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Therapeutic Potential of *Allium sativum* Essential Oil in Attenuating Biochemical and Oxidative Stress Dysregulation in Lead Nitrate-Induced Cardiovascular Toxicity in Swiss Albino Mice

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Abstract

Lead nitrate exposure induces oxidative stress and biochemical alterations, contributing to cardiovascular damage. This study evaluated the cardioprotective potential of *Allium sativum* Essential Oil (ASEO) against lead nitrate-induced oxidative damage in male Swiss albino mice. Olive oil and silymarin were included for comparative insights. Thirty-six male Swiss albino mice were divided into six groups (n=6). The control group received double-distilled water, while Group II was exposed to lead nitrate (50 mg/kg) for 30 days. From day 12, Groups III and IV received low (50 mg/kg) and high (80 mg/kg) doses of ASEO, respectively, while Group V was treated with silymarin and Group VI with olive oil, all alongside lead nitrate. Oxidative stress markers (SOD, GPx, GST, CAT, LPO) and biochemical parameters (ALT, AST, ALP, total protein) were assessed. Lead nitrate exposure significantly reduced antioxidant enzyme activities (SOD, GPx, GST, and CAT) and increased LPO levels. High-dose ASEO (80 mg/kg) significantly restored antioxidant enzyme activities, with SOD, GPx, GST, and CAT increasing up to 2.5-fold compared to controls, while LPO was reduced by 50%. Silymarin and olive oil also demonstrated protective effects, though less pronounced. Biochemical analysis showed that lead nitrate exposure elevated ALT, AST, and ALP levels, which were significantly reduced by high-dose ASEO and silymarin treatments. High-dose ASEO also significantly increased total protein content (15.99 µg/ml) compared to lead nitrate-treated mice (7.45 µg/ml).

Major Findings: High-dose ASEO effectively mitigated Pb-induced oxidative stress and cardiovascular damage, outperforming olive oil and silymarin in restoring antioxidant enzyme levels and reducing lipid peroxidation. These findings highlight ASEO's potential as a protective agent against heavy metal-induced cardiovascular toxicity, warranting further investigation into its mechanisms and optimal therapeutic dosing.

Keywords: Cardiovascular Damage, Cellular Homeostasis, Lead Nitrate, Oxidative Stress.

1. Introduction

Lead is an extremely hazardous metallic contaminant that is pervasive in the environment due to extensive industrial processes, such as mining, battery manufacturing, and the production of pigments and explosives[1]. Its widespread presence results from industrial emissions, improper waste disposal, and contamination of natural resources, leading to its accumulation in soil, water and air. This environmental distribution significantly increases the risk

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of human exposure through polluted air, contaminated water supplies, and food chains, making lead a major public health concern[2]. Long-term exposure to this compound is associated with a range of toxic effects, particularly affecting the nervous, hepatic, renal, and cardiovascular systems, and contributing to oxidative stress and cellular damage[3]. Its deleterious effects on human health have been extensively documented, particularly regarding its cardiovascular implications, as chronic exposure to lead is associated with an elevated risk of hypertension, atherosclerosis and a range of cardiovascular disorders. Lead toxicity stems from its capacity to disrupt crucial cellular functions, such as the induction of oxidative stress, promotion of inflammatory response, and disturbance of calcium homeostasis, all of which collectively contribute to cardiovascular dysfunction[4]. Recent studies have further indicated that chronic exposure to lead disrupts mitochondrial function, impairs nitric oxide bioavailability, and alters lipid metabolism, thereby exacerbating cardiovascular risk factors. Moreover, lead is known to interfere with essential enzymatic processes by displacing calcium and zinc ions, leading to metabolic dysfunction and cellular apoptosis[5]. The cardiovascular system is predominantly vulnerable to the deleterious impacts of lead due to its central role in regulating blood pressure, vascular tone, and cardiac contractility. Lead-induced oxidative stress, characterised by a disparity between the generation of Reactive Oxygen Species (ROS) and the body's capacity to neutralise them with antioxidant defences, results in endothelial dysfunction, arterial stiffening, and the advancement of atherosclerosis[6,7]. Additionally, lead stimulates the secretion of pro-inflammatory cytokines and chemokines, initiating a cascade of inflammatory events that foster vascular inflammation and plaque formation in the cardiovascular system[5].

