The Applied Biology & Chemistry Journal
https://www.theabcjournal.com
eISSN: 2582-8789

Original Research

Garlic essential oil confers shielding against nephrotoxicity elicited by lead nitrate in Swiss albino mice

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Received May 15, 2023
Revised September 26, 2023
Accepted October 22, 2023
Published December 12, 2023

Abstract: Lead is an immensely poisonous metal that can infiltrate the human body through various natural processes and human activities, therefore it possesses a significant risk to human health. Garlic (*Allium sativum*), a widely recognized medicinal plant, is employed to diminish a diverse array of health issues. While investigating the potential curative properties of the garlic essential oil (GEO) derived from fresh garlic bulbs, researchers explored its impact on the mice renal tissue subjected to lead nitrate. In the present research work, a sum of 36 healthy male Swiss albino mice were randomized into one control group (I) and five treatment groups: lead nitrate (II a), lead nitrate + low dose of GEO (II b), lead nitrate + high dose of GEO (II c), lead nitrate + silymarin (II d) and lead nitrate + vehicle olive oil (II e). Lead nitrate exposure resulted in elevated levels of alanine transferase (ALT), aspartate aminotransferase (AST), lipid peroxidation (LPO) and decreased levels of antioxidant enzymes, thus contributing to the oxidative stress and adversely affected the normal structure of renal tissues. Conversely, treatment with garlic essential oil (GEO) resulted in upsurge in these antioxidant levels and depletion in ALT, AST, and LPO levels. The findings support the notion that a higher dosage of garlic essential oil is more effective in mitigating lead nitrate-induced nephrotoxicity than a lower dosage. Consequently, garlic essential oil holds promise as a novel therapeutic agent for alleviating nephrotoxicity induced by lead nitrate exposure.

Keywords: lead nitrate; nephrotoxicity; oxidative stress; histopathology; garlic essential oils

1. Introduction

Heavy metals perturb the ecosystem as they are the foremost menace to human health and can pervade everywhere. The human body can get exposed to them via multiple natural and industrial activities. These heavy metals are contaminated naturally by soil erosion, volcanic activity, and airborne particles; industrial pollution results from the metal, textile, and nuclear industries [1]. Additionally, these noxious metals possess hefty atomic weight and have a five-fold higher density than water [2]. Lead is a formidable environmental polluter with potentially disastrous consequences for human health. Exposure to lead causes a wide range of health issues, including neurological, cardiovascular, reproductive, and nephrotoxicity ailments [3]. Lead nitrate is a common form of lead contamination that can majorly affect the renal tissues [4]. The human body encounters lead either directly or indirectly through the intake of drinking water and tainted food including chocolates, peas, and sweet potatoes as well as beverages like fruit juices and protein shakes [5].

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According to previous studies, the lead's harmful effects have increased over the past few decades, particularly for children, pregnant women, and workers in poor nations [6]. Therefore, it is vital to lower the hazards to one's health related to this metal in both the workplace and the general population. The nephrotoxicity is a serious public health issue and hence, there is an urgent need for a new herbal cure. Garlic (*Allium sativum*) is a well-known medicinal herb recognized for millennia for its health-enhancing attributes including antifungal, anticancer, anti-inflammatory, antioxidant, anti-diabetic, and renal protective actions [7]. This perennial plant contains a high concentration of polyphenols, flavonoids, and organic sulfur compounds like sulfides (DAS), disulfides (DADS), diallyl trisulfides (DATS), S-allyl-cysteine (SAC), and ajoenes [8]. Garlic essential oil, derived from fresh garlic cloves has been shown to possess several medicinal benefits, including the capacity to guard against chemical toxicity [9]. However, the underlying implementation of its protective effect is not fully acknowledged. Keeping the health-promoting medicinal properties of garlic under consideration, recent research work is outlined in Figure 1. The purpose of the current investigation was to ascertain whether garlic essential oil could have any protective effects against lead nitrate-induced nephrotoxicity by evaluating oxidative stress markers, biochemical changes,

Citation: Sharma S, Sharma K, Sharma V (2023). Garlic essential oil confers shielding against nephrotoxicity elicited by lead nitrate in Swiss albino mice. *T Appl Biol Chem J*; 4(4):102-112. https://doi.org/10.52679/tabcj.2023.0010

