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RESEARCH ARTICLE

Administration of Garlic Essential Oil Restored the Altered Enzymatic and Non-enzymatic Parameters and Pulmonary Histoarchitecture in Mice Subjected to Lead Nitrate

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Abstract

The focus of the current research work was to unfold the therapeutic potential of Garlic Essential Oil (GEO) in altered oxidative stress, biochemical parameters and histoarchitecture of pulmonary tissue of mice intoxicated with the inorganic salt of Lead. Thirty six (36) mice were used in the experiment, and they were divided into 6 groups, with 6 mice in each group. The experimental groups were as: control/untreated, Lead Nitrate (LN), LN + low dose of GEO, LN + high dose of GEO, LN with standard drug (silymarin) and LN with vehicle olive oil. The total duration of the experimental study was of 30 days. The outcome of the study showed downstream levels of SOD, CAT, GPx, GSH and TPC and upstream levels of LPO, total level of cholesterol, LDH, ADH and GGT. Toxicant exposure also de-structured the pulmonary tissue and on the other side both low and high doses of GEO, standard compound silymarin and vehicle olive oil improved the altered enzymatic and non-enzymatic parameters and re-structured the distorted pulmonary tissue to a greater extent. Thus, it is concluded that GEO plays a vital role in imparting protection to lung tissue from lead poisoning.

Keywords: *Allium sativum*, Lead Nitrate, Lung

Abbreviations: BALF- Bronchoalveolar lavage fluid; DAS- Di allyl sulphide; DADS- Diallyl di sulphide; GEO- Garlic essential oil; LN- Lead Nitrate; ROS- Reactive oxygen species

1. Introduction

Heavy metals are constant pollutants which are present in the environment and cause harmful and numerous dysfunctions to the specific organs of the body. They cannot be broken down into the human body but rather get stored and inhibit the normal functions of the cells and tissues after communicating with several macromolecules such as proteins and enzymes[1]. It is believed that lead (Pb) is the primary heavy metal that finds its way into the environment from the production of coal, iron, steel, oil, batteries, smelters, solid waste, and tobacco smoke. The gastrointestinal and respiratory systems are the primary routes of lead exposure[2]. According to the Toxic Substances and Disease Registry (ATSDR 2017) Lead is considered as the second most common toxic substance. Lungs are the main and sensitive organ of the human body which gets exposed to heavy metals like Lead. They are made up of several types of cells with different metabolic and immune functions[3]. A variety of parameters, including antioxidants and non-antioxidant indices like Lipid Peroxidation Level (LPO), Total Protein Content (TPC), Total Cholestrol Level (TCL), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), and lung toxicity biomarkers like Lactate Dehydrogenase (LDH), Alcohol Dehydrogenase (ADH), and Gamma Glutamyl-Transpeptidase (GGT), were employed to evaluate the lead-induced toxicity in

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the lungs of male Swiss albino mice. Exposure to lead triggers the production of Reactive Oxygen Species (ROS), which through molecular and biochemical pathways induce oxidative stress in the target cell. This further causes DNA damage and cell death[4]. Oxidative stress playsa main role in several respiratory diseases like asthma, pulmonary fibrosis, ischemia-reperfusion and chronic obstructive pulmonary disease. Oxidative stress causes antioxidant–oxidant imbalances in Broncho-Alveolar Lavage Fluid (BALF) and lung tissues which contribute to respiratory diseases[5]. Throughout history, medicinal plants have been utilized by all societies as a means of treating a wide range of illnesses. In recent years, *Ayurvedic* herbs with their biological activities align themselves in focus for their cost effectiveness, natural origin and lesser side effects. *Allium sativum* is one of the most well-known herbal medicines. It has been used for ages and has a special place in history. It is used to treat several dysfunctions likecolds, diabetes, lowering blood pressure, heart disease, lung infections, glucose concentration, and arteriosclerosis. It can be used to treat lead-related illnesses due to its antitumor, anti-diabetic, anti-inflammatory, antioxidant, immunomodulatory and other beneficial properties. Garlic has two main classes of antioxidant components:sulphur-containing compounds (diallyl sulphide, tri sulphide and allyl-cysteine) and flavonoids. These components play an important role in treating several dysfunctions against Lead toxicity[6]. So, after recognizing the medicinal properties of garlic, this study was designed to measure the oxidative stress, biochemical parameters, lung biomarkers and histoarchitecture of lung tissue in mice to investigate the protective potential of garlic essential oil against lead toxicity.

2. Material and Methods

2.1 Chemicals Used in the Experimental Study

Lead nitrate was acquired for this study from the Central Drug House in India. Sisco Research Laboratories (India), SD Fine Chemicals (India), HIMEDIA (India), Qualigens (Germany/India), and Central Drug House (India) provided the otherremaining chemicals and reagents needed for the investigation. These chemicals were of the analytical grade. Microlabs Ltd. was the source of the silymarin suspension.

2.2 Plant Specimen

The plant employed in this experimental study was garlic *(A. sativum*), which was sourced from the premises of Banasthali Vidyapith (BV), Rajasthan, India. It was identified and authenticated as a local variety from Krishi Vigyan Kendra (BV) and submitted in the Herbarium section with authentication number no. BURI-1710/2022 in Bioscience and Biotechnology Department at Banasthali Vidyapith, Rajasthan, India.

2.3 Preparation of Garlic Essential Oil

2.3.1 Hydro-Distillation Technique

The garlic essential oil was separated with the help of aglass apparatus called Clevenger. The garlic bulbs were first cleaned, peeled and crushed with the help of distilled water and then crushed bulbs were taken into the round bottle flask which was further connected to the Clevenger extractor. Then the crushed bulb mixture was heated for 4-5 hours until no more essential oil was extracted. After the extraction procedure, the oil was dried over anhydrous sodium sulphate. The oil yield (%) was calculated by the formula given below and the extraction was done in triplicates[7]. After the calculation of % oil yield, it was stored at 4ºC in a vial for further experiments.

$$oil\ yield\ \% = \frac{volume\ of\ oil\ obtained\left(ml\right)}{weight\ of\ sample} \times 100$$

2.4 Experimental Animal Specimen

The study employed male Swiss albino mice which were collected from Lala Lajpat Rai University (Hisar), Haryana, India. They were in the weight category of 20-30g and the ethical committee of Banasthali Vidyapith approved the experimental study (approval no. BV/IAEC/January/2020/10). The animals were housed in an air-conditioned room at a temperature of 25°C ± 3°C, 50% ± 5% humidity, and a 12-hour light/dark cycle in cages made of polypropylene. The mice were given a chow meal and an endless supply of water to drink during the experimental study. Before the experiment commencement, animals were also acclimatized for a week.