Given the profound health risks posed by lead-induced cardiovascular toxicity, the exploration of effective therapeutic interventions is paramount. *Allium sativum*, generically known as garlic, has garnered considerable scientific interest for its diverse spectrum of pharmacological effects[8]. Recent studies have highlighted the therapeutic potential of ASEO, obtained through the hydro-distillation of crushed garlic[9]. ASEO is rich in bioactive compounds, with thiosulfates—particularly allyl thiosulfates—being the most prominent. These thiosulfates are formed via the enzymatic conversion of S-alkyl-l-cysteine-S-oxide within the cytoplasm, followed by a C-S lyase-catalysed reaction that generates sulfite intermediates, which then condense to form highly reactive thiosulfates[10]. These intermediates undergo further rearrangement, producing a diverse range of sulfur-containing compounds with significant biological activity[11]. The essential oil is predominantly composed of sulfides, with allyl polysulfides being the major bioactive constituents. The five principal allyl sulfides in garlic oil include Diallyl Sulfide (DAS) (1.9%–9.5%), Diallyl Disulfide (DADS) (20.8%–27.9%), Diallyl Trisulfide (DATS) (16.8%–33.4%), allyl methyl disulfide (4.4%–8.3%), and allyl methyl trisulfide (14.5%–19.2%), all of which contribute to its potent antioxidant, anti-inflammatory, and cardioprotective properties[12]. The rationale for investigating ASEO as a potential therapeutic agent against Lead Nitrate ($Pb(NO_3)_2$induced cardiovascular toxicity stems from its capacity to counteract oxidative stress, mitigate inflammation and modulate vascular function. Several studies have suggested that the bioactive compounds in *A. sativum* can scavenge ROS, enhance antioxidant defenses, and inhibit pro-inflammatory pathways[13]. These compounds serve a central role in the broad spectrum of biological activities attributed to garlic, including antioxidant, antibacterial, antithrombotic, immunomodulatory, and anticancer effects. The medicinal potential of these compounds is ascribed to their capacity to regulate various biochemical pathways and mechanisms, including those associated with oxidative stress, inflammation, and cell proliferation[14]. The high metabolic demand and constant oxygen consumption of cardiac cells render them particularly vulnerable to oxidative damage[4]. Consequently, the cardioprotective effects of *Allium sativum* have gained significant attention due to its rich composition of bioactive sulfur compounds[15]. The present investigation examines the cardioprotective effects of ASEO in the setting of $Pb(NO_3)_2$ induced oxidative damage. Additionally, the roles of olive oil and silymarin, a well-known standard drug for Lead toxicity, are evaluated. Olive oil and silymarin were chosen for comparison to ASEO in this study due to their established therapeutic properties. Olive oil, a staple in the Mediterranean nutrition regimen, is renowned for its free radical scavenging and anti-inflammatory effects, attributed to its rich polyphenol content[16]. Silymarin, derived from milk thistle, is a well-recognised standard treatment for liver disorders and oxidative stress, providing a benchmark against which ASEO's efficacy can be evaluated[17]. Notably, while silymarin and olive oil have been studied in various oxidative stress models, their comparative efficacy against lead-induced cardiac toxicity in conjunction with ASEO remains largely unexplored. This study strives to address this

gap by providing a thorough evaluation of these interventions in a lead-exposed murine model.

The investigation aims to elucidate the impact of these treatments on antioxidant enzyme activity, systemic oxidative damage indicators in lead nitrate-exposed mice. Specifically, this study examines the alterations in key antioxidant enzymes like Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx) and Catalase (CAT) as well as the levels of Lipid peroxidation (LPO) products. Disruption of these enzyme activities due to lead nitrate exposure underscores the severe oxidative imbalance and subsequent cellular dysfunction[18]. Furthermore, the study investigates the biochemical changes associated with lead nitrate toxicity, including alterations in Alanine Transferase Activity (ALT), Aspartate Aminotransferase Activity and Total Protein Content (TPC) in cardiac tissues. By highlighting the protective effects of ASEO, the study advocates for the inclusion of antioxidant-rich dietary supplements in strategies to mitigate heavy metal-induced oxidative stress and associated health risks. This research is novel in its systematic comparison of ASEO with both silymarin and olive oil, thereby providing new insights into natural antioxidant interventions for heavy metal toxicity. The findings are expected to contribute significantly to the field of cardioprotection and support the development of antioxidant-based strategies for mitigating heavy metal-induced oxidative stress and associated health risks.

