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# Prophylactic Efficacy of *Allium sativum* Essential Oil on Hepatic Tissues of Mice Model Exposed to Inorganic Lead Salt

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## Abstract

**Hypothesis:** Lead is a pervasive environmental pollutant that is major threat for human health. *Allium sativum* essential oil could impart possible protection from Lead Nitrate (LN) as it contains organosulfur compounds which possess various pharmacological potential including antioxidant, anti-inflammatory, anticancer, anti-apoptotic and other activities as well. **Parameters Studied:** The ameliorative role of *Allium sativum* essential oil on hepatic tissue damage caused by Lead nitrate was evaluated through oxidative stress, biochemical parameters, oxidative Stress Index (OSI) and hepatic biomarkers. **Methodology and Results:** In this study, six groups of animals were taken. These groups were: control animals, toxicant treated animals (LN), LN + plant oil low and high dose treated animals, LN + silymarin treated animals and LN + vehicle oil control group. Lead nitrate exposure significantly decreased the antioxidant molecules mainly SOD, CAT, Gpx, GSH, GST, TPC and increased the lipid peroxidation content, Oxidative Stress Index (OSI), liver indices, Total Cholesterol Content (TCC) and biochemical parameters [ALT, AST, ALP] In addition to this, Lead nitrate increased the level of hepatic biomarkers such as cytp4502E1, 5'-nucleotidase, and γ-glutamyltranspeptidase and decreased the cytb5 content in hepatic tissues. **Conclusion:** Antioxidant activity of *Allium sativum* Essential Oil (ASEO) prevented oxidative stress and restored the level of liver indices, biochemical parameters, and hepatic biomarkers in Lead nitrate-intoxicated mice. Therefore, ASEO can be considered as a promising protective strategy against Lead nitrate-induced hepatotoxicity.

**Keywords:** *Allium sativum* Essential Oil (ASEO), Hepatic Biomarkers, Lead Nitrate, Oxidative Stress

## 1. Introduction

A worldwide increment of environmental pollutants increases the incidence of severe health disorders. Long term exposure to these toxicants may directly cause tissue injury[1]. Heavy metal exposure is considered to be carcinogenic due to its interaction with proteins related to the cell cycle, apoptosis, DNA synthesis and repair. Among various heavy metals compounds, Lead nitrate is believed to be a potent environmental pollutant and bioaccumulates from various industrial sources to natural sources (water, air and food)[2,3]. The majority of toxicants including Lead affects the liver and causes alterations at the tissue level mainly by inducing oxidative stress, cholestasis, necrosis and also cause ultrastructural changes in organelles like mitochondria, endoplasmic reticulum, and vascular lesions[4]. Liver is a vital organ for detoxification and metabolism[5]. If liver damage occurs due to these environmental pollutant, then it may result in the downregulation of antioxidants and upregulation of inflammatory markers[6,7]. So, in order to manage structural integrity of hepatic tissues,

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now-a-days use of natural products in our diet is becoming popular.

Natural product mainly *Allium sativum* commonly known as garlic, belongs to the *Alliaceae* family and is considered as an edible plant since ancient times for its medicinal properties[8]. Due to its various pharmacological potential, it has been considered as most valuable nutraceutical in most countries for its health-promoting effects and disease prevention. Essential oil extracted by chopping garlic bulbs activates the enzyme alliinase that converts allicin into organosulfur compounds mainly diallyl disulfide, diallyl sulfide, diallyltrisulfide, diallyltetrasulfide[9]. Mechanisms of hepatoprotective effects by herbal treatment include antioxidant, anti-inflammatory, antiviral, anticancer, anti-apoptotic, inhibition of hepatic stellate cell activation and cell cycle arrest[10]. Although extensive research has been conducted on the *Allium sativum*, but still there is an utmost demand to explore the novel drugs from this plant so that it can be used as a new therapeutic agent for liver ailments.

# 2. Material and Methods

## 2.1 Chemicals

All the reagents used in the current work were purchased from Himedia, Sigma, Qualigens and were of analytical grade.

## 2.2 Experimental Plant

*Allium sativum,* commonly known as garlic was used as an experimental plant in the present study and was collected from the premises of Banasthali Vidyapith, Rajasthan. The garlic bulbs were peeled out and smooth paste was made in grinder. The essential oil was extracted from the paste of bulbs of *Allium sativum* by hydrodistillation using clevenger apparatus. The flask containing garlic extract was heated for 3-4 h. As a result, the generated condensed vapors were separated through an oil-water separator and continuously flowing water with low pressure was provided to cool down the condenser temperature. After this, extracted oil was dried over anhydrous sodium sulphate and kept in a vial at 4°C for experimental studies[11].

## 2.3 Animals

Male Swiss albino mice were collected from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (India), weighing between 25-30 g (2-2.5 months). The animals were kept in propylene bags in a room with air conditioning, set at 25°C, 50% relative humidity, and 12-hour cycles of light and darkness. The mice were given access to chow food (Ashirwad Industries, Chandigarh) and drinking water *ad libitum*. The Banasthali Vidyapith ethical committee granted approval for experimental activity (Approval no. BV/IAEC/January/2020/10).

## 2.4 Experimental Design

For experimental purpose, the mice were divided into six groups with six mice in each group. The total duration of the study was of 30 days. The doses of *Allium sativum* essential oil, silymarin and vehicle oil were given to the animals from 12$^{th}$ day after beginning of Lead nitrate exposure to the end of the experiment.

The treated groups were as follows:

1. Group I: Control group (provided with distilled water).
2. Group II: Lead nitrate (50mg/kg) treated group.
3. Group III : Lead nitrate + Low dose of *Allium sativum* essential oil (50mg/kg)
4. Group IV: Lead nitrate + High dose of *Allium sativum* essential oil (80mg/kg)
5. Group V : Lead nitrate + Silymarin(25mg/kg)
6. Group VI: Lead nitrate + Vehicle control (olive oil treated group)

After completion of the experiment, animals were allowed to rest overnight and then on the following day, they were sacrificed by cervical dislocation. The animals were dissected and liver lobules were removed immediately, followed by washing in ice-cold saline. The liver tissues were further examined for biochemical, oxidative stress parameters, and hepatic biomarkers. Toxicant dose and *Allium sativum* essential oil doses were decided on the basis of previous studies[12,39].