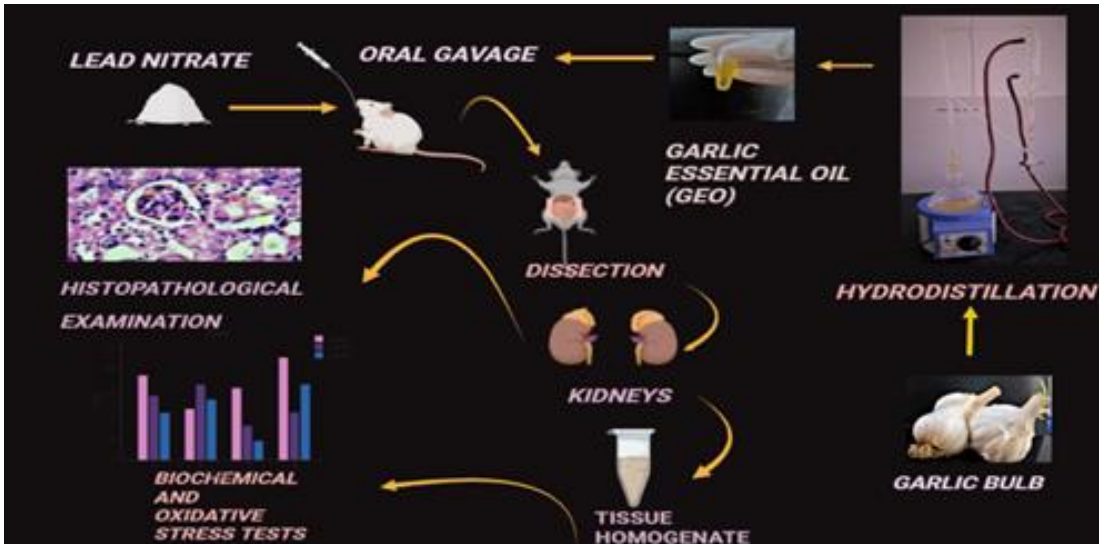

Figure 1. Schematic representation of complete study design.

and histological deformations in the renal tissues of Swiss albino mice.

2. Experimental

2.1 Chemicals

Lead nitrate was obtained from HiMedia Laboratories Pvt. Ltd (India), while all other chemicals of high analytical quality were purchased from Sisco Research Laboratories (India), Qualigens (India/Germany), SD Fine Chemicals (India), and HiMedia (India).

2.2 Experimental animals

For this investigation, the male Swiss albino mice (Mus musculus) weighing between 25-30g and aged 2-2.5 months were chosen and were acquired from the Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana (India). The animals were housed in a pathogen-free enclosure that was well-maintained with air conditioning to maintain a temperature of 23-25°C and humidity at 50 ± 15%. They were provided with an adequate pellet diet composed of wheat flour, roasted Bengal gram flour, skim milk powder, casein, refined groundnut oil, a salt mixture, and a vitamin mixture. Additionally, they had unrestricted access to water (ad libitum) [10]. Before the trial, the animals had one week of acclimatization. The Institutional Animal Ethical Committee, at Banasthali Vidyapith also provided its clearance to conduct the experiments in terms of ethics. The ethics approval letter number is (Approval No. BV/IAEC/January/2020/10). Every experiment was carried out in compliance with accepted practices and moral standards. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines were carefully followed in all animal procedures.

2.3 Sample collection

The bulbs of garlic were randomly rolled up from the native premises of Banasthali Vidyapith, Rajasthan, India.

2.4 Preparation of garlic essential oil through hydrodistillation

Garlic essential oil is extracted from fresh and raw garlic bulbs using a conventional hydrodistillation technique, as shown in figure Figure 1. Prior to hydrodistillation, fresh garlic bulbs (250-300 g) were precisely cleaned and peeled. Afterwards, the bulbs were cut into small pieces followed by converting them into a smooth paste. This paste was cooked in a round bottom flask. Using a clevenger device connected to a flask containing the garlic paste, the essential oil was extracted. The content was cooked at 60-70°C for 4-5 hours, or until the last drop of the essential oil was extracted. An oil-water separator is used to separate the condensed vapour produced as a result. In order to lower the condenser temperature, a low-pressure water pipe was concurrently turned on. The extracted oil was placed in a vial and stored at 4°C. It was dried over anhydrous sodium sulphate. The extraction procedure was conducted in triplicates, and the fresh weight of the sample was used to compute the oil yield (v/w) [11].

$$Oil\ yield\ (\%) = \frac{Volume\ of\ garlic\ essential\ oil\ (ml)}{Garlic\ sample\ weight\ (g)} \text{ x } 100$$

2.5 Study design

The purpose of this study is to investigate the potential preventative effects of garlic essential oil against lead nitrate-induced nephrotoxicity. In the current study, a total of 36 male Swiss albino mice weighing between 25 and 30 g were carefully chosen and then split into six separate groups of six mice each. Group I was used as the control group, which was considered to be untreated, and the animals of this group received double-distilled water. Group II was used as the lead nitrate-treated group, which included 30 mice. This group is divided into five sub-groups. All the subgroups were 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 along with lead nitrate to the animals of groups II b, II c, II d, and II e except group II a from 12th day to the end of the experiment. A detailed description of these experimental groups is provided in Figure 2.

In the context of the ongoing research, it is noteworthy to emphasize that the entirety of the administered doses to the Swiss albino mice were exclusively through the method of oral gavage [12]. All the animals were dissected by cervical dislocation thereby, the kidneys were removed and the the outer fatty layer present on the tissue was taken off with the help of forceps. To eliminate any blood, the renal tissues were thoroughly cleaned in ice-cold saline (0.9% sodium chloride solution), blotted off, and weighed to perform the renal redox status parameter and histopathological examination. Previous studies were used to decide the toxicant and plant dosages [13, 14].