2.5 Experimental Design

In this thirty-day study, thirty-six adult male Swiss albino mice were used. These mice were subsequently divided into

six groups, each group consisting of six animals. During the experimental trial, all the animals except the control mice were given lead nitrate (50mg/kg body weight) orally which was dissolved in double-distilled water for 30 days. From the 12th day until the end of the experiment, the animals of groups III, IV, V, and VI were also given garlic (*A. sativum*) essential oil prepared in vehicle oil, silymarin, and olive oil along with LN up to the end of the experiment. Here, GEO was used as a therapeutic agent to counteract the toxicity of lead nitrate and was given at two different concentrations which were: a low dose of 50 mg/kg and a high dose of 80 mg/kg. Silymarin was selected as the standard drug (25mg/kg)[8], and olive oil served as the vehicle control group. Based on laboratory experiments, the dosage of LN was determined[9] and previous research served as the basis for the collection of plantdoses[10]. Animal grouping was as follows: Table 1.

Table 1. Grouping of animals for experimental study

Groups	N = 6 in each group	Treatment
I	Control / untreated	Double distilled water
II	Toxicant treated (LN)	50mg/kg body weight
III	LN+ GEO low dose	LN 50mg/kg body weight + low dose GEO (50mg/kg)
IV	LN+ GEO high dose	LN 50mg/kg body weight + high dose GEO (80mg/kg)
V	LN+ Silymarin	LN 50mg/kg body weight + 25mg/kg of silymarin
VI	LN+ Olive oil	LN 50mg/kg body weight + Olive oil

The mice were provided with an overnight break after the completion of the work. Then on the next day, their weight was determined before sacrifice. The lung tissues were quickly removed, washed in an ice-cold buffer, weighed, and then stored in a buffer for use in other experimental studies.

2.6 Organ Weight

After sacrifice, the lungtissue of the mice was removed and their relative weight was determined using a formula:

$$Relative\ organ\ weight = \frac{O}{T} \times 100$$

Where 'O' represents a change in organ weight while 'T' represents a change in body weight taken at the time of sacrifice[11].

2.7 Homogenate Preparation

In this process, lung tissue was taken into the centrifugal tube along with the homogenate buffer (pH-7.4), (0.1M) and then minced with the help of the homogeniser (Yorco high-speed tissue homogeniser). After that, centrifugation was performed at 10,000 rpm for 15 to 20 minutes at 4°C. After that, the supernatant was collected and stored at -20°C for further use[12].

2.8 Oxidative Stress Parameters

2.8.1 Superoxide Dismutase (SOD)

A reaction mixture was prepared using the following reagents: 1.5 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 ml of Nitro Blue Tetrazolium (NBT) (2.25 Mm/L), 0.2 ml of sample homogenate, 0.1 ml of Na_2CO_3, 0.2 ml of methionine (200µM), 0.1 ml of EDTA (3mM/L), and 0.1 ml of riboflavin (60µM). In a separate test tube blank was prepared into which all these reagents were poured exceptthe tissue sample. After 60 minutes of light incubation at room temperature, the volume was raised to 3 millilitres using distilled water and the absorbance at 560 nm was then measured[13]. The following formula is used to calculate activity expressed in U/min/mg of protein.

Here A represents SOD activity.

$$A = \frac{1}{100} \times absorbance \times \left(\frac{volume\ of\ reaction\ mixture}{volume\ of\ sample} \right)$$

$$specific\ activity\ of\ SOD = \frac{absorbance}{protein\ content}$$

2.8.2 Catalase (CAT)

The reaction mixture consisted of 1.9ml phosphate buffer (50Mm/L) (pH7), 1ml H_2O_2 (0.09 M) and 0.1 ml homogenate. Blank contained 2.9 ml buffer and 0.1 ml homogenate without H_2O_2 and were mixed properly. Then the absorbance was recorded at 240nm[14]. The activity was estimated using the following formula and represented as µmole of hydrogen peroxide consumed/min/mg of protein:

$$A = \left((A2 - A1) \times \frac{1}{0.436} \right) \times \left(\frac{volume\ of\ reaction\ mixture}{volume\ of\ sample\ taken} \right)$$

Here A represents catalase activity.

A2 = absorbance taken with H_2O_2 consumed

A1 = absorbance taken without H_2O_2 consumed

$$specific\ activity = \frac{Absorbance}{protein\ content}$$

2.8.3 Glutathione Peroxidase (GPx)

The reaction mixture contained 0.4 ml of Tris-HCl buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of H_2O_2 (10 mM), and 0.1 ml of tissue homogenate. A second test tube was made as a blank, to which all the chemicals were introduced except tissue sample. Distilled water was used to make the volume 1 ml. The mixture was incubated at 37°C for 10 minutes. After that, centrifugation was performed for ten minutes at 3000 rpm meanwhile 0.5 ml of 10% TCA (trichloroacetic acid) was added to it. Later on, 0.5 millilitres of the supernatant was taken and combined with 0.5 millilitres of DTNB (0.04%) and 2 millilitres of Na_3HPO_4 (0.3 M). At 420 nm, the absorbance was determined[15]. The formula below was used to compute the enzyme activity, which is expressed as nmol NADPH oxidized/ minute/ milligram of protein:

$$GPx = \left(A \times \left(\frac{1}{6.22} \right) \right) \times \left(\frac{total\ volume\ of\ reaction\ mixture}{sample\ volume} \right)$$

Here, A represents the absorbance of the sample.