## 2.5 Liver Indices

The mice were weighed after completion of the experiment. Mice were sacrificed and liver lobules were removed to analyze the weight. Finally, liver indices were calculated by the liver weight to body weight ratio which was further multiplied by 100[13].

## 2.6 Tissue Homogenate Preparation

The liver lobules were removed, blood was washed off with 0.9% saline and then they were blotted individually on

filter paper. After that, liver tissue was weighed and 10% (w/v) tissue was homogenized by means of a homogenizer (Remi Motor RQ-122) using ice-cold phosphate buffer saline and then centrifuged at 10,000 rpm for 15-20 minutes at 4°C. The supernatant was collected and stored at -20°C in a deep refrigerator for oxidative stress, biochemical parameters and hepatic biomarkers[14].

## 2.7 Oxidative Stress Parameters

### 2.7.1 Superoxide Dismutase (SOD)

It is mainly based on the auto-oxidation of pyrogallol into superoxide radicals which in turn dismutated by SOD to produce $H_2O_2$. In this assay, 0.1ml of supernatant was thoroughly mixed with 2.85ml of Tris-HCl (0.05mol/L containing 1mM $Na_2EDTA$) and 0.05ml of pyrogallol solution (60mM in 1mM HCl) and reading was taken at 325nm. The absorbance was recorded and the difference between 0 and 5 min was calculated. Control was also prepared by mixing 2.9ml of Tris-HCl buffer and 0.05ml of pyrogallol solution. The enzyme activity was determined as U/ml/min/mg protein where 1U denotes auto-oxidation of pyrogallol by 50%, calculated by the difference in absorbance between control and test per min[15].

$$\text{SOD activity: } \frac{\text{\% inhibition x Volume of reaction mixture}}{\text{Volume of sample x mg of protein}}$$

### 2.7.2 Catalase (CAT)

This experiment was carried out in compliance with the protocol provided by Aebi *et al.* The reaction tubes contained 1.9 ml of Phosphate Buffer Saline (PBS) (0.1mol/L, pH = 7.4), 1 ml of hydrogen peroxide and 0.1ml of sample homogenate against sample blank, which only contained buffer (2.9ml) and sample homogenate (0.1ml). The absorbance was taken at 240nm at an interval of 0 and 60 s until 3 min and the molar extinction coefficient was used to calculate enzyme activity (43.6$mole^{-1}cm^{-1}$) which was expressed as µmole/min/mg protein[16].

### 2.7.3 Lipid Peroxidation (LPO)

The procedure described by Okhawa *et al.*, was followed during the experiment. In this assay, 0.2ml of KCl (1.15%) and SDS (8.1%) were mixed with 1.5ml of acetic acid (20%) and thiobarbituric acid which was followed by the addition of 0.2ml of tissue homogenate and then volume was built upto 4ml with distilled water. The reaction tubes were heated at 95°C in a water bath for 60 min to obtain a pink color. After complete incubation, tubes were cooled down with the addition of n-butanol: pyridine (5ml) in the ratio of 15:1 and centrifuged at 1000rpm for 10min. The supernatant was taken and the absorbance was recorded at 532nm spectrometrically. The LPO level was calculated by the molar extinction coefficient of 1.56 x $10^{-5}$ $mole^{-1}cm^{-1}$ and expressed as nmole of MDA formed/mg protein. The change in color of the sample in tubes is due to a reaction between Thiobarbituric Acid (TBA) with Malondialdehyde (MDA[17].

### 2.7.4 Glutathione Peroxidase (GPx)

The methodology outlined by Mohandas *et al.*, was followed in conducting this experiment. In this assay, 1.44ml of PBS (0.1 mol/L, pH=7.4), 0.1ml of EDTA (1mmol/L), 0.1ml of sodium azide (1mmol/L), 0.05ml of glutathione reductase (2%), 0.1ml of GSH (1mmol/L), 0.1ml of NADPH (0.02mmol/L) and 0.01ml of $H_2O_2$ (0.25mmol/L) were mixed. Further, 0.1 ml of sample homogenate was added to start the reaction, and readings at 340 nm were recorded at every 30 seconds for two minutes. The enzyme activity was determined by the molar extinction coefficient of 6.22 x $10^3$ $mole^{-1}$ $cm^{-1}$ and expressed as nmole NADPH oxidized/min/mg protein[18].

### 2.7.5 Glutathione-S-Transferase(GST)

GST was performed according to the procedure of Habig *et al.* The reaction mixture contained 1.675ml of sodium phosphate buffer saline (0.1mol/L, pH=7.4), 0.2ml of reduced glutathione (1mmol/L), 0.025ml of CDNB (1-Chloro-2,4-Dinitrobenzene) (1mmol/L) and sample homogenate (0.1ml). After 1min, absorbance was taken at 340nm and enzyme activity was calculated by considering the molar extinction coefficient of 9.6 × $10^3$ $mole^{-1}cm^{-1}$. The enzyme activity was expressed as nM/min/mg protein[19].

### 2.7.6 Reduced Glutathione (GSH)

The protocol was based on the method described by Ellman, 1959 with some modifications. In this assay, the sample homogenate (0.5ml) was first treated with 2ml of TCA (5%) to precipitate out the protein and then centrifuged at 5000 rpm for 10min at 4°C. The supernatant was used for further experiments. The reaction tubes were prepared by the addition of 2.5ml of sodium phosphate buffer (0.1mol/L, pH=7.4), 0.8ml of EDTA (1 mmol/L),

0.1 ml of 5'5'-Dithiobis -2-Nitrobenzoic acid (DTNB) (10 mmol/L) and supernatant (0.1ml). Then, absorbance was taken at 412nm and activity was expressed in terms of µmole of GSH/ g tissue. Reduced Glutathione used as a standard compound to prepare the standard curve for calculating the GSH concentration in sample[20].