2.6 Determination of renal weight

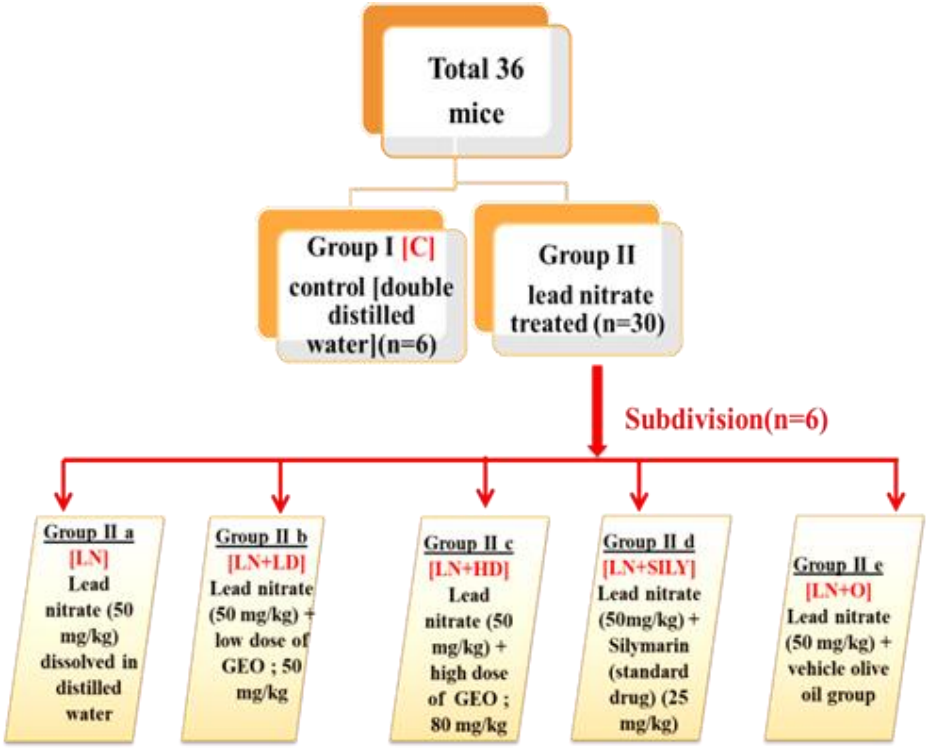

Figure 2. A schematic representation of the animal groups and their treatment regimen.

The weight of the kidneys was measured with precision using a sensitive weighing balance (Docbel-Braun), and from this measurement, the kidney index was determined by applying the subsequent formula [15]:

$$Kidney\ Index\ (KI) = \frac{\text{Weight of the kidney}}{\text{Body mass}} \text{x } 100$$

2.7 Homogenate preparation

Renal tissues were homogenized (10% W/V) in 0.1 M sodium phosphate buffer (pH=7.4) by using a homogenizer (YS1-471 York Tissue Homogenizer). Further, the homogenized mixture was centrifuged at 10,000 rpm for 10-15 min at 4°C. Then, the supernatant was collected to examine the oxidative stress and biochemical parameters.

2.8 Oxidative stress and biochemical assays

According to standard procedures, the following parameters were analyzed after homogenate preparation: superoxide dismutase (SOD) activity [16], catalase (CAT) activity [17], glutathione peroxidase (GPx) [18], reduced glutathione (GSH) [19], glutathione-s-transferase (GST) [20], lipid peroxidation (LPO) [21], total protein content (TPC) [22]. Alanine transferase (ALT) and aspartate aminotransferase (AST) [23].

2.9 Histopathological examination

After the mice were dissected, the renal tissue was collected, and the blood was completely removed with 0.9% saline. The tissue was then sliced into 2 mm thick slices and fixed in 10% buffer formalin (pH 7.4; 0.1 mol/L) for 24 hours. The tissues were dehydrated using a graded sequence of ethanol, including 30, 50, 70, 90, and 100%. After that, tissues were placed in a clearing agent; xylene to remove the ethanol. Paraffin wax was melted and utilized to create tissue blocks. Then, using a digital microtome, tissues were cut into tiny pieces (3-5 µm). Water-floating ribbons or sections were put on glass slides and given a coat of Meyer's albumin (egg albumin with glycerol) and then the slides were stretched on a hot plate. Subsequently, slides were immersed in xylene, alcohol: xylene (1:1), and then in decreased order of alcohol content (absolute to 30%; 5 min each), followed by rinsing with distilled water. After each staining, slides were passed through ascending order of

alcohol up to 70% in the case of Haematoxylene whereas 90 and 100% in the case of eosin. The stained slides were then placed in xylene: alcohol and then in pure xylene. Finally using DPX to mount the slides, they were then covered with a cover slip. Under a light microscope equipped with 10x, 20x, and 100x magnification objective lenses, the histopathological alterations were semi-quantitatively evaluated [24].

2.10 Statistical analysis

Experiments were carried out in triplicates, and the mean ± SEM was used to represent the findings. Statistical analysis was performed using the one-way analysis (ANOVA) followed by Tukey's range test, utilizing the SPSS 20.0 statistical software. A significance level of $p<0.05$ was considered statistically significance.

3.2 Determination of renal redox status parameters

3.2.1 Superoxide dismutase, catalase, and glutathione peroxidase activity

The lead-intoxicated group declined the antioxidant level significantly ($p<0.05$) mainly the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) in the renal tissues of Swiss albino mice when examined against the control. Treatment with garlic essential oil at different dosages (50 mg/kg and 80 mg/kg) restored the antioxidant concentrations in contrast with lead-impaired groups in the case of SOD statistically favorable ($p<0.05$) whereas in the CAT and GPx non-significantly favorable ($p>0.05$). The standard drug, silymarin was found to be significantly effective in augmenting the enzymatic levels as opposed to the lead nitrate group ($p<0.05$) in all these