$$specific\ activity = \frac{GPx\ activity}{protein\ content}$$

2.8.4 Reduced Glutathione (GSH) Content

In this parameter, 2.5ml of 0.1 M phosphate buffer, 0.8ml EDTA (1mM), 0.1 ml DTNB (10mM) and 0.1ml of tissue sample were taken while in another test tube blank was prepared by mixing all the reagents except tissue sample and all were mixed properly. Distilled water was used to acquire a volume up to 4 millilitres. The formation of a yellow colour because of DTNB's reaction with the sulfhydryl-containing molecule was obtained and GSH activity was then measured at 412 nm[16]. The activity of GSH was expressed as µmole GSH/g wet tissue and is calculated by using the formula given below:

$$GSH = \left(\frac{1}{13.6} \times A \right) \times \left(\frac{Volume\ of\ reaction\ mixture}{volume\ of\ sample} \right)$$

Here, A represents absorbance.

$$Specific\ activity = \frac{absorbance}{protein\ content}$$

2.8.5 Lipid Peroxidase Activity (LPO)

The reaction mixture contained the following components: 0.2 ml tissue homogenate, 0.4 ml distilled water, 1.5 ml TBA (0.6%), 0.2 ml KCL (1.15%), 0.2 ml SDS (8.1%), and 1.5 ml acetic acid (20%, pH-3.5). On the other hand, a blank test tube was prepared, and all the chemicals were introduced except tissue sample. With distilled water, the volume was adjusted to 4 ml in each tube. This was followed by mixing the reaction mixture and heating it in a water bath for 60 minutes at 95°C to get the pink hue. The pink colour was formed due to the reaction of MDA with TBA the secondary product of lipid peroxidation. It was extracted in a butanol: pyridine (5ml) layer after centrifugation at 3000rpm and absorbance was recorded at 532nm[17].

Calculation: Using the molar extinction coefficient of 1.56×10^{-5}/mole/cm, the calculation was done, and the result is expressed as nmol MDA formed/mg tissue.

$$LPO = \left(\frac{O.D\ of\ unknown}{156 \times mg\ of\ protein} \right) \times 1000$$

2.9 Biochemical Parameters of Lungs

2.9.1 Total Protein Content

The reaction mixture contained 0.1 ml of lung tissue homogenate and 2.5 ml of Bradford reagent. In contrast, the blank was prepared with 2.5 ml of Bradford reagent free from tissue sample and then the volume was built up to 3 ml with distilled water. The absorbance at 595 nm was measured at room temperature after 5 min incubation. In addition, the BSA (1 mg/ml) standard was also prepared[18]. Using the BSA standard curve, the quantity of protein was measured in milligrams per gram of wet lung tissue.

2.9.2 Total Cholesterol Content

Different aliquots of standard curve tubes were taken and on the other hand, another tube was taken with 0.05ml tissue homogenate. In all the tubes, volume was built up to 4ml with 8% glacial acetic acid. Then 2ml of the fresh colouring agent was added to all the test tubes followed by the incubation of 15 minutes and then the absorbance was recorded at 550nm[19]. The calculation was done by making a standard plot between the concentration of standard cholesterol (µg) and absorbance. Cholesterol amount is expressed as mg/g of tissue homogenate.

2.10 Lung Biomarkers

2.10.1 Lactate Dehydrogenase (LDH)

In this parameter, three test tubes were taken and labelled as test, blank and control. In the blank 1.2 ml phosphate buffer and 1 ml DNPH solution were added while in control 1 ml of working pyruvate solution (as buffered substrate), 0.2 ml phosphate buffer and 1 ml of DNPH solution were added. One ml of buffered substrate and one ml of tissue homogenate were used in the experiment. The three test tubes were then submerged in a water bath and kept at 25°C for 10 to 15 minutes. Then 0.1 ml of NADH solution was added to start the reaction. One ml of DNPH solution was also added to the tubes, which were then vortexed and kept at room temperature for twenty minutes. Then the final volume was made by adding 10ml of NaOH solution and was mixed properly. Lastly, the absorbance was recorded at 510nm[20].

$$LDH\ activity\left(\frac{U}{L}\right)=\left(\frac{control-test}{control-blank}\right)\times 50$$

2.10.2 Alcohol Dehydrogenase (ADH)

The reaction mixture contained 1.30 ml of sodium pyrophosphate buffer (50 mM, pH 8.8), 0.10 ml of 95% ethanol, 1.50 ml of NAD (15 mM), and 0.1 ml of tissue homogenate. After that, 340 nm wavelength was used to measure the absorbance[21].

$$ADH = Absorbance\times\frac{3.21}{amount\ of\ protein}$$

2.10.3 Gamma Glutamyl Transpeptidase (GGT)

The reaction mixture contained the following reagents: 1.425 ml of phosphate buffer (pH-6.5, 0.1M), 0.05 ml of tissue homogenate, 0.2 ml of reduced glutathione solution (1 mM), and 0.025 ml of CDNB solution (1 mM). Following the successful construction of each component, the absorbance was measured at 340 nm in comparison to a blank, which lacked the tissue sample[22]. Using distilled water, the volume was adjusted to 2 ml. Activity was computed using the following formula:

In the calculation, a molar extinction coefficient of 9.63×10^{3}M/cm was utilized and expressed as: min/mg of protein/mole of CDNB conjugate produced.

$$\frac{\Delta A}{min}=\frac{final-initial}{total\ reation\ mixture}$$

$$GGT\left(\frac{IU}{L}\right)=\left(\frac{\Delta A}{minute}\right)\times 2201$$

2.11 Histopathological Examination

The lung tissue of each group was subjected to histopathological investigation using Hematoxylin and Eosin (H and E) staining method. In this analysis, the lung parts were fixed in 10% formalin. After that the thin paraffin sections of 3-4mm of lung tissue were cut into pieces. Further,these sectioned pieces were examined under a light microscope after stained with H and E (Nikon H600L, made in Japan) for the histopathological examination[23].