## 2.8 Oxidative Stress Index (OSI)

The OSI was observed in terms of the Prooxidant (P) versus Antioxidant (A) ratio, which was further calculated by using the following formula[21]:

OSI = level of LPO activity (P) / level of activity of SOD + CAT + GPx + GSH + GST (A)

## 2.9 Biochemical Parameters

### *2.9.1 Alanine Aminotransaminase (ALT) and Aspartate Aminotransfearse (AST)*

This method was based on the protocol described by Reitman and Frankel[22]. In this method, 0.5ml of substrate solution for AST: aspartic acid with α-ketoglutarate, For ALT: alanine with α-ketoglutarate was mixed with tissue homogenate (0.2ml) which was further incubated for 60 min (AST) and 30 min (ALT) at 37°C. After completion of incubation, 0.5ml of 2,4-DNPH solution was added to stop the process and then again incubated for an additional 20 minutes. After this, 5ml of sodium hydroxide (0.4N) was added to each tube and left for 10min. The absorbance was taken at 510nm. The control was similarly set up by adding all of the ingredients, but it was not incubated. The activity was described as µmole of pyruvate formed/ min/mg protein. Sodium pyruvate was used as an internal standard.

### *2.9.2 Alkaline Phosphatase (ALP)*

ALP was performed by the kit purchased from Accurex Biomedicals Pvt. Ltd. Activity was described as IU/L.

## 2.10 TPC (Total Protein Content)

TPC was performed in accordance with the methodology established by Lowry *et al.*, using BSA (Bovine Serum Albumin) as a reference compound. In this method, tissue homogenate (0.1ml) and standard (20-100 µg/ml) were mixed with an analytical reagent (4.5ml) prepared by using $Na_2CO_3$ (2%) in 0.1N sodium hydroxide, 1% copper sulfate and 1% sodium potassium tartrate in the ratio 48:1:1. At room temperature, test tubes were incubated for 10 minutes. After that, 0.5ml of Folin's reagent (prepared in a 1:1 ratio) was added, and the mixture was left at room temperature for 30 minutes. Then, the OD was recorded at 660 nm[23].

## 2.11 TCC (Total Cholesterol Content)

TCC was performed according to the protocol described by Zak, using cholesterol as an internal standard. In this approach, tubes containing the sample (0.05ml) and standard (2mg/ml) were properly mixed with Glacial Acetic Acid (GAA) to make the final volume upto 4ml. Further, 2 ml of coloring reagent (conc. $H_2SO_4$ with $Fecl_3$-acetic acid reagent in the ratio 99:1) was added and tubes were kept at room temperature for 15min. Then, the OD was recorded at 550nm in a UV-VIS spectrophotometer[24].

## 2.12 Hepatic Biomarkers

### *2.12.1 Microsome Preparation*

The preparation of the microsome fraction was on the basis of the method given by Omuro and Sato[25]. Tissue homogenate (0.5 g) was prepared by using 2ml of ice-cold homogenizing buffer (10mM Tris-HCl, 0.25 M sucrose pH = 7.4) followed by centrifugation at 13,000g for 10min at 4°C. Pellet was removed and calcium chloride (10mM) in a 1:5 ratio was added to the supernatant which was then centrifuged at 25,000g for 10min at 4°C. The pellet obtained was then resuspended in a 0.5 ml of homogenization resuspending buffer (100mM Tris-HCl, 20% w/v glycerol, 10mM EDTA). Before proceeding with the experiment, the microsome fraction was diluted with diluent buffer. (0.1M sodium phosphate buffer pH = 7.4, 0.5% Triton X-100, 1mM EDTA in 1:9 ratio).

### *2.12.2 Total Protein Content*

The method outlined by Lowry *et al.,* was used to assess the level of protein concentration in microsomes[23].

### *2.12.3 Cytochrome P450 2E1*

The procedure outlined by Watt *et al.*, was used to accomplish the cytochrome P450 2E1 analysis. The reaction mixture contained 0.2ml of microsome fraction, 0.7ml of phosphate buffer saline (0.1 mol/L), 0.1ml of p-Nitrophenol (40mmol/L) in test tubes and then the reaction was initiated by adding 0.01ml of NADPH (10mmol/L). Further, the

reaction tubes were incubated at 37°C for 1 hour. The reaction was terminated by adding 0.2ml of TCA (20%) which was followed by centrifugation at 1000rpm for 5 min. After that, 0.01ml of sodium hydroxide (10mmol/L) was added to the supernatant (1ml), and absorbance was taken at 450nm by using a UV-VIS spectrophotometer. The concentration of cytochrome P4502E1 in the sample was calculated by taking the standard curve of P-nitrocatechol (10mmol/L) and expressed as mmole p-Nitrocatechol formed/min/mg protein[26].

### 2.12.4 Cytochrome $b_5$

The procedure was performed in accordance with the approach outlined by Omura and Sato. In reaction tubes, the 0.5ml of microsomal fraction was mixed with 4.3ml of Tris-HCl buffer (50mmol/L) which was further equally divided into two parts with equal proportion. One proportion with 0.1ml of NADPH (4mg/ml) was considered as test sample and the other tube without NADPH was considered as a reference sample. These tubes were incubated for 2 min and then absorbance was measured at 424nm and 409nm. Its calculation was done by considering the molar extinction coefficient of 185$mM^{-1}cm^{-1}$ and expressed in nmole/mg protein[25].

### 2.12.5 5'- nucleotidase

This experiment was conducted using the approach described by Hyder *et al*. The reaction mixture contained 1 ml of Tris-HCl buffer (184mmol/L), 0.1 ml of $MgSO_4.7H_2O$ (50mmol/L), 0.1 ml of KCl (650mmol/L), 0.1 ml of EDTA(1mmol/L), 0.1 ml of 5'-AMP (40mmol/L) and 0.1 ml of distilled water. The addition of liver homogenate (0.2 ml) initiated the reaction, which was further left for 15 min at 37°C. Following incubation, the addition of 2 ml of TCA (10%) terminated the reaction and tubes were centrifuged at 5000rpm for 5 min. Further, the supernatant was analyzed to estimate the liberated phosphorous in the sample by adding 1 ml of ammonium molybdate reagent and 0.4 ml of amino naphthol sulphonic acid reagent. The reaction tubes were further incubated for 10 min to develop blue color. Absorbance was recorded at 660nm and liberated phosphorous content was calculated by the standard calibration curve of $KH_2PO_4$[27].