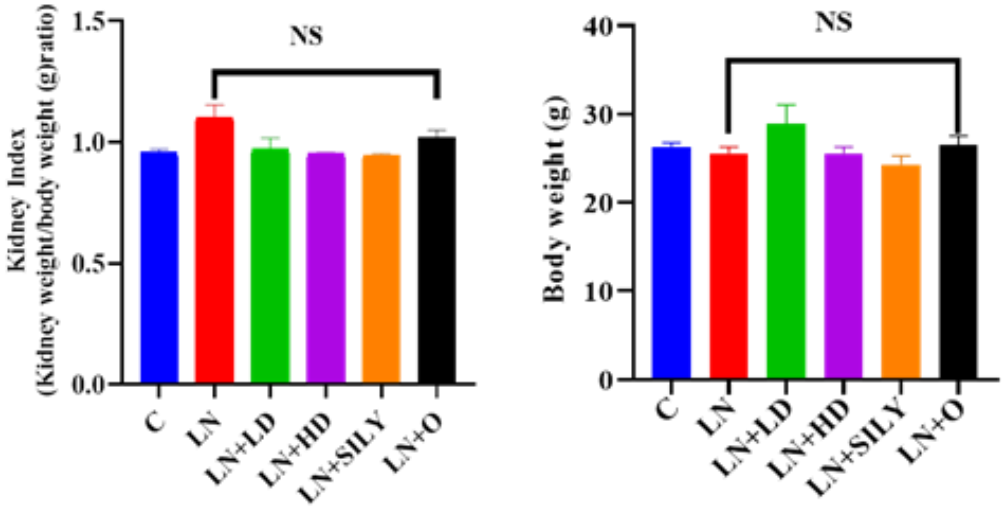

Figure 3. Estimation of kidney index (KI) and the body weight (in grams) after receiving lead nitrate (LN, group II a), lead nitrate + low dose of garlic essential oil (LN+LD, group II b), lead nitrate + high dose of garlic essential oil (LN+HD, group II c), lead nitrate + standard drug silymarin (LN+SILY, group II d), lead nitrate + vehicle olive oil (LN+O, group II e) in the renal tissues of male Swiss albino mice for 30 days. The gathered data was presented as mean ± SEM (n = 6), which are statistically favorable at $p<0.05$. NS stands for non-significance.

3. Results

3.1 Kidney index

At the end of the course, mice intoxicated with lead nitrate (group II a) showed a non-significant decline in their final body weight and elevation in the relative kidney index ($p>0.05$) in comparison with the control animals (group I). In garlic essential oil-treated groups (group II b and c), restoration of body weight and down-regulation of the kidney index was noticed non-significantly ($p>0.05$) when compared to the lead nitrate-treated group (group II a). Along with that, silymarin (group II d) and vehicle (olive oil treated) (group II e) also enhanced the final body weight and reduced the kidney index when compared to the lead intoxicated group (group II a) non-significantly (Figure 3). Thus, we can conclude that garlic essential oil is beneficial in recovering the body weight and kidney index that were disturbed by the lead nitrate intoxication.

parameters. Along with that enzymatic activities were also found to be escalated in the vehicle olive oil group in contrast to the lead nitrate intoxicated group significantly in the case of SOD ($p<0.05$) and non-significantly in the case of CAT and GPx ($p>0.05$) respectively depicted via bar graphs in Figure 4 (A, D, and F). Consequently, via raising the levels of SOD, CAT, and GPx, garlic essential oil has an inhibitory effect on lead nitrate-induced oxidative stress.

3.2.2 Reduced glutathione and glutathione-S-transferase

The toxicant group showed significant down-regulation in the reduced glutathione (GSH) and glutathione-S-transferase (GST) levels examined alongside the control group ($p<0.05$). Further treatment with garlic essential oil at different doses (50 mg/kg and 80 mg/kg) restored the antioxidant levels as opposed to the lead-intoxicated group ($p<0.05$). The standard drug, silymarin, and the vehicle