2.12 Statistical Analysis

The experimental data was analysed using S.P.S.S. 20, a statistical tool for social science software. For the results in this experimental work, which is provided as mean ± S.E.M., the threshold of significance (% of O.D. change) between the groups was established at $P<0.05$, $P<0.01$, and $P<0.001$. One-way analysis of variance (ANOVA) and post hoc pair-wise analysis of homogeneous variances (Tukey's test) were also used to compare the experimental groups.

3. Results

Results showan increase and decrease in enzymatic and non-enzymatic parameters of lung tissues in mice on exposure to the inorganic salt of lead. Apart from the toxicant, GEO, standard drug and vehicle oil oral administration shows improved levels of these parameters in animals exposed to LN. The results of this study are depicted below.

3.1 Organ Weight Changes in Mice

The organ weight of the animals in the lead nitrate-intoxicated group was decreased when compared to the control group. In contrast to the mice group treated with LN, weight gain was seen in groups III and IV animals administered with varying dosages of garlic bulb oil. The changes in tissue weight unequivocally show how the toxicant has affected the mice's lungs (Table 2).

3.2 Pulmonary Oxidative Stress Parameters

The effect of LN, GEO, standard drug and vehicle oil on enzymatic and non-enzymatic levels in the lung tissue

Table 2. Mean relative organ weight of experimental animals on exposure to lead nitrate

GROUPS	CONTROL I	LEAD NITRATE (50mg/kg) II	LN+ GARLIC ESSENTI-AL OIL LOW DOSE (50mg/kg) III	LN+ GARLIC ESSENTI-AL OIL HIGH DOSE (80mg/kg) IV	LN+ SILYMARIN (standard drug) (25mg/kg) V	LN+ OLIVE OIL (vehicle) VI
Final organ weight (gm)	0.36±0.03	0.26±0.04	0.33±0.01	0.37±0.007	0.29±0.02	0.27±0.03
Relative organ weight (gm)	0.2±0.04	0.05±0.02*	0.04±0.00*	0.22±0.01	0.13±0.02	0.12±0.03

Values are presented in Mean ± SEM. (n=6/group)
Relative organ weight Organ weight/body weight (gm)
P<0.05* represents the significance value of the control group with all other groups.
Except for the asterisk mark ones all other values are non-significant (NS).

of mice are illustrated in Figure 1 (A, B, C, D). The level of SOD, CAT, GPx and GSH decreased with the significance value of P<0.01, P<0.05, P<0.01, P<0.05 in LN treated group in comparison with control group of mice.

The levels of SOD, CAT, GPx, and GSH were elevated by oral administration of both the low and high doses of GEO. A high dose of GEO was found to be more effective than a low dose in enhancing the effect of these parameters. As it significantly elevated the activity of SOD (P<0.01), CAT (P<0.01), GPx (P<0.01) and GSH (P<0.05) in group IV in comparison to the group II treated animals. Silymarin the standard drug was found to be ineffective in almost all the parameters except SOD and GPx having significant values of P< 0.01 and P<0.05 while vehicle olive oil did not show any recovery in all these parameters in comparison to LN exposed mice. In addition to these parameters, LPO level was also determined, and results conclude that the toxicant significantly increased its activity in group II mice in comparison to the control group animals with a significance value of P<0.05. However, the low dose of GEO was able to treat the toxicity to a moderate level (P<0.05), but the high dose of GEO significantly improved the altered LPO level in group IV animals with a significance value of P<0.01 in comparison with the group II animals. Here, standard drugs and vehicle oils played insignificant roles in treating the toxicity in group V and VI animals (Figure 1: E).

3.3 Pulmonary Biochemical Parameters

The results of pulmonary biochemical parameters in mice against LN-induced toxicity and the therapeutic role of GEO are presented in Figure 2. When compared the mouse model of group II to the animals of group I, a significant regression (P<0.01) in the total protein content was observed. In group III (low dose) insignificant rise of TP was observed while in group IV animals significant rise in TPC level was observed with a significance value of P< 0.05 (high dose). However, the GEO high dose tried to restore the protein content in the lung tissue of mice when compared to group II animals. That means a high dose was more efficient thana low dose in raising the protein level. When compared the animals of groups V and VI to those of group II animals, an insignificant rise in protein content was found (Figure 2: A).

In cholesterol estimation, its level was elevated (P<0.05) in the LN-treated group in comparison with the animals of the control group. Here, the GEO high dose (P<0.01) was more effective than the low dose in declining the effect of lead nitrate in comparison to group II mice models. In Group V and VI animals the standard drug and vehicle oil were both found to be ineffective in declining the toxicant level in comparison to the Group II animals (Figure 2: B).

3.4 Pulmonary Lung Biomarkers Parameters

The observations related to lung biomarkers in mice against LN-induced toxicity and the therapeutic role of GEO are presented in Figure 3. On exposure to lead nitrate in group II mice the activity of LDH witha significance value of P<0.01, ADH with P<0.001 and GGT (P<0.05) elevated in comparison to the group I animals. A low dose of GEO in group III resulted in the decline of the LDH activity (P<0.01), ADH activity (P<0.01) and GGT activity (P<0.05) in comparison to group II animals. Whereas, on administration with