### 2.12.6 Gamma-Glutamyltranspeptidase(GGT)

GGT was performed by the kit purchased from Accurex Biomedicals Pvt. Ltd. and activity was expressed as IU/L.

### 2.12.7 Statistical Analysis

The entire tests were conducted in triplicates and data was expressed as mean±SEM. Statistical analysis was performed by SPSS software, using one-way ANOVA followed by Tukey test to observe p values. The data was considered significant if the p value was less than 0.05.

## 3. Results

Oral administration of lead nitrate significantly elevated the hepatic injury, which was subdued by the administration of *Allium sativum* Essential Oil (ASEO). In order to assess the therapeutic role of *Allium sativum* essential oil, various assays mainly the SOD, CAT, GPx, GST, GSH and LPO were performed. It was found that *Allium sativum* Essential Oil possess capacity to alter the liver functioning by regulating the enzymatic and non-enzymatic levels. This property of ASEO showed that it has effective role in inflammatory conditions. Other parameters like hepatic biomarkers and biochemical parameters mainly cytochrome P450 2E1, cytochrome $b_5$, 5'-nucleotidase and Gamma-Glutamyltranspeptidase (GGT), Alanine transaminase, Aspartate transaminase, Alkaline phosphatase were also studied. The bulb oil tried to restore the biochemical and oxidative stress parameters to a greater extent in mice treated with lead nitrate. In this study, it was noticed that lead nitrate administration altered the body weight of albino mice which ultimately increased the liver indices.

Toxicant given to animals altered the mice body weight is depicted in Table 1. The experimental data showed that there was decrease in mice body weight in lead-intoxicated mice when compared to the control group. This might be due to less growth and development of mice. Treatment with *Allium sativum* Essential Oil, standard drug and vehicle oil increased the mice weight in comparison to lead nitrate-intoxicated mice (Group 2). The results of the oxidative stress parameters are depicted in Table 2. In SOD activity, a reduction in enzymatic level in group 2 animals was observed in comparison to group 1 animals ($p<0.001$). Animals treated with plant oil dose ($p>0.05$) and with standard compound ($p>0.05$) showed an insignificant rise in SOD level whereas vehicle oil group mice showed significant rise in SOD level ($p<0.01$). Similarly, a decrease in the catalase enzyme level in the lead nitrate-treated group (group 2) was recorded in comparison to group 1 mice ($p<0.001$). Treatment with *Allium sativum* Essential

**Table 1.** Effect on body weight (g) following 30 days exposure with Lead Nitrate (LN), LN + Low dose of ASEO, LN + High dose of ASEO, LN + Silymarin (standard), LN+ Vehicle control (olive oil only) in hepatic tissues of male Swiss albino mice.

| Group | Dose | Body weight (g) | | Liver indices |
|---|---|---|---|---|
| | | 0 day | 30day | |
| Control | - | 20 ± 1.00 | 26.3 ± 1.45 | 6.25 ± 0.05 |
| Lead nitrate intoxicated group | 50 mg/kg | 22.5 ± 2.5 | 25.5 ± 3.5 | 8.95 ± 0.19NS* |
| Lead nitrate intoxicated group + ASEO low dose | 50 + 50 mg/kg | 25 ± 3.51 | 29 ± 2.08 | 6.47 ± 0.08NS# |
| Lead nitrate intoxicated group + ASEO high dose | 50 + 80mg/kg | 20 ± 2.51 | 25.5 ± 1.60 | 6.01 ± 0.17 NS# |
| Lead nitrate intoxicated group + Silymarin | 50 + 25 mg/kg | 20.6 ± 1.45 | 24.3 ± 2.33 | 6.58 ± 0.12 NS# |
| Lead nitrate intoxicated group + Vehicle control | 50 + olive oil | 22 ± 2.0 | 26.5 ± 1.5 | 6.20 ± 0.05 NS# |

Each data point shows mean ± SEM of 6 mice. Significant differences in data are expressed as *p<0.001, **p<0.01, ***p<0.05 compared to control group; ap<0.001, bp<0.01, cP<0.05 compared to Lead nitrate treated group. NS stands for non-significant. NS* = Comparison of Lead nitrate treated group with control group and NS# = Comparison of Lead nitrate treated group with ASEO, Silymarin and Vehicle control.

**Table 2.** The outcomes of 30 days oral administration of Lead Nitrate (LN), LN + Low dose of ASEO, LN + High dose of ASEO, LN + Silymarin (standard), LN+ Vehicle control in oxidative stress parameters of liver in male Swiss albino mice.

| Parameters | Group I Distilled water | Group II Lead nitrate (50mg//kg body weight) | Group III Lead nitrate + ASEO low dose (50 mg/kg) | Group IV Lead nitrate + ASEO high dose (80mg/kg) | Group V Lead nitrate + Silymarin (25mg/kg) | Group VI Lead nitrate + vehicle control |
|---|---|---|---|---|---|---|
| SOD | 4.69 ± 0.0005 | 3.91 ± 0.026* | 4.25 ± 0.0003 NS# | 4.36 ± 0.0001 NS# | 4.04 ± 0.0004 NS# | 4.11 ± 0.05 b |
| CAT | 104.64 ± 0.001 | 64.42 ± 0.002* | 74.7 ± 0.002a | 77.92 ± 0.007a | 81.03 ± 0.05a | 73.05 ± 0.003a |
| LPO | 298.64 ± 0.03 | 526.12 ± 0.05 NS* | 157.86 ± 0.029 NS# | 298.37 ± 0.007 NS# | 283.21 ± 0.056 NS# | 211.4 ± 0.053 NS# |
| GPx | 8.46 ± 0.007 | 2.95 ± 0.003* | 3.25 ± 0.007 NS# | 3.51 ± 0.005 NS# | 5.0 ± 0.007 NS# | ± 0.002 NS# |
| GSH | 1.10 ± 0.002 | 0.93 ± 0.001NS* | 1.58 ± 0.001a | 1.33 ± 0.001b | 1.024 ± 0.003b | 1.08 ± 0.001 NS# |
| GST | 2.35 ± 0.04 | 0.21 ± 0.02* | 0.47 ± 0.006 NS# | 2.03 ± 0.038 NS# | 0.59 ± 0.026 NS# | 0.61 ± 0.01 NS# |

Each data point shows mean ± SEM of 6 mice. Significant differences in data are expressed as *p<0.001, **p<0.01, ***p<0.05 compared to control group; ap<0.001, bp<0.01, cP<0.05 compared to Lead nitrate treated group. NS stands for non-significant. NS* = Comparison of Lead nitrate treated group with control group and NS# = Comparison of Lead nitrate treated group with ASEO, Silymarin and Vehicle control.