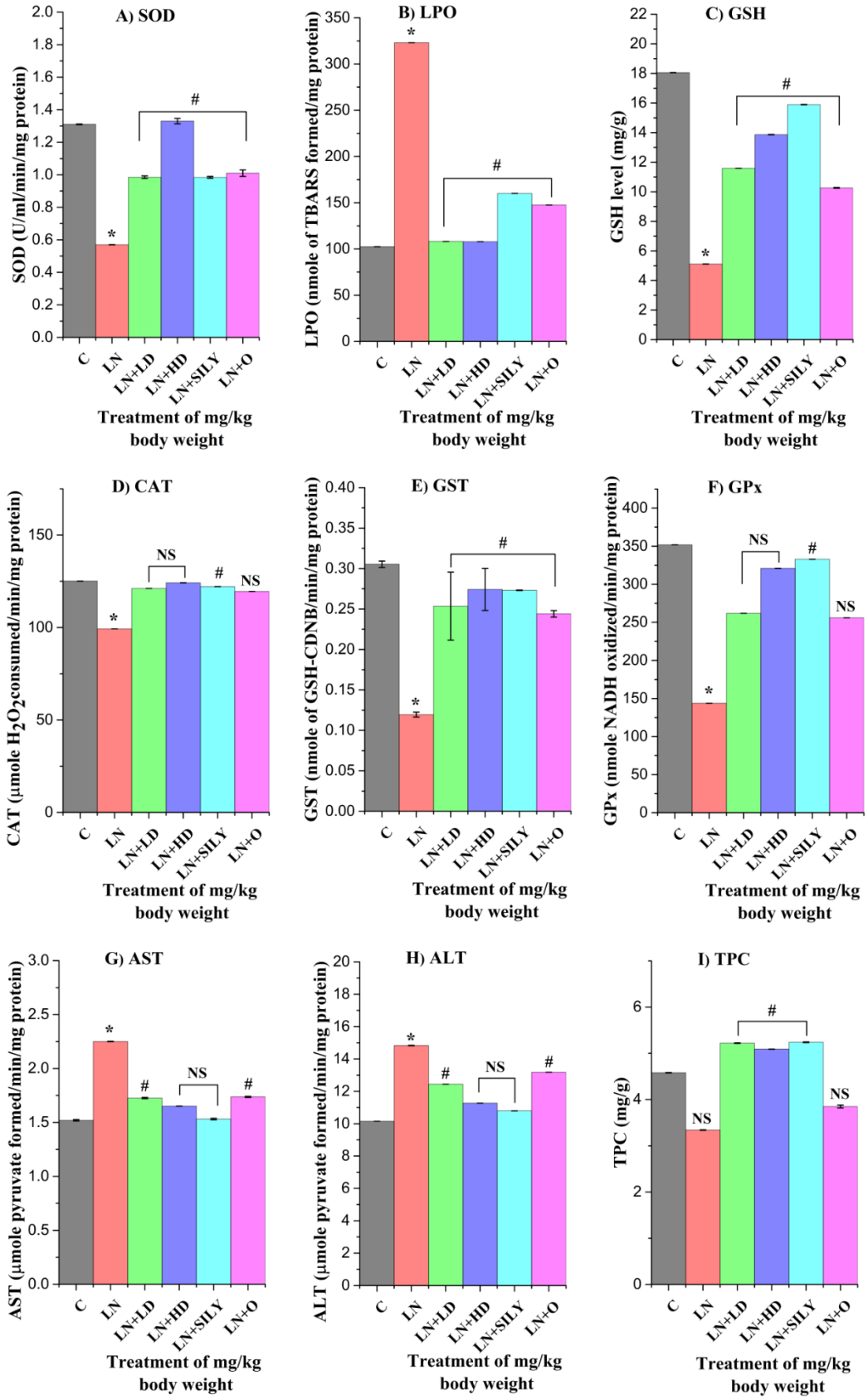

Figure 4. Bar graphs (A-I) shows the renal redox and biochemical associated parameters in the study groups and the values were illustrated as mean ± SEM (n=6), which are statistically favorable at $p<0.05$.*$p<0.05$ depicts the lead nitrate treated (LN, group II a) when relative to the group under control (group I). # $p<0.05$ depicts the lead nitrate group (LN, group II a) in comparison with lead nitrate + low dose of garlic essential oil (LN+LD, group II b), lead nitrate + high dose of garlic essential oil (LN+HD, group II c), lead nitrate + standard drug silymarin (LN+SILY, group II d) and with lead nitrate + vehicle olive oil (LN+O, group II e), respectively. NS stands for non-significant.

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olive oil group were found to be significantly effective in up-regulating the GSH and GST extents evaluated in the bar graphs in Figure 4 (C and E). By this, we can conclude that garlic essential oil has a positive impact in reducing lead-induced stress by elevating the GSH and GST levels respectively.

3.2.3 Lipid peroxidation level

Thiobarbituric acid reactive substances (TBARS) assay is the lipid peroxidation (LPO) measure as an outcome of free radical damage. The mice inebriated with lead nitrate caused a significant rise in TBARS extent when evaluated against the control group ($p<0.05$). Conversely, however, garlic essential oil therapy at different dosages (50 mg/kg and 80 mg/kg) obstructs lipid peroxidation in renal tissues in contrast to the lead nitrate group ($p<0.05$). In addition, the silymarin and the vehicle olive oil group also recovered the LPO extent significantly ($p<0.05$) when examined against the lead nitrate intoxicated group, depicted in Figure 4 (B). Thus, the garlic essential oil was also found to recover the membrane viability by reducing the LPO level increased due to lead nitrate intoxication.

3.3 Determination of renal biochemical parameters

3.3.1 Alanine transferase and aspartate aminotransferase

In this study, the sequel of lead nitrate and garlic essential oil therapy on a few biochemical indices were evaluated. Lead nitrate showed a significant up-regulation in the alanine transferase (ALT) and aspartate aminotransferase (AST) levels which were measured in contrast with the control group ($p<0.05$). A low dose of garlic essential oil; 50 mg/kg was seen to decline ALT and AST levels significantly ($p<0.05$) contrasted with the toxicant group (group II a), along with this the high dose of garlic essential oil;80 mg/kg and silymarin (25 mg/kg) also down-regulated the enzymatic levels non-significantly ($p>0.05$) in comparison to the toxicant group (group II a), moreover in comparison to mice intoxicated with lead nitrate, the vehicle olive oil group exhibited a notable decline in the amounts of ALT and AST, which was statistically significant ($p<0.05$) Figure 4 (G and H). By this, we can conclude that the garlic essential oil contributes significantly to recovering the renal biochemical parameters by decreasing the ALT and AST levels that were increased by the lead nitrate intoxication. These findings suggest that lead nitrate-induced alterations in the oxidative stress indices and biochemical parameters support the pathological changes in the renal tissue.

3.3.2 Total protein content

The toxicant group revealed a non-significant down-regulation in the TPC in opposition to the control group ($p>0.05$). Further treatment with the garlic essential oil at different doses (50 mg/kg and 80 mg/kg) restored the protein levels evaluated against the lead-intoxicated group ($p<0.05$). Moreover, the standard drug (silymarin) was found to be significantly effective in up-regulating the TPC levels in contrast to the lead nitrate inebriate group ($p<0.05$), and in the case of the vehicle, olive oil group showed insignificant restoration of the TPC level ($p>0.05$) as opposed to the group that received lead nitrate treatment Figure 4 (I). Lead nitrate treatment found to be harmful to the renal tissue as a reduction in the protein content was noticed, on the other hand garlic essential oil showed its beneficial effect by enhancing the TPC levels.