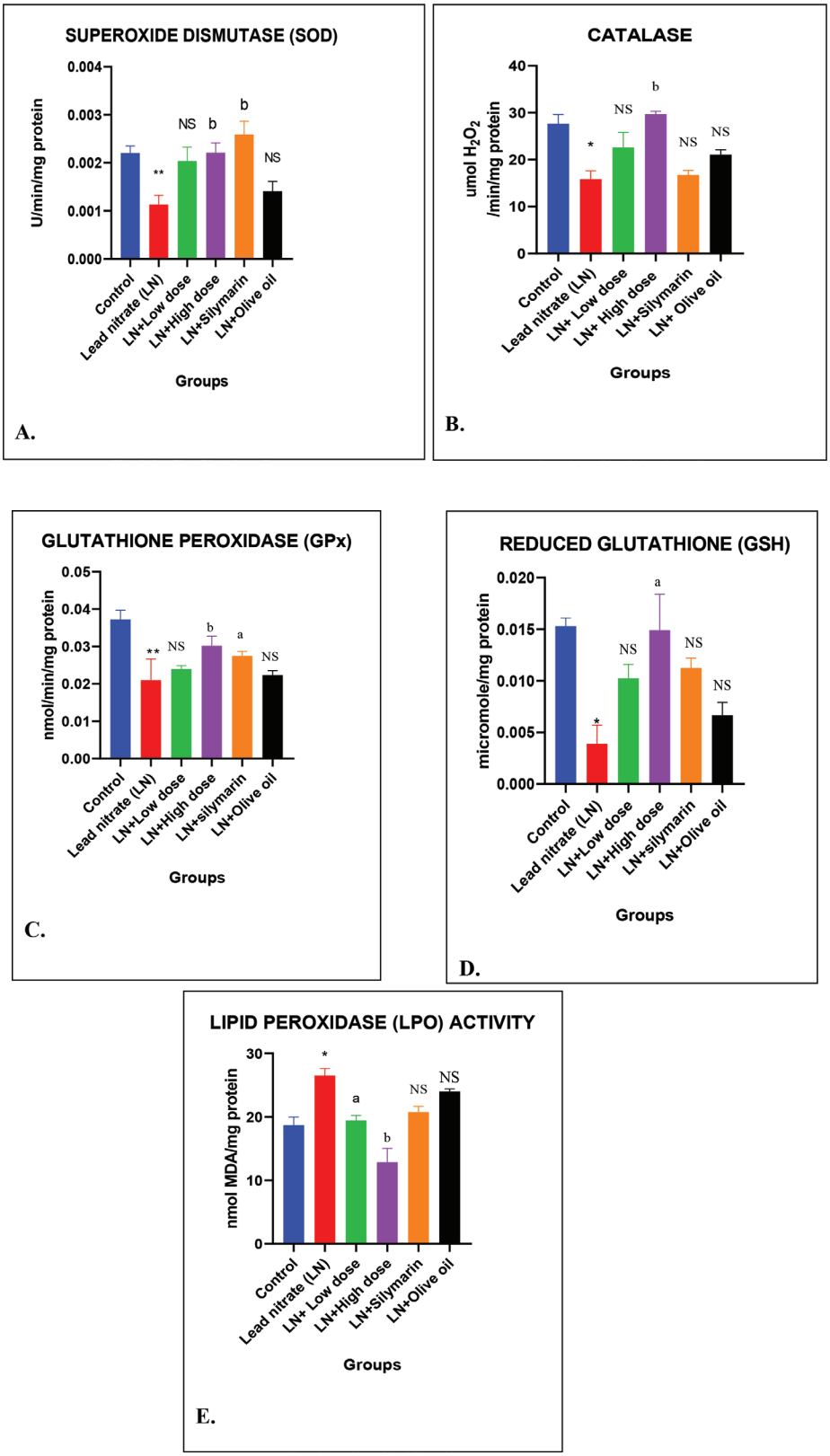

Figure 1. Graphs showing the level of lead nitrate and garlic essential oil in different groups of the experimental study. **A.** SOD, **B.** CAT, **C.** GPx, **D.** GSH, **E.** LPO. NS stands for non-significant, n=6. Significance differences in data are expressed as - $P<0.05^{*}$, $P<0.01^{**}$, $P<0.001^{***}$ lead nitrate compared to the control group. $P<0.05^{a}$, $P<0.01^{b}$, $P<0.001^{c}$. When other treated groups are compared with LN treated group.

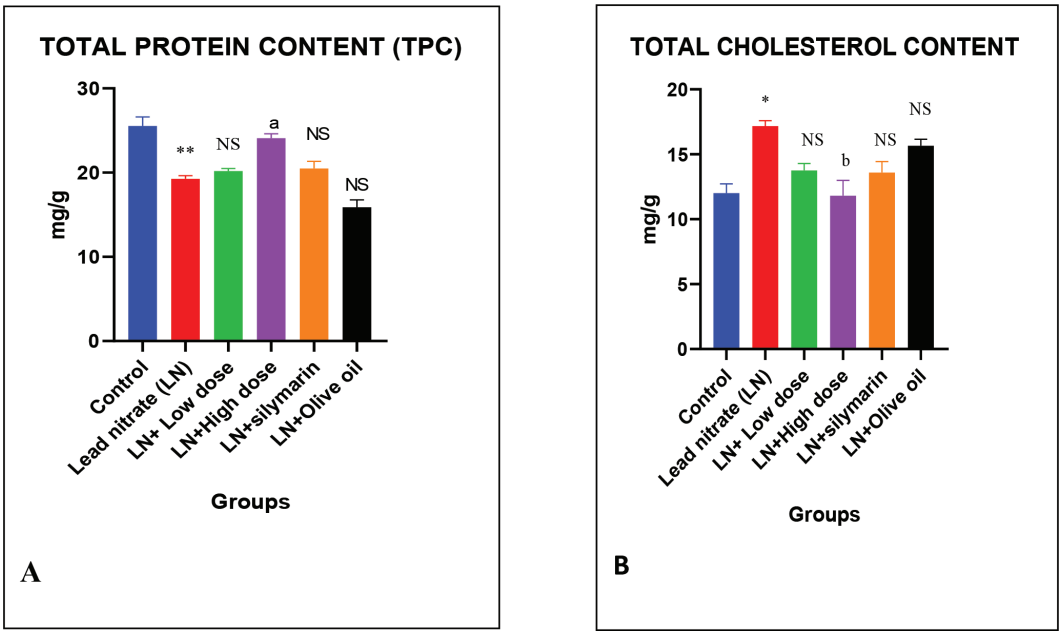

Figure 2. Graphs showing the level of lead nitrate and garlic essential oil in different groups of the experimental study. **A.** TPC, **B.** Cholesterol. NS stands for non-significant, n=6. Significance differences in data are expressed as - $P<0.05^{*}$, $P<0.01^{**}$, $P<0.001^{***}$ lead nitrate compared to the control group. $P<0.05^{a}$, $P<0.01^{b}$, $P<0.001^{c}$. When other treated groups are compared with LN treated group.

the high dose of GEO the activity of LDH (P<0.001), ADH (P<0.001) and GGT (P<0.01) declined in a more efficient way than the low dose in group IV animals in comparison to group II mice. Significant decreases of LDH (P<0.01), and ADH (P<0.01) content were observed in group V while insignificant decreases of these enzymatic parameters were recorded in group VI in comparison with the group II animals. Further GGT level recovery was found to be insignificant in groups V and VI animals (Figure 3. A, B, C).