Oil , standard compound and vehicle oil upregulated the catalase activity when compared to lead nitrate-treated group (p<0.001). High dose of *Allium sativum* essential oil is found to be more effective in elevating the catalase activity than low dose of garlic oil.

An insignificant increase in LPO level was observed in toxicant-treated group (group2) (p>0.05) in comparison to control group (group1). Mice that received two different doses of *Allium sativum* Essential Oil, standard and vehicle oil showed an insignificant decline in LPO level in comparison to group 2 animals (p>0.05).

In case of glutathione peroxidase, a decrease in the enzymatic level in the lead nitrate-treated group was seen in comparison to the control group (p<0.001). Further, treatment with both plant oil dose, standard compound and vehicle oil insignificantly upregulated the enzymatic level in mice model (p>0.05) as compared to Group 2 toxicant exposed animals.

GSH concentration was also decreased insignificantly (p>0.05) in toxicant exposed animals in comparison to untreated animals. *Allium sativum* Essential Oil doses (p<0.001 and p<0.01), standard compound (p<0.01), and vehicle oil control group animals (p>0.05) showed an increment in enzymatic level when compared to group 2 animals. Thus, we conclude that low dose of *Allium sativum* Essential Oil can be better approach in elevating

**Table 3.** The outcomes of 30 days oral administration of Lead Nitrate (LN), LN + Low dose of ASEO, LN + High dose of ASEO, LN + Silymarin (standard), LN+ Vehicle control in oxidative stress index which is categorized as prooxidant (P)/antioxidant (A) ratio of liver tissue in male Swiss albino mice.

| Parameters | Experimental groups | | | | | |
|---|---|---|---|---|---|---|
| P/A | Group I Distilled water | Group II Lead nitrate (50mg//kg) | Group III Lead nitrate + ASEO low dose (50mg/kg) | Group IV Lead nitrate + ASEO high dose(80mg/kg) | Group V Lead nitrate + Silymarin (25mg/kg) | Group VI Lead nitrate + vehicle control |
| | 2.46 | 7.26 | 1.87 | 3.34 | 3.09 | 2.51 |

GSH level in comparison to high oil dose, standard compound and vehicle oil ($p<0.05$).

It was examined that GST activity was significantly declined ($p<0.001$) in the lead nitrate-treated group (Group 2) when compared with the control group animals. Treatment with low and high dose of ASEO (Group 3 and Group 4), standard compound and vehicle oil showed an insignificant increment in enzymatic level when compared with Group 2 animals ($p>0.05$). All the treated groups when compared with the lead nitrate treated group (Group 2) indicated that a high dose of ASEO could be potent source for elevating GST level.

Table 3 illustrates the outcome of OSI which is based on the Prooxidant (P)/Antioxidant (A) ratio in mice liver tissue homogenate. The measurement of the P/A ratio in the liver was more prominent in the case of lead-nitrate exposed mice. However, administration of ASEO to lead nitrate-exposed mice attenuated the elevation of P/A ratio in liver tissues. Similarly, in the case of standard and vehicle oil, a reduction in the oxidative stress index in mice exposed to lead nitrate was observed.

Table 4 represents the results of biochemical parameters. It was noticed that the total protein content was insignificantly reduced ($p>0.05$) in the lead nitrate-treated group in comparison to the control group. Treatment with the low and high dose of *Allium sativum* Essential Oil ($p<0.001$ and $p>0.05$), standard compound ($p<0.01$), and vehicle oil ($p>0.05$) indicated normalization in TPC level when compared to the lead nitrate treated group. Total Cholesterol Content (TCC) observed for Group 1 and Group 2 animals showed an insignificant elevation of TCC in Group 2 mice in comparison to Group 1 (untreated animals) ($p>0.05$). Comparison of lead nitrate treated group with low and high dose of *Allium sativum* Essential Oil, standard compound and vehicle oil control group explained insignificant reduction in cholesterol level ($p>0.05$). The high dose of *Allium sativum* Essential Oil (ASEO) ($p>0.05$) exhibit a good potential to downregulate the cholesterol level in comparison to low dose of ASEO, silymarin and vehicle oil.

The activity of ALT and AST was examined to evaluate the effectiveness of ASEO in diminishing the enzymatic

**Table 4.** The outcomes of 30 days oral administration of Lead Nitrate (LN), LN + Low dose of ASEO, LN + High dose of ASEO, LN + Silymarin (standard), LN+ Vehicle control in biochemical parameters of liver in male Swiss albino mice.