3.4 Histopathological changes

Histopathology of kidney tissues after lead nitrate and garlic essential oil treatment was investigated in the present work. This was demonstrated in Figure 5 (A-J) at different magnifications 100x with a scale bar of 10µm, 20x with 50 µm and 10 x with 100 µm. In the control group, shown in Figure 5 (A and B), no substantial change in the pathophysiology of the renal tissues was seen and glomerulus cells and collecting tubules (CT) were observed to be normal. In the case of the lead nitrate intoxicated group (group II a), shown in Figure 5 (C, D, and E), deformities like renal tubular atrophy, glomerular shrinkage, and necrosis of the renal tubules' epithelium in the collecting ducts. Two different doses of garlic essential oil treatment group II b and group II c indicate an approximate recovery in the alterations in histopathology demonstrated in Figure 5 (F, G, and H). Group II d (silymarin) and group II e vehicle olive oil group, shown in Figure 5 (I and J), have no degradation in the medullar region (MR) and the cortex shows near normal renal architecture when relative to the group that was treated with lead nitrate (group II a). Hence, by the results, we can conclude that garlic the essential oil found to be beneficial in reducing the pathological deformities that are enhanced by the lead nitrate.

4. Discussion and Conclusion

Lead poisoning is an emerging global issue that can result in a wide range of clinical outcomes, including nephrotoxicity, neurotoxicity, hepatitis, inflammation, and immune suppression, and is also frequently linked to hypo-hemoglobinemia, high blood pressure, and cardiovascular diseases [25, 26]. It can participate in both organic and inorganic redox pathways, specifically targeting sulfhydryl and electron donor groups within bio-molecules. Furthermore, it initiates the production of free radicals, leading to an imbalance in antioxidant levels, potentially resulting in cell death [27]. Kidneys mainly eliminate most of the toxins, pesticides, drugs, and other unwanted substances from our bodies [28]. As lead is a toxic heavy metal that has been associated with several health problems, including kidney damage, it is clear from several studies that prolongation of lead (Pb) exposure worsens chronic kidney disease (CKD) and nephrolithiasis. Headache, stomach pain, and cognitive issues are just a few of the signs of lead poisoning. To safeguard our kidneys and general health, it is crucial to take action to prevent lead exposure [29]. Lead exposure disrupts physiological and metabolic

