in renal tissues. Comets-structures resembling tails-in diverse experimental groups are shown in different sections: **(A)** Control Group (I): This group has normal cells without any DNA damage, as seen by the lack of a tail-like appearance. **(B)** Lead-nitrate intoxicated group: This group's cells exhibit more comet-like formations, which may indicate severe cell damage. Cells in this group show increment in molecular damage when exposed to Lead nitrate **(C)** Lead nitrate + low dose of garlic essential oil: This group cells exhibit minor damage with some sort of recovery as well, as shown by the tail in the picture. **(D)** Lead nitrate + high dosage of essential oil of garlic: This group of cells exhibit less damage and a greater percentage of normal cells. **(E)** Lead nitrate + silymarin: These cells show almost normal morphologies with only minor DNA damage. **(F)** Lead nitrate + olive oil: This group displays some normal cell structures, albeit to a lesser extent.

Table 1. Effects of Lead nitrate and garlic essential oil on renal DNA damage parameters levels

Groups	Tail Length (px)	% DNA in Tail	Tail Moment (µm)
Group I	3.01±0.06	15.14±0.57	45.61±1.24
Group II a	5.72±0.19*	22.22±0.51*	127.53±7.08*
Group II b	3.56±0.08$	13.87±0.26$	49.42±1.71$
Group II c	3.28±0.06$	11.36±0.49$	37.26±1.07$
Group II d	3.12±0.09$	10.68±0.31$	33.21±1.98$
Group II e	3.82±0.26$	17.02±0.51$	65.45±6.33$

Table 1. Mean values of comet assay parameters in the renal tissues. The results underwent comparisons; Group II a (Lead nitrate treated) was compared (*variations) with the control mice (Group I), while comparisons with Group II a ($ signs) included low dose GEO (Group II b), high dose GEO (Group II c), Silymarin (Group II d), and olive oil treated mice (Group II e).

concentration of garlic essential oil could lower the parameters associated with DNA damage more effectively.

4. Discussion

Global public health is always threatened by exposure to heavy metals because of their widespread uses across several industrial and human-caused activities. Lead is an exceptionally dangerous heavy metal that impairs a variety of cellular and molecular processes of the body systems[19,20]. Among all the organs the renal system is considered one of the most important systems as it is primarily used for filtration and elimination of toxins. Previously conducted studies indicated that soft tissues like kidneys are the most prevalent site for Lead (Pb^{2+}) bio-accumulation[5]. This may cause nephrotoxic injuries that lead to oxidative stress and inflammatory reactions which are the most hazardous effects[5,21,22]. Further, these reactions can increase the release of pro-inflammatory mediators and decrease the effectiveness of anti-inflammatory cytokines which suggest complex processes underlie the substance's activities in the renal tissue of mice. Lead nitrate has been shown to activate NF-κB signalling pathways, which in turn encourages the production of pro-inflammatory mediators such as Nitric Oxide (NO), IFN-γ, NF-κB, TNF-α, and IL-6. Increased synthesis of these mediators is associated with inflammation that is ultimately implicated in cell survival, growth, apoptosis and also in many immunoreactions of the bodily systems[31,32] (Figure 1). Additionally, it has the ability to cause oxidative stress and decrease the effectiveness of cytokine-like IL-10. A statistically significant positive correlation value between the Lead nitrate bioaccumulation and alterations in cytokines levels is consistent with our ELISA data. NF-κB a crucial family of inducible nuclear transcription factors is linked to inflammatory alterations in a variety of human disorders, including liver, kidney, cardiac and brain[28]. Targeting NF-κB as a potential mechanism for modulating inflammation suggest that the medications are capable of inhibiting the NF-κB signaling pathway hold promise as alternative therapeutic options for managing inflammatory conditions in the body[29]. Furthermore, the phosphoinositide 3-kinase (PI3K) and the Mitogen-Activated Protein Kinase (MAPK) pathways have been causally found in connection with oxidative stress, inflammation and cell death program[30]. Reactive Oxygen Species (ROS), when generated during oxidative stress results in inflammation and eventually damage DNA in the renal tissues. Since ROS can cause double-stranded breaks, it may lead to mutations or cell death[17]. The comet test is a convenient method for identifying DNA strand breaks, damage and repair at the single-cell level[18]. Additionally, comet results from the kidney tissues of mice under the oxidative stress and inflammation in our study showed higher comet parameter values (Table 1) which indicates that the molecular damage has occurred due to the metal toxicity. Mice given lead nitrate showed this harm particularly well. However, mice treated with garlic essential oil showed fewer DNA fragments, lowered DNA's sensitivity to oxidative damage and protected the cell from the negative consequences of heavy metal intoxication as shown by the reduction in the comet tail length (Figure 6). The exposure to Lead nitrate disrupts the delicate equilibrium within renal tissues, resulting in an imbalance between pro-oxidants, such as nitric oxide and intrinsic antioxidants, thereby exacerbating the toxic impact of lead on the renal system[33]. Following Lead intoxication, the presence of elevated Nitric Oxide (NO) extent in the biological tissues, is indicative of the Reactive Nitrogen Species (RNS) generation. Notably,