| Parameters | Group I Distilled water | Group II Lead nitrate (50mg//kg body weight) | Group III Lead nitrate + ASEO low dose (50mg/kg) | Group IV Lead nitrate + ASEO high dose(80mg/kg) | Group V Lead nitrate + Silymarin (25mg/kg) | Group VI Lead nitrate + vehicle control |
|---|---|---|---|---|---|---|
| TPC | 8.54 ± 0.009 | 7.06 ± 0.017 $^{NS*}$ | 12.75 ± 0.004$^{a}$ | 11.94 ± 0.075 $^{NS\#}$ | 14.67 ± 0.022$^{b}$ | 11.28 ± 0.053 $^{NS\#}$ |
| TCC | 1.01 ± 0.028 | 1.7 ± 0.019 $^{NS*}$ | 1.51 ± 0.032 $^{NS\#}$ | 1.19 ± 0.019 $^{NS\#}$ | 1.51 ± 0.062 $^{NS\#}$ | 1.49 ± 0.07 $^{NS\#}$ |
| ALT | 86.47 ± 0.034 | 127.95 ± 0.047 $^{NS*}$ | 30.7 ± 0.039 $^{NS\#}$ | 15.54 ± 0.004 $^{NS\#}$ | 14.77 ± 0.005 $^{NS\#}$ | 32.73 ± 0.027 $^{NS\#}$ |
| AST | 83.73 ± 0.029 | 450.2 ± 0.125 $^{NS*}$ | 156.6 ± 0.020 $^{NS\#}$ | 136.2 ± 0.029 $^{NS\#}$ | 117.5 ± 0.013 $^{NS\#}$ | 127.2 ± 0.003 $^{NS\#}$ |
| ALP | 5.44 ± 0.01 | 8.88 ± 0.028 $^{NS*}$ | 7.97 ± 0.016$^{c}$ | 6.71 ± 0.001$^{b}$ | 7.43 ± 0.002$^{c}$ | 7.25 ± 0.01$^{b}$ |

Each data point shows mean ± SEM of 6 mice Significant differences in data are expressed as *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to control group; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}P<0.05$ compared to Lead nitrate treated group. NS stands for non-significant. NS* = Comparison of Lead nitrate treated group with control group and NS# = Comparison of Lead nitrate treated group with ASEO, Silymarin and Vehicle control.

**Table 5.** The outcomes of 30 days oral administration of Lead Nitrate (LN), LN + Low dose of ASEO, LN + High dose of ASEO, LN + Silymarin (standard), LN+ Vehicle control in hepatic biomarkers of liver in male Swiss albino mice.

| Parameters | Group I Distilled water | Group II Lead nitrate (50mg//kg body weight) | Group III Lead nitrate + ASEO low dose (50mg/kg) | Group IV Lead nitrate + ASEO high dose(80mg/kg) | Group V Lead nitrate + Silymarin (25mg/kg) | Group VI Lead nitrate + vehicle control |
|---|---|---|---|---|---|---|
| Cytochrome P450 2E1 | 6.85 ± 0.043 | 18.04 ± 0.067*** | 13.39 ± 0.075 $^{NS\#}$ | 14.05 ± 0.014 $^{NS\#}$ | 5.95 ± 0.041 $^{NS\#}$ | 4.88 ± 0.028 $^{NS\#}$ |
| Cyt $b_5$ | 0.037 ± 0.0003 | 0.018 ± 0.0006* | 0.036 ± 0.0002$^{a}$ | 0.045 ± 0.0004$^{a}$ | 0.029 ± 0.0003$^{a}$ | 0.020 ± 0.0001$^{b}$ |
| 5'-nucleotidase | 0.046 ± 0.0025 | 0.072 ± 0.0023* | 0.015 ± 0.005$^{a}$ | 0.0064 ± 0.0009$^{a}$ | 0.008 ± 0.0009$^{a}$ | 0.010 ± 0.0017$^{a}$ |
| GGT | 2.3 ± 0.007 | 5.39 ± 0.001$^{NS*}$ | 2.31 ± 0.006 $^{NS\#}$ | 3.85 ± 0.006 $^{NS\#}$ | 3.3 ± 0.002 $^{NS\#}$ | 1.54 ± 0.007 $^{NS\#}$ |
| TPC | 5.24 ± 0.018 | 4.23 ± 0.011 $^{NS\#}$ | 5.31 ± 0.014 $^{NS\#}$ | 4.26 ± 0.033 $^{NS\#}$ | 10.89 ± 0.124 $^{NS\#}$ | 13.8 ± 0.039$^{b}$ |

Each data point shows mean ± SEM of 6 mice. Significant differences in data are expressed as *p<0.001, **p<0.01, ***p<0.05 compared to control group; $^{a}$p<0.001, $^{b}$p<0.01, $^{c}$P<0.05 compared to Lead nitrate treated group. NS stands for non-significant. NS* = Comparison of Lead nitrate treated group with control group and NS# = Comparison of Lead nitrate treated group with ASEO, Silymarin and Vehicle control.

content. Comparison of Lead nitrate treated animals with the control group showed an increment in ALT and AST enzymatic level that indicates the sign of inflammatory condition in hepatic tissues. However, the enzymatic level was increased insignificantly (p>0.05). Further, treatment of mice with ASEO, standard compound and vehicle oil showed that standard compound to some extent exhibit a good potential for decreasing the enzymatic level (p>0.05) as compared to Group 2 mice. In the case of plant dose, a high dose of ASEO (Group 4) showed better results in decreasing the enzymatic level than low dose of ASEO (Group 3) with a significant value at p>0.05.

Insignificant increase of Alkaline Phosphatase (ALP) activity in hepatocytes was recorded in mice treated with lead nitrate when compared with control group (p>0.05). This activity of ALP was declined by the action of low and high doses of ASEO at the significant level of p<0.05 and p<0.01 respectively when compared to lead nitrate exposed mice. Silymarin treated group (Group 5) and vehicle group (Group 6) possess the ability to reduce the enzymatic level with significance levels of p<0.05 and p<0.01. Comparison between treated groups showed that high dose of ASEO exhibit better potential than other treatments for diminishing alkaline phosphatase level.

The results of hepatic biomarkers are tabulated in Table 5. Cytochrome p450 2E1 activity was calculated in the microsomal fraction of hepatic tissues by observing the regression equation ($y = 0.120x - 0.054$; $r^2 = 0.977$) of the standard compound p-nitrocatechol. The enzyme activity was elevated in Group 2 (lead nitrate treated group) as compared to Group 1 (control group) with a significant value of p<0.05. This indicated a pathological condition in hepatic tissues. Decline in enzymatic level (p>0.05) was observed on treatment with ASEO, silymarin, and vehicle oil in comparison to the lead nitrate-treated group (Group 2). Although the decrease was insignificant but it can be assumed that ASEO can also be an alternative approach to restore cytochrome P450 2E1 levels in hepatic tissues.