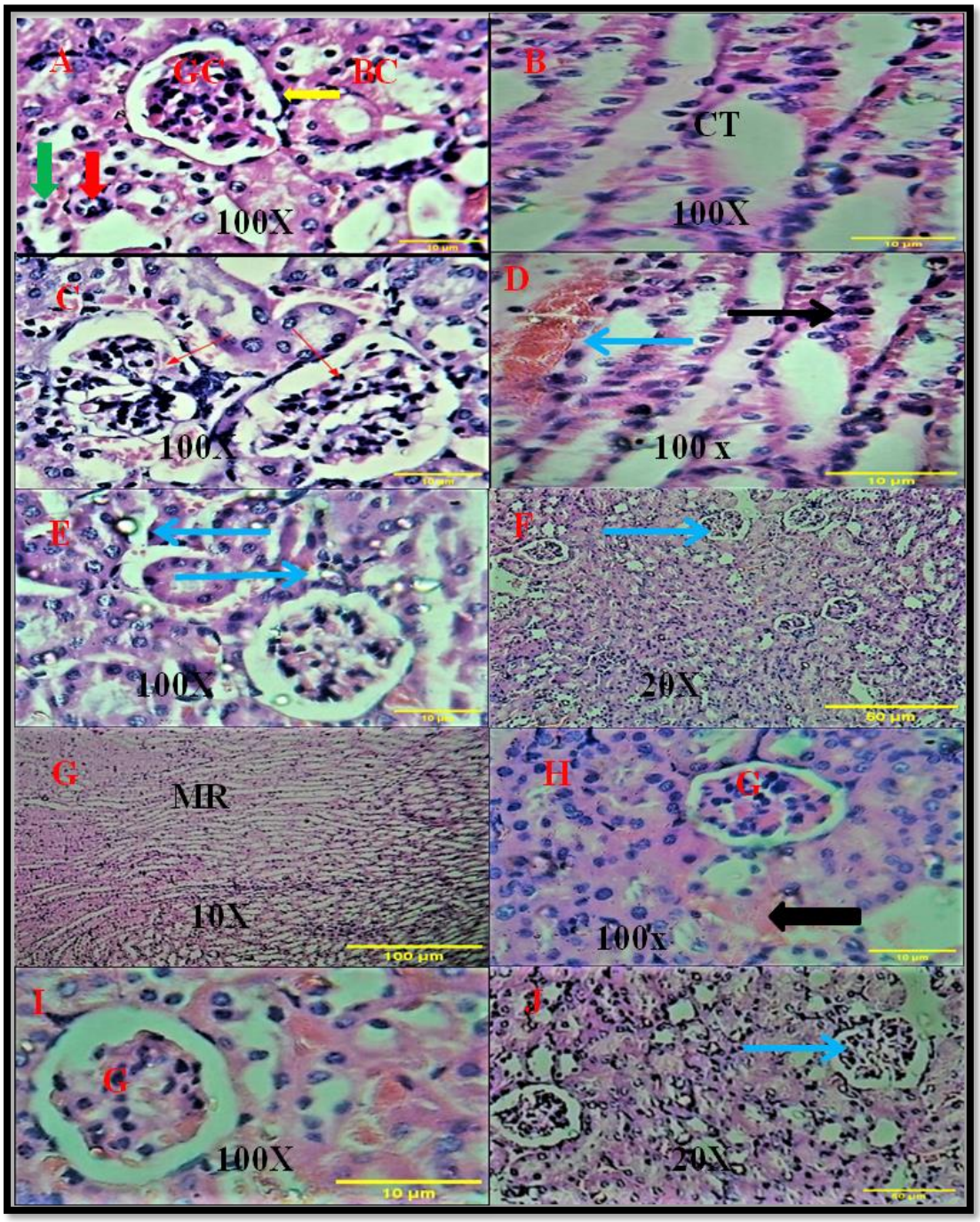

Figure 5. Photomicrographic representation of the transverse sections of mice renal tissues. A-B depicts the control (group I) displaying the standard architecture of renal tissue and shows the normal ordinary glomerulus cell (GC), Proximal tubules (PT) (red arrow), distal tubules (DT) (green arrow), Bowmen's Capsule (BC) (yellow arrow), and kidney medulla of the control group showing well defined collecting tubules (CT). It also shows prominent renal cell nucleus with regular sizes. Photomicrograph of lead nitrate treated renal groups (group II a) shown in figures C-E; C shows the shrinkage of the glomerulus, D indicates necrosis of the renal tubule epithelium in the collecting ducts (sky blue arrow), the black arrow pointing to the clusters of the deformed cells. E shows the focal segmental glomerulosclerosis and the epithelial cast in the urinary space. Lead nitrate (50 mg/kg) + low dose of GEO; 50 mg/kg treated group (group II b) is represented by F and G; F demonstrates nearly standard form with intact glomeruli and glomerulus membranes. G shows the near normal medullar region (MR). Photomicrography representation of the lead nitrate (50 mg/kg) + high dose of GEO; 80 mg/kg treated group (group II c) is shown in H depicts near normal architecture of kidney with no significant pathological changes having ordinary glomerulus cell (G) and with slighter necrosis of blood vessel as indicated by black arrow. I represent photomicrography of the kidney administered with lead nitrate (50mg/kg) + Silymarin (25 mg/kg) (group II d) with no remarkable histopathological changes and well-organized normal glomerulus (G), and bowmen's capsules. J represents photomicrography of the lead nitrate (50 mg/kg) + vehicle olive oil (group II e) showing a well-organized glomerulus with arrow pointing to flattening of tubular epithelial.

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activities by substituting essential cofactors such as copper, zinc, calcium, iron, selenium, and magnesium ions. This substitution results in organ-level disorders. The significance of these cofactors lies in their pivotal roles within antioxidant systems [9, 25, 30]. Interestingly, garlic essential oil contains organosulfur compounds that enhance the content of these cofactors in animal tissues, further highlighting its potential to mitigate the negative consequences of exposure to lead [31]. In renal tissues of mice, exposure to lead alters the body's anatomical functions which in turn decreases the body weight and increases the kidney index. This change in body and kidney weights among lead nitrate-exposed mice could potentially arise from factors like reduced food intake, hormonal imbalances, and diminished protein levels. Treatment with garlic essential oil in the present study reduced the kidney index by restoring the body weight and eliminating the pathological stress in renal tissues. Lead can produce several highly reactive species, including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and lipid peroxides. All of these compounds initiate radical chain reactions that negatively impact nucleic acids, proteins, carbohydrates, and lipids, which are the frequent targets of oxidative damage, resulting in permanent cell damage [32, 33]. Although the decisive implementation of lead-persuade oxidative stress is unclear, numerous studies indicated that ROS damages the cell membrane and depletes the antioxidant enzymes such as SOD, CAT, and Gpx in the biological system.

During lead metabolism, an increase in superoxide radicals can be blamed for the decline in Superoxide dismutase (SOD) activity, which is regarded as the most powerful antioxidant enzyme. Superoxide and hydroxyl radicals produced from molecular oxygen by SOD turn these superoxide radicals into hydrogen peroxide which gives rise to hydroxyl groups via the Fenton reaction that additionally serves as a root cause of ROS generation. Other enzymes, catalase and Gpx break down hydrogen peroxide into water and oxygen. Lead exposure exerts a multifaceted impact on enzymatic activities within renal tissues. Specifically, catalase, a vital enzyme with iron at its catalytic core, experiences diminished activity as a result of lead binding to iron [34]. Simultaneously, the essential element selenium (Se), a key component of GPx, also encounters depletion under the influence of lead nitrate. This depletion contributes to the reduction in GPx activity, creating a cascade of enzymatic disruptions due to lead's intricate interactions [35]. Studies on dietary plants with antioxidant qualities including garlic have been popular from past eras. Essential oil extracted from garlic is believed to confer protection upon animal tissues against the deleterious impact of lead intoxication [36].