3.5 Histopathological Examination of Lung Tissues

The results of the histopathological examination of mice in all experimental groups which were exposed to lead nitrate alone and in combination with the GEO low dose, GEO high dose, silymarin and vehicle olive oil are shown in Figure 4. Control group animals show the normal cytoarchitecture of the lung with well-defined alveolar space, fine interstitium along the alveolar walls and the capillaries with RBCs all together formingthe air blood barrier and the respiratory membrane seen in the pulmonary tissues of mice, seen in Figure 4(1). Figure 4 (2A and 2B)shows destroyed lung tissuesin the LN-treated group in which an array of pathological manifestations including alveolar congestion with Moderate Lymphocyte Hyperplasia (MLH) is seen along with the alveolar septal thickening. In addition to these, infiltration of mononuclear cells are also observed in the pulmonary tissue of mice. In addition, Figure 4 (3 and 4) low and high doses of GEO groups demonstrate favourable outcomes against metal toxicity because the DADS (di-allyl di-sulphides) in the GEO may contribute to the preservation of pulmonary parenchyma cells, Alveolar Septa (AS), in the Pulmonary Alveoli (PA) restored to a greater extent than that of the animals in the control group. However, in Figure 4(5), which is the silymarin-treated group, used as a standard drug maintained the altered structure of lung tissue to some extent. On the other hand, in Figure 4(6); olive oil the vehicle in this study shows lesser impact in declining the lead-induced toxicity in mice lung, as it was unable to maintain the alveolar septa intact but to some extent reduced inflammation in the tissue.

4. Discussion

Lead is a heavy metal that contaminates the environment in various forms. It causes severe effects on human health resulting in alteration of the metabolism and physiology of the lung tissues. As lungs are the first organ of the body

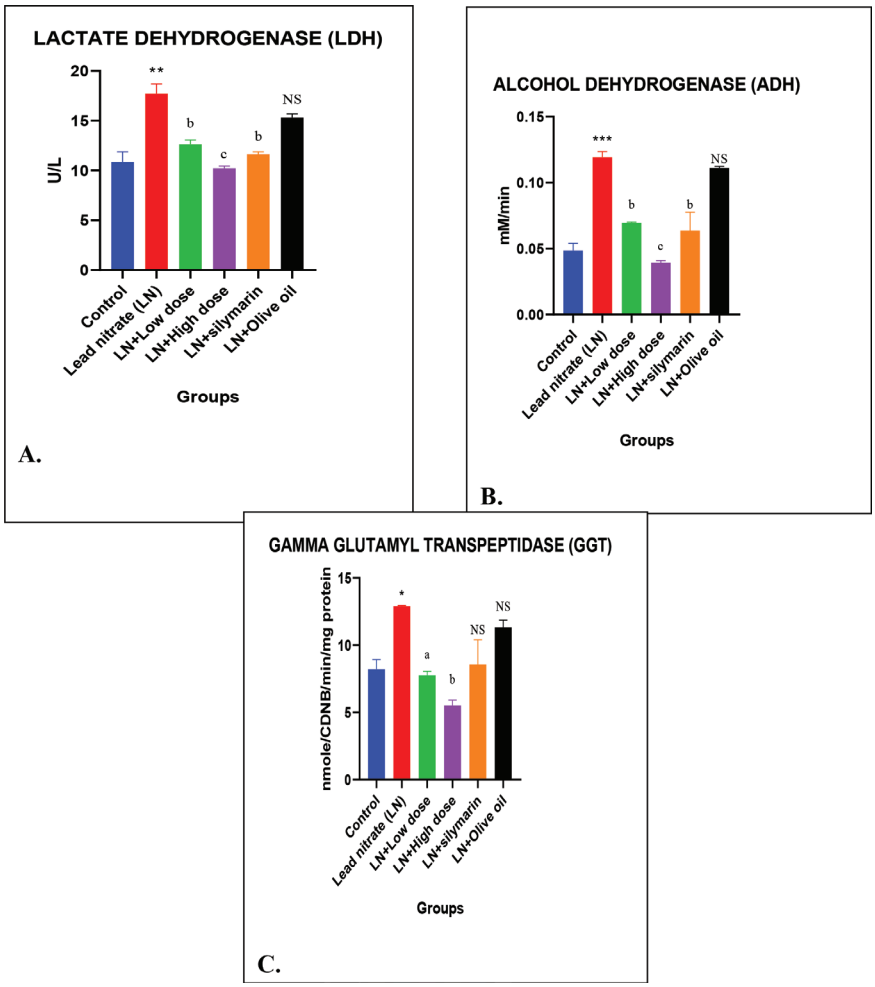

Figure 3. Graphs showing the level of lead nitrate and garlic essential oil in different groups of the experimental study. **A.** LDH, **B.** ADH, **C.** GGT. NS stands for non-significant, n=6. Significance differences in data are expressed as - P<0.05*, P<0.01**, P<0.001*** lead nitrate compared to the control group. P<0.05[a], P<0.01[b], P<0.001[c]. When other treated groups are compared with LN treated group.

which gets affected by metal toxicity. The findings of this study showed that when lead nitrate was administered orally to mice of group II then the antioxidant and non-antioxidant enzymes level (SOD, CAT, GPx, and GSH) in the lung tissue of the mice showed a decreased pattern in comparison to group I mice. Down regulation of these indices therefore results in oxidative stress, which damages DNA and modifies proteins by causing lipid peroxidation of the cell membrane. These deleterious changes affect the structure and function of the lung tissues leading to the progression of several respiratory diseases[24]. SOD serves as a primary defence against ROS as it catalyses the conversion of ROS to hydrogen peroxide and molecular oxygen. The most common toxic by product of several metabolic processes is hydrogen peroxide. CAT protect the cells from the harmful effects of hydrogen peroxide by breaking it into water and oxygen. Later, GPx also maintains the redox balance by converting the hydrogen peroxide into water and alcohol (Figure 5).