The cytochrome $b_5$ level in the microsomal fraction of hepatic tissues was decreased in Group 2 (lead nitrate treated group) when compared to group 1 (control group) with a significant value of p<0.001. An increase in enzymatic level was noticed in groups treated with low and high dose of ASEO (p<0.001), standard compound (p<0.001) and vehicle oil (p<0.01), when compared with lead nitrate treated group (Group 2). The 5'-nucleotidase activity was determined in different groups of hepatic tissues of mice by the regression equation $y = 1.671x + 0.161$, $r^2 = 0.958$ of the calibration curve of $KH_2PO_4$ considered as reference compound. The enzymatic level was increased in group 2 (p<0.001) when compared to the control group (Group 1). The low and high dose of ASEO (p<0.001), standard compound (p<0.001), and vehicle oil-treated animals (p<0.001) remarkably downregulated the enzymatic level. Furthermore, comparison among ASEO, standard compound and vehicle control indicated that a high dose of ASEO (Group 4) was found to be more effective in decreasing enzymatic level than other treated groups. This indicates that ASEO can be considered as a potent and alternative approach for the treatment of hepatic disorders.

Gamma-glutamyltranspeptidase activity was insignificantly increased in the lead nitrate-treated group (Group 2) when compared with control animals (Group 1). On administration of low and high dose of ASEO, standard compound and vehicle oil represented a decline in enzymatic level ($p>0.05$). Further, observation revealed that low dose of ASEO possesses greater activity in decreasing enzymatic level than high dose of ASEO. Total Protein Content (TPC) in the microsomal fraction was calculated in all treated groups of hepatic tissues by the regression equation $y = 0.033x + 0.057$, $r^2 = 0.973$ of BSA, considered as standard compound. Comparison of the lead nitrate-treated group (Group 2) with the control group (Group 1) showed a remarkable but insignificant decline in TPC level in Group 2 ($p>0.05$). Treatment with plant oil doses ($p>0.05$), standard compound ($p>0.05$) and vehicle oil ($p<0.01$) indicated an increment in TPC level. To deduce the ASEO potency, observation between different plant doses revealed that low dose expressed better results than high dose ($p>0.05$). Thus, a low dose of ASEO is effective in elevating the TPC level in the microsomal fraction of the liver tissues of Swiss albino mice.

## 4. Discussion

Liver is the major and vital organ for the clearance of toxic chemicals. Continuous exposure to heavy metals such as lead, chromium, arsenic and cadmium induces ROS production that might result in chronic hepatic injury[28]. If chronic liver injury is not treated for a longer duration, then it may lead to inflammation and degeneration that implicate liver cirrhosis, fibrosis and Hepatocellular Carcinoma (HCC)[29]. Reactive oxygen species such as superoxide, hydroxyl ions enhance pathological changes in hepatic tissue by altering the biological membrane[30]. Major sources of ROS generation are kupffer cells, neutrophils, mitochondria and cytochrome P450 in the hepatic cell, which participate in the chronic inflammatory response. Kupffer cells induce oxidative stress and enhance hepatic stellate cells to activate the proliferation and synthesis of the extracellular matrix that leads to Hepatocellular Carcinoma (HCC)[31]. Severe side effects include Polyunsaturated Fatty Acids (PUFAs) oxidation in the cell membrane that reduces membrane fluidity, disturbs membrane integrity, causes cholestasis, cirrhosis etc. This can be overpowered by different antioxidant enzymes that decrease oxidation status at the cellular level[32]. There is a correlation of dietary supplements mainly natural products, which combat the toxicity of environmental pollutants and various other toxicants. Natural products can be considered as free radical scavengers that modulate the antioxidant defense system. These natural compounds prevent substrate oxidation mainly DNA, lipids and proteins. Also, lower the oxidative stress, declines malignant transformation and other parameters of cellular damage[33,34].

Alteration in body weight is a key marker of toxicity. After 30 days of LN (Lead Nitrate) treatment, diminished body weight was observed in group 2 mice when compared with group 1 mice[35,36]. This may be due to impaired digestion and absorption of essential nutrients, which in turn decreased the metabolic activities and thus reduced the body weight. On treatment with *Allium sativum* Essential Oil, silymarin and vehicle oil, increase in body weight was observed. This demonstrates that ASEO has capacity to improve metabolic activities and thus can increase the body weight. Free radicals and Lipid Peroxidation (LPO) are known markers of oxidative stress. In Lead nitrate-treated animals (Group 2), the LPO level was markedly raised. The elevated level of LPO results in increase in MDA content that damages the liver. In brief, an alteration in PUFAs, membrane integrity and permeability results in increase in LPO concentration that may lead to increased tissue complications[37]. In a prior investigation, an increment in MDA level in hepatic tissue after lead nitrate exposure was observed in Swiss albino mice[38]. In this study, *Allium sativum* Essential Oil reduced the LPO level in mice exposed to lead nitrate. This could be due to the presence of volatile components in ASEO[39,40].

The glutathione antioxidant system enhances defense mechanisms against free radicals and xenobiotic detoxification. It is a major scavenger of hydrogen peroxide and lipid hydroperoxides in a reaction that converts GSH to GSSG. Glutathione peroxidase which acts as a natural antioxidant in the cellular system was significantly decreased in the hepatic tissues of mice treated with lead nitrate. Reduced Glutathione (GSH), low molecular weight thiol play a role in defensive mechanisms mainly free radical scavenging, drug metabolism and $H_2O_2$ reduction by providing reducing equivalents to ROS. The attribute of lead binding to the thiol group of both oxidized and reduced glutathione forces the cell towards oxidative damage. In this study, lead nitrate elevated the concentration of hydrogen and

lipid peroxides due to ROS production, which depressed the GPx and GSH activity whereas treatment with ASEO elevated the GpX activity, which in turn increased the conversion of reduced glutathione into oxidized glutathion. Another enzyme, GST, a phase II enzyme, is involved in the detoxification of xenobiotics in order to scavenge toxicants, which in turn offer defense against chemicals that can cause cancer and mutation. The reaction mechanism involves GSH and CDNB (acting as substrate) producing dinitrophenyl thioether, which is detected at 340nm using a UV-VIS spectrophotometer. A decrease in GST level explains the hepatic toxicity in mice treated with lead nitrate. Further treatment with ASEO elevated the GST concentration, which indicates superior protection against the lead nitrate-treated group[41]. This protection might be due to presence of organosulfur compounds in ASEO that possess potent antioxidant power and also act as a valuable precursor of glutathione biosynthesis.