The results of the current investigation showed that administering garlic essential oil to mice could improve levels linked to renal oxidative stress. During oxidative stress, cell membrane damage promotes lipid peroxidation which in turn produces malondialdehyde. Lead toxicity is correlated with the elevation in lipid peroxidation (LPO), as it is an ongoing process that fragments membrane lipids in mice's renal tissues. Concurrently, garlic essential oil administration showed down-regulation of lipid peroxidation (LPO) levels to a more normalized state, this indicates that garlic essential oil prohibits the membrane damage and enhances the tissue viability. Free radicals act as electron donors, creating covalent bonds with the sulfhydryl groups within antioxidant enzymes. Sulphur-rich metal-sequestering peptides like GSH are extremely important because they are a crucial part of cells' antioxidant defense in opposition to the overproduction of free radicals, and cellular defenses [37, 38]. Glutathione is intimately related to glucose metabolism via the reduced nicotinamide adenine dinucleotide phosphate (NADPH) of the pentose phosphate pathway.

Several studies have shown that diminished glucose-6-phosphate dehydrogenase (G6PD) activity reverses the hexose monophosphate shunt [39]. Redox between GSSG/2GSH is linked to GSH's capacity to produce primary antioxidants. Exposure to lead nitrate lowered GSH levels, which in turn decreased GSH/GSSG ratio followed by an increase in the oxidative stress conditions. This results in glutathione efflux from the cell which may weaken the cellular antioxidant barrier [40, 41]. A drop in GSH concentration increases the origination of pro-oxidants (hydrogen peroxide and superoxide anion) and oxidative burden, which has a cascade of repercussions that impact both the structural integrity and function of cell and organelle membranes. Administration of garlic essential oil increased the GSH content in renal tissues of mice intoxicated with lead. This may be because garlic essential oil targets free radicals which reduces oxidative stress and increase the GSH content in cells.

Lead nitrate inhibitory function in translation could be attributed to decreased polynucleotide levels [42]. In the lead nitrate-intoxicated group increased amounts of AST and ALT, imply toxicity which can lead to organ damage, cell lysis, enhanced permeability, or cell necrosis [43]. The abnormal extent of AST and ALT were restored to a possible extent after garlic essential oil treatment. The observed AST and ALT levels reduction suggests that garlic essential oil is capable of safeguarding the renal tissues' structural integrity and preventing lead nitrate poisoning. Garlic's improved action on antioxidant enzymes can be viewed as a generalized electrophilic counteractive reaction [44]. Moreover, it has been confirmed that the lead concentrations in renal tissues decreased in the lead nitrate + garlic essential oil-treated group as well. Lead also interferes with intracellular calcium ion (Ca^{2+}) signaling and endoplasmic reticulum damage and significantly reduces protein production [45, 46].

According to Yuan et al., histopathological findings are commonly used as markers for toxicological research and heavy metal intoxication [47]. The renal tissues in this investigation showed a variety of histopathological alterations, including significant renal interstitial fibrosis and renal tubular atrophy (Figure 5 (C-E). These pathological alterations in the lead nitrate-treated group (group II a) might be a result of up-regulated ROS generation, which arises due to increased redox imbalance,

thus reducing the antioxidant defense system. Nonetheless, it is hypothesized that the presence of sulfur compounds in garlic essential oil, known for their high electron affinity, leads to the production of charged ions, which contributes to its antioxidant activity. Consequently, these compounds form stable complexes [13, 48].

Furthermore, the outcomes of the oxidative and biochemical analysis validate the light microscopy findings. Previous research showed that garlic extract rich in flavonoid compounds is known to decrease the level of lead in tissues, suggesting the possibility of garlic essential oil chelating effect and its capacity to lessen lead toxicity [13, 49]. More significantly, it has been shown that the organosulfur components of garlic essential oil, particularly diallyl trisulfides, may bind metal ions and reduce their negative impact on the biological system [50]. As a result of the current research, the bioactive components of garlic essential oil include diallyl trisulfide, diallyl tetra sulfide, and allyl disulfide, residues which may facilitate the excretion of divalent metal cation lead from the body and prevent its absorption in the renal tissue. Therefore, it implies that organosulfur compounds possess the ability to act as a chelating agent that can lower the lead traffic in renal tissues [44].

In conclusion, our discussion reveals the beneficial effects of administering garlic essential oil to mice and mitigates the harmful effects of lead toxicity to the best possible extent. Our findings show that administering a high dose of garlic essential oil substantially reduces the adverse effects of lead, further supporting its potential as a valuable therapeutic intervention.

Overall, this study has yielded significant and promising results. This research has demonstrted that GEO possesses potent antioxidant properties, which effectively counteract the oxidative stress triggered by lead nitrate. This shielding impact was further proved by evidence through recovering the histopathological observations, where the administration of GEO exhibited marked reductions in renal tissue damage and cellular injury caused by lead nitrate exposure. Furthermore, this study holds significant implications in both scientific and practical domains. First and foremost, the study sheds light on the protective effects of GEO, expanding our understanding of its therapeutic potential in safeguarding against lead-induced renal damage. Given the alarming global prevalence of lead pollution and its detrimental effect on huma health, this research serves as a stepping stone for future investigations for developing natural and cost-effective interventions.

Declarations

Author Contribution: SS performed literature survey, and experiments and wrote the manuscript. KS helped in the statistical analysis of the results and VS supervised, designed, and helped in preparation of the manuscript.

Acknowledgment: The authors would like to acknowledge the Bioinformatics Centre, Banasthali Vidyapith, supported by DBT and DST, for providing computation and networking support through the FIST and CURIE programs at the Department of Biosciences and Biotechnology, Banasthali Vidyapith, Rajasthan, India.

Conflict of Interest: The authors declare no conflict of interest.

Funding: Not applicable.

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