However, GEO helps in re-attaining the normal levels of antioxidant and non-antioxidant parameters to a greater extent, due to the antioxidant properties[25] of Organo-sulphur compounds present in GEO. These components also exhibit metal chelating activity and help in scavenging free radicals from the cell.

GSH is an intracellular, multifunctional, non-enzymatic antioxidant and a major thiol-disulphide redox buffer of the cell[26]. The results showed that reduction in GSH is another important mechanism

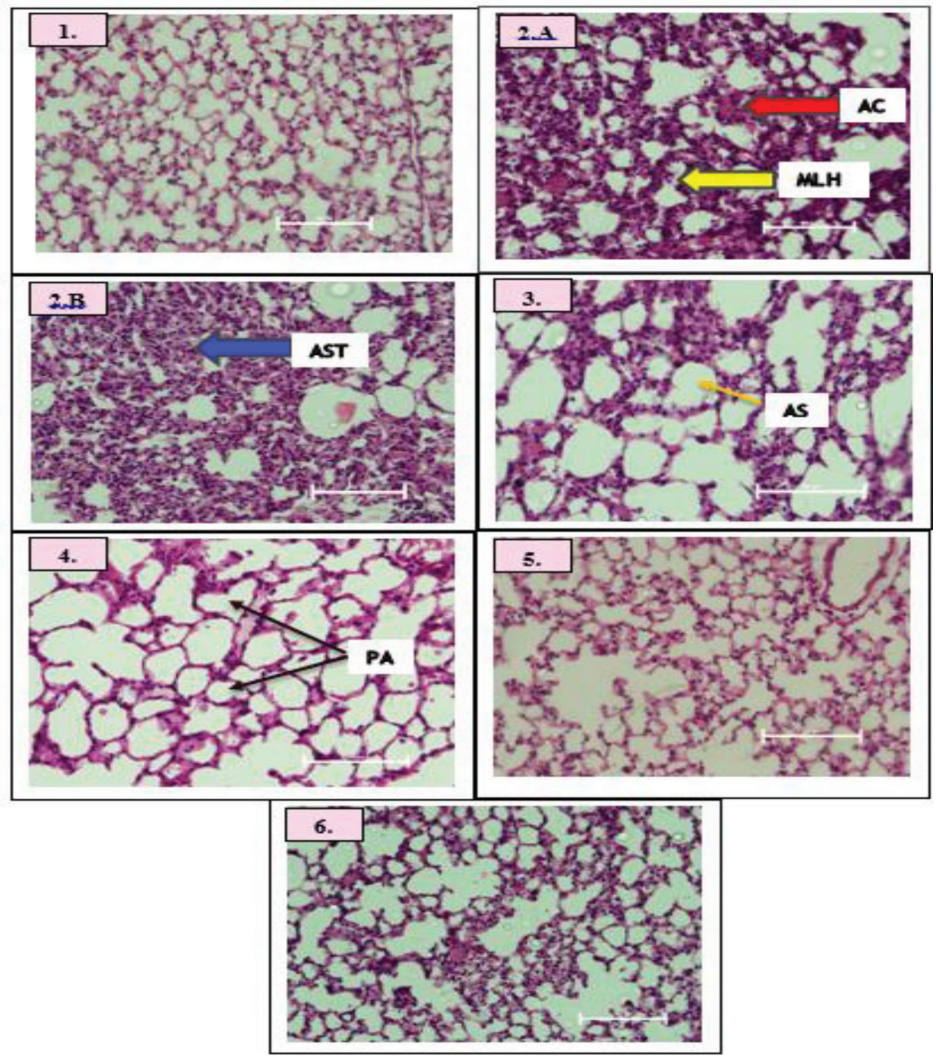

Figure 4. Histopathological examination of all the groups in the experimental study. **1.** Represents the normal architecture of lung tissue of control group animals. **2A.** Represents Alveolar Congestion (AC) (red arrow) and the yellow arrow represents the MLH, **2B.** (blue arrow) Representsthe Alveolar Septal Thickening (AST), haemorrhages, diffuse mild type II pneumocyte proliferation and infiltration of mononuclear cell infiltration of 30 days exposed mice with lead nitrate through oral administration. **3.** The toxicity in the lungs declined in this group because of garlic essential oil (low dose) on lead nitrate toxicity as it maintained the AS intact to some extent shown by the orange arrow. **4.** Toxicity level declined in a more significant way shown by the high dose of GEO by maintaining the PA structure intact shown by black arrows. **5.** Silymarin treated group used a standard drug. **6.** The oliveoil-treated group had less effect on the toxicity and was used as a vehicle. Magnification 20X and 40X were used for the interpretation of the data.

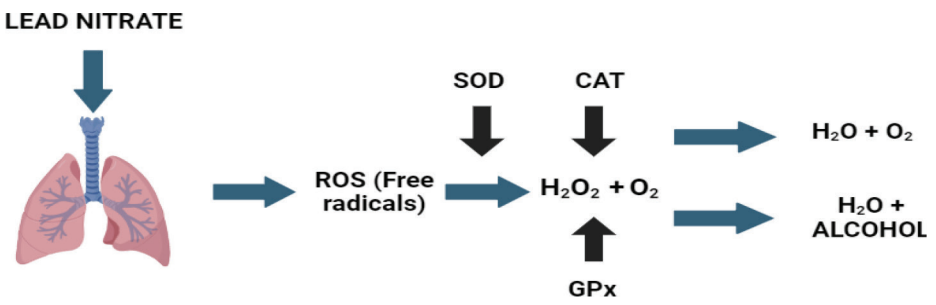

Figure 5. The conversion of ROS into hydrogen peroxide, oxygen, water and alcohol.