Superoxide Dismutase (SOD) provides the first line of defense and is effectively involved in the dismutation of superoxides to $H_2O_2$ and $O_2$, which is further catabolized by catalase and glutathione peroxidase[42]. At the intra and extracellular levels, it plays a crucial role against disease generated by oxidative bursts, thereby preventing cellular toxicity. Besides this, Catalase (CAT) is a hemoprotein and ubiquitous antioxidant enzyme that reduces non-reactive ROS as its substrate i.e., $H_2O_2$, a toxic product of aerobic metabolism into hydrogen and water[43]. It was observed that mice treated with lead nitrate produced a decline in superoxide dismutase and catalase activity in hepatocytes. This may be due to the outcome of an elevation in oxidative tension that results in free radical formation, which ultimately causes auto-oxidation of hepatic tissues. Mice treated with ASEO showed increased antioxidant potential by elevating SOD and catalase levels which indicates possible protection of hepatic disorders.

Upregulation of biochemical parameters such as ALP, AST and ALT in hepatic tissue is a sensitive indicator of liver injury and cholestasis. The lead nitrate treated group indicated an elevation in enzymatic concentration due to damage in the structural integrity of the liver or altered cell membrane permeability that lead to enzymatic leakage from hepatocytes. This can be due to an increment in cell membrane permeability, which affects $K^+$- $Ca^{2+}$ channels, an alteration in the cytoskeleton followed by hepatic injury[44]. Mice treated with ASEO showed lower enzymatic concentration in comparison to the lead-treated group. This suggests that ASEO can be used as a powerful agent for reduction in hepatocellular injury which generally occurs from oxidative damage of the cell membrane.

TPC content is a marker of hepatic functioning, which guides the severity of hepatic damage. A major increase in protein content enhances tissue damage, whereas a decrease in protein content shows hepatic toxicity[45]. The reduced TPC content in liver tissue was observed in mice treated with lead nitrate when compared to the control group. These results are in agreement with the study conducted by Sharma *et al.,*[38] who also analyzed the decline in TPC content in mice intoxicated with lead nitrate. Reduction in protein content may be due to an increased protein excretion or alteration in DNA and RNA content by base pair mutation, singlet oxygen attack on nucleic acid during cell lysis. In one study, it was concluded that increased excretion of proteins may cause a decrease in protein concentration at the cellular level. Effects of heavy metals on protein concentration may be due to ribosomal death, which leads to a decrease in protein synthesis[46-48]. ASEO treatment increased the TPC in LN-treated mice that might be due to reduction in tissue damage and protein excretion from the cell.

Cholesterol, vital component of cell membranes, participates in the production of bile acids, vitamin D, and steroid hormones. An abnormal increment in cholesterol content may increase the risk of cardiovascular diseases and atherosclerosis. It can be due to the inhibitory effects of fatty acid β-oxidation and a reduction in lipid excretion[49,50]. Cholesterol content was elevated in mice intoxicated with lead nitrate. This may be due to the inhibitory activity of cholesterol catabolic enzymes and the stimulatory effect of enzymes involved in the biosynthesis of cholesterol[39]. However, treatment with ASEO reduced the cholesterol content. This might be due to decreased level of enzymes involved in fatty acid and cholesterol biosynthesis in hepatic tissues.

Phase I detoxification system is mainly involved in the enhancement of metabolic activities. Cytochrome P450 2E1 is a phase I enzyme, a member of the oxidoreductase family, mainly expressed in the liver that alters in numerous pathological and physiological conditions. Factors responsible for alteration in enzymatic level are inflammation, infection and other factors such as age, sex and hormonal rhythms[51,52]. The majority of cytochrome P450 isoforms are responsible for the metabolism of 90% of drugs. Alteration in the level of cytochrome P450 2E1

and $cytb_5$ can induce oxidants, which in turn decreased the phase II enzymes and impaired the cellular functions. Subsequent treatment with ASEO tried to restore the enzymatic level, which might be due to reduction in oxidative burst.

Gamma-GT is a well-known marker of hepatic damage. This enzyme is mostly found in the canalicular region of the hepatic plasma membrane, therefore when it is released, it causes damage to the membrane. Its major role is to transport amino acids for GSH synthesis. It is hypothesized that binding with free radicals promotes the oxidation of PUFAs, resulting in an induction of enzyme leakage from the cell membrane[53,54]. Treatment with ASEO attenuated the increased gamma-GT level produced by lead nitrate intoxication, results in subsequent recovery of the enzyme. This suggests membrane stability through the acceleration of hepatocytes followed by diminishing the enzyme concentration in the circulation.

5'-NT is a sialoglycoprotein, one of the best indicators for hepatocyte destruction which is involved in AMP hydrolysis into adenosine and inorganic phosphate. It is generally increased in hepatobiliary diseases mainly intrahepatic obstruction[55]. The rise in 5'-nucleotidase level increased glycoprotein synthesis, which in turn provoked malignant transformation. In the lead nitrate-treated group, the amount of 5'-NT was increased, which may be an early indication of carcinogenesis. Further treatment of the lead nitrate-treated animals with ASEO minimized 5'-nucleotidase activity. This suggests that garlic oil may be an effective tool in the suppression of cancer[56,57].

## 5. Conclusion

To the best of our knowledge, this is a novel work that explains the protective role of *Allium sativum* Essential Oil in mitigating lead nitrate hepatotoxicity. The findings of this investigation shows that ASEO supplementation modulates the activities of phase I and phase II enzymes in hepatic tissues treated with lead nitrate. Therefore, it paves the way to consider *Allium sativum* Essential Oil as a potent alternative and futuristic approach to explore therapeutic agents against chronic diseases.

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