nitric oxide exerts its cytotoxic effects through its active byproducts, peroxynitrite radicals. This cytotoxic action underscores the intricate interplay between NO and RNS in the context of Lead poisoning and its impact on renal function[34,35]. Herbal therapies are the preferred option to counteract the detrimental consequences of these toxic metals as they have hardly any side effects on the human bodily systems[25,26]. In the context of the present study, garlic was specifically selected due to its potential to mitigate nephrotoxicity resulting from inflammatory reactions in the tissue[12,14]. It is noteworthy that the organo-sulfur components present in garlic essential oil have been recognised for their ability to bind metal ions, including Pb^{2+} [23,24]. Beyond their chelating properties, these compounds exhibit beneficial effects in terms of anti-inflammatory and genotoxicity-reducing properties. Furthermore, the study underscores the possible therapeutic potential of garlic essential oil in mitigating the adverse effects of lead exposure, through its broader health benefits. This is especially noteworthy in view of previous research that showed a connection between the onset of oxidative stress and inflammation after heavy metal exposure[22]. The present study supports this relationship by demonstrating an elevated expression of pro-inflammatory cytokine genes, contributing to the generation of some inflammatory indicators in the renal tissue when exposed to Lead nitrate. The generation of these pro-inflammatory cytokines, on the other hand, was found to be restored with the administration of garlic essential oil. More importantly, garlic essential oil therapy to some extent aids in maintaining renal function which eventually helps to maintain the cell's viability at the cellular as well as molecular level as shown by the comet assay results depicted in Table 1 and Figure 6[27,35]. It has been previously shown that garlic essential oil has anti-inflammatory features by inhibiting the inflammatory marker expressions which avoids the development of renal inflammation in response to the heavy metals[36-38]. It is evident from the current study that the garlic essential oil specifically the high dose of GEO was found to be more potent in the restoration of the NF-κB and other pro-inflammatory marker levels as illustrated in Figures 3, 4 and 5. Along with that garlic essential oil was also found to be effective in reducing the DNA damage parameters which were enhanced due to the Lead nitrate intoxication as illustrated in Figure 6 and Table 1. These findings suggest that the use of garlic essential oil could potentially reduce the risk of inflammation and genomic damage caused by Lead nitrate.

5. Conclusion

This present work underscores the possible potential effect of garlic essential oil in mitigating the nephrotoxic effects induced by Lead nitrate in mice, as evidenced by its effectiveness in reducing inflammation and genotoxicity. Future perspectives include delving deeper into the underlying mechanisms and developing targeted formulations, to validate these findings. This research not only holds promise for enhancing renal protection but also contributes to a broader understanding of natural compounds' roles in safeguarding vital organs, potentially informing public health strategies to counter the impact of environmental toxins on human health. Moreover, efforts are needed for the optimisation of the appropriate dose, duration of treatment, and possible side effects on major organs.

6. Acknowledgments

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7. Author Contributions

The entire experimental work was conceptualised by S.S. and V.S. The manuscript's principal author S.S. contributed the majority of writing activities but support was also provided by V.S. A Literature search was done by S.S. Figures were developed by S.S. The study design was framed by V.S. The experimentation and data analysis were carried out by S.S. The statistics and data interpretation were done by S.S. The final manuscript writing was done by S.S. and V.S. Authors have read and approved the final manuscript.

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