2. Materials and Experimental Procedures

2.1 Study Rationale

This investigation sought to comprehensively evaluate the prospective protective effects of ASEO against cardiotoxicity induced by lead nitrate administration in male Swiss albino mice. By analysing a range of biochemical and oxidative changes, the research aims to offer an in-depth elucidation of the mechanisms underlying how ASEO may counteract the detrimental impact of lead nitrate on the heart.

2.2 Reagents

The Lead nitrate [$Pb(NO_3)_2$] utilised in this present research was procured from HiMedia Laboratories Private Limited, bearing CAS No. 10099-74-8. All other chemicals utilised were of high analytical grade and obtained from various commercial suppliers within the country. These chemicals were carefully selected to ensure the accuracy and reliability of the experimental procedures.

2.3 Plant Material

Mature, disease-free specimens of *A. sativum* L. were procured from the medicinal plant garden curated by the Banasthali Vidyapith, India. Selection of these bulbs followed stringent criteria to ensure optimal quality for subsequent essential oil extraction. This rigorous selection process maximised the productivity and quality of extracted essential oil by prioritising the garlic bulbs with optimal physiological characteristics for essential oil production.

2.4 Preparation of *A. sativum* Essential Oil Using Hydrodistillation

Raw *A. sativum* L. bulbs were carefully selected and utilised for essential oil extraction via conventional hydrodistillation. Before extraction, the bulbs underwent thorough cleaning and peeling, followed by crushing into a uniform paste with distilled water. This prepared garlic paste was then placed into a round-bottomed flask for hydrodistillation. The distillation process was conducted using a Hydrodistillation Clevenger Apparatus System, which was connected to the flask containing the garlic paste. The system was heated to a controlled temperature range of 65-75°C and maintained for 3-4 hours, or until the essential oil was fully extracted. Throughout the process, steam carried volatile components, including the essential oil, from the plant material, and the resulting vapours were condensed. The essential oil, separated from the aqueous phase using an oil-water separator, was further anhydrously treated with sodium sulfate (Na_2SO_4) to remove any residual moisture. Finally, the oil was stored under refrigeration at 4°C in a light-protected and contaminant-free environment to preserve its stability throughout the research period[9].

2.5 Experimental Animals

Male Swiss albino mice (*Mus musculus*) were used as the experimental subjects in this study. Obtained from Lala Lajpat Rai University of Veterinary and Animal Science, Hisar, Haryana (India), the mice were aged 2-3 months and weighed between 20-30 grams. Housing adhered to a strict pathogen-free protocol within a well-maintained air-conditioned environment, ensured a constant

temperature of 23-25°C, with controlled relative humidity maintained at 50 ± 15% and a 12-hour light/dark cycle to simulate natural conditions. The mice were fed a nutritionally balanced pelleted diet and had unlimited access to fresh drinking water. To minimise stress and ensure proper acclimation, the animals underwent a one-week habituation period before the commencement of the experiment. Subsequently, a randomisation process was implemented to allocate the mice into designated experimental groups.

2.6 Ethical Clearance

Animal care and management procedures strictly adhered to the directives set forth by the Institutional Animal Ethics Committee (IAEC), which operates in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) established by the Ministry of Environment, Forest and Climate Change, Government of India. All experimental protocols received prior approval from the IAEC (BV/IAEC/January/2020/10) of the Department of Bioscience and Biotechnology at Banasthali Vidyapith. The conduct of each experiment adhered to established ethical principles and recognised best practices.

2.7 Animal Allocation and Treatment Regimen

Following a one-week acclimatisation period housed in individually ventilated cages, thirty-six male Swiss albino mice were randomly assigned to six groups (n=6 per group). To ensure consistency and precise dosing, all treatment solutions were administered to the mice via oral gavage throughout the experiment. The control group (Group I) was administered double-distilled water orally throughout the entire study duration.