of lead nitrate toxicity. The antioxidant ability of sulfur atoms, which are found in thiol compounds, allows them to readily compensate for the loss of one electron. By binding itself to the GSH–SH group, lead reduced the GSH activity[27]. DADS (diallyl disulphide) in garlic act as an enzyme activator for SOD, CAT and GPx, a stimulator of GSH synthesis and an inhibitor of cytochrome P450 family, restricts the production of ROS and carcinogens. In addition to antioxidant properties, DADS also have anti-inflammatory effects, boost antioxidant enzymes, reduce cytokine production, and decrease oxidative stress indicators. In the current research,it was observed that the high dose of GEO helped the tissues to get rid of oxidative damage caused by lead nitrate. In the current investigation decrease in the TPC level in the LN-treated groupwas also noticed. Lead tends to bind with the DNA, and thus interferes with the transcription process affecting the mRNA and ribosomal protein synthesis. In the present study, GEO's sulphur compound protected the lung from the deleterious effect of LN by increasing the TPC level. By further activating the M1 or M3 receptor, GEO sulfur components strengthen intracellular Ca2+ by interacting with G protein and causing the receptor to undergo conformational changes. PIP2 then hyd rolysed(phosphatidylinositol– (4, 5)-bisphosphate) to yield DAG (dactyl-glycerol) and IP3 inositol (1, 4, 5)-triphosphate. IP3 enters the endoplasmic reticulum and releases calcium ions (Ca2+), which compete with lead and shield the cell from lead toxicity[28]. However, silymarin also to some extent increased the total protein level as it is a polyphenolic flavonoid known for its antioxidant properties which play a central role in scavenging free radicals, chelating free elements, maintaining redox balance of the cell, prohibiting ROS-producing enzymes, boosting the integrity of mitochondria, declining inflammatory responses and maintaining GSH level in the cell. Apart from silymarin, olive oil was also found to be beneficial in the recovery of total protein as it possesses vitamin E and polyphenols that help in stabilizing lung injuries by neutralizing free radicals therefore inhibiting oxidative damage to pulmonary tissues of mice.

On the other side of the study, there was an increase in cholesterol levels in the lead-intoxicated group in comparison with other groups. Cholesterol plays a vital role in maintaining the cell membrane structure and function. After exposure to lead nitrate, the key enzymes involved in cholesterol metabolism, transport and biosynthesis are affected. Thereby, causing hypercholesterolemia, which in turn activates the enzymes that synthesize cholesterol (3-hydroxy-3methyglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase, CYP51), and suppress the enzymes that catabolize cholesterol, including 7α-hydroxylase. The results of the current investigation demonstrated that the anti-inflammatory and antioxidant characteristics of garlic changed the metabolism of cholesterol. By inhibiting HMG-CoA reductase, a thiol group enzyme that limits the production of cholesterol, and CoASH, a multi-enzyme complex that facilitates the creation of fatty acids, garlic helps to lower blood levels of cholesterol, triglycerides, and total lipid. Garlic's allyl-disulphide or allyl-sulfhydrl group are accountable for inhibiting cholesterol synthesis and the principal mechanism of garlic to inhibit cholesterol levels is by inhibition of 4α-methyl oxidase[29]. Here, the high dose of GEO played a vital role in regulating lead-induced toxicity in the lungs of mice. Further olive oil administration also reduced the cholesterol content as it contains monounsaturated fats that can positively affect cholesterol levels in group VI animals. Hence, according to these findings, olive oil can also treat Pb toxicity to some extent.

Besides all these parameters, the study also emphasised the lung biomarkers namely: LDH, ADH and GGT. The two primary enzymes involved in the metabolic pathways are Lactate Dehydrogenase (LDH) and Alcohol Dehydrogenase (ADH). While LDH catalyses the conversion of pyruvate to lactate, ADH catalyses the conversion of alcohol to ketones or aldehydes. Here, higher levels of LDH and ADH were observed in the LN-treated mice when compared to control group animals. This is because lead binds to the –SH groups of LDH and ADH proteins thereby disrupting their catalytic activity. Disruption of LDH activity by lead resulted in disruption of the balance of pyruvate and lactate and affect ATP production which is followed by an overall energy imbalance in the lung tissue while ADH-impaired activity caused cellular damage[30]. In contrast to the Pbtoxicity, GEO treatment tried to restore the levels of both biomarkers as it contains Allicin which is the main active component, exhibit the ability to bind with the free sulfhydryl

group of enzymes and form the disulphide bond which results in inactivation of the enzyme activity[31]. Apart from the LDH and ADH enzymatic activity, the study was also focused on the activity of GGT in pulmonary tissues. Here the increased level of GGT in LN treated mice group was significantly decreased in groups III and IV on the treatment of GEO. However, GGT activity is localised in the alveolar epithelium of Clara cells and on the luminal surface of type II cells. The primary role of GGT was to recover extracellular GSH, which the cell loses. It is important for the glutathione cycle, which takes place at the plasma membrane and ensures cysteine homeostasis, intracellular GSH levels, and the redox status of the cell[32]. GEO may be due to its sulphur compound (DAS) played an important role in declining the level of GGT in group III and IV animals. The increased level of GGT activity in LN-treated group II animals may be caused by the carcinogenic polycyclic hydrocarbons. However, olive oil has oleocanthal compound with anti-inflammatory properties which also retained the function of LDH, ADH and GGT enzymes in the lungs. Thus, this may be due to monounsaturated fats which get incorporated into the cellular membrane and provided stability and protection against lead nitrate in group VI mice.

From the results of the histopathological examination of lung tissue, we can summarize that GEO helped in returning the tissue alveoli and bronchioles to their natural form except for congestion of accompanied blood vessels in animals of some groups. With these findings, we can conclude that GEO plays a vital role in ameliorating lead-induced pulmonary toxicity in mice efficiently.

5. Conclusion

This experimental study provides significant information about the medicinal properties of the garlic essential oil that plays a therapeutic role in declining lead nitrate-induced toxicity in pulmonary cells. In this study, pathological changes were seen in lung alveoli and bronchioles, and these were efficiently reduced by different bioactive components present in GEO. Therefore, garlic has the potential to deal with respiratory illness after exposure to lead nitrate and it should be taken into consideration for further medicinal research and drug developmental studies.

6. References

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