A $[Pb(NO_3)_2]$ dose of 50 mg/kg body weight was prepared in distilled water and given continuously for 30 days (Group II)[19]. Two doses of ASEO, 50 mg/kg for Group III and 80 mg/kg for Group IV[20] along with silymarin (Group V) and olive oil (Group VI) were administered

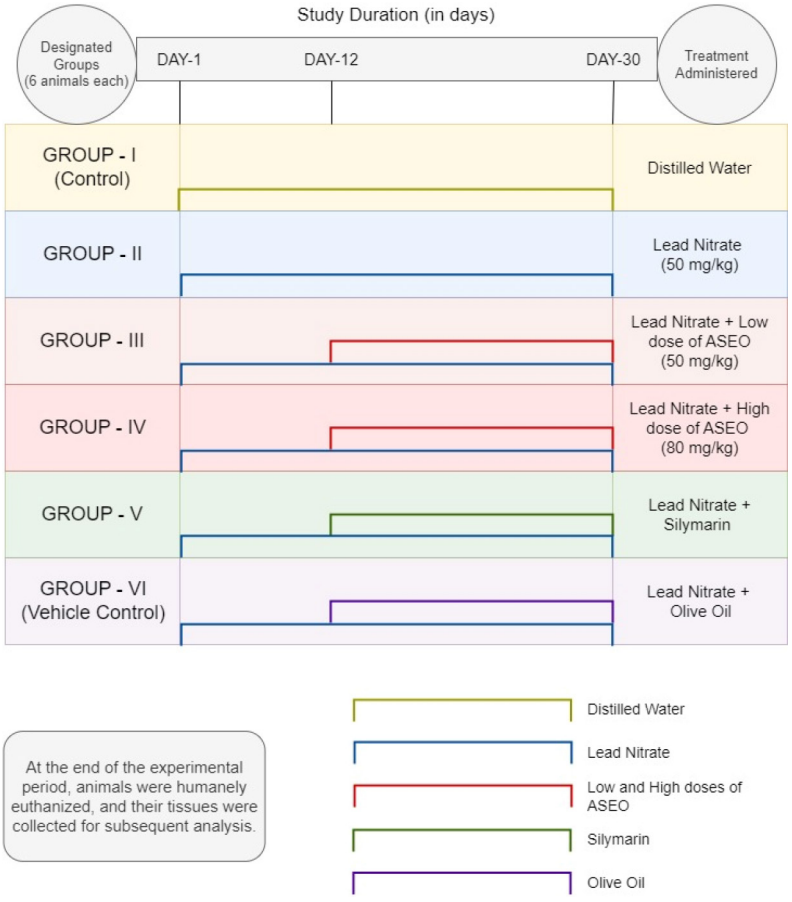


Figure 1. Treatment groups and dosing regimen for Swiss albino mice exposed to lead nitrate.

starting from the 12th day in conjunction with [$Pb(NO_3)_2$] exposure, continuing until the experiment's conclusion[21]. The assignment of these two different doses was intended to critically analyse the dose-dependent protective efficacy of ASEO relative to the standard therapeutic compound, silymarin (Group V) and the vehicle control olive oil (Group VI). A comprehensive illustration of the treatment regimen for each subgroup is presented in Figure 1.

2.8 Animal Dissection and Tissue Homogenate Preparation

All animals were dissected via cervical dislocation on the 31st day of the study, after which their hearts were removed post-perfusion. The adhering cardiac adipose tissue was carefully excised using sterilized forceps. To remove any blood, the cardiac tissues were thoroughly rinsed in ice-cold saline (0.9% sodium chloride solution), blotted dry and weighed for redox and biochemical parameter analysis. The cardiac tissue was minced into small pieces to increase the surface area for homogenisation. A 10% (w/v) homogenate was prepared by blending the minced heart tissue with pre-chilled 0.1M sodium phosphate buffer (pH 7.4). This ratio ensures sufficient tissue is available for subsequent analyses while maintaining a workable suspension. The tissue homogenate subsequently underwent centrifugation at 10,000 rpm for 15-20 minutes at 4°C with a centrifugal force of 8,000 x g (8g). This step separated the cellular components from the buffer. The obtained supernatant with soluble cellular components was carefully employed for subsequent redox and biochemical assays.

2.9 Biochemical and Oxidative Assays Employed in the Study

The aforementioned assays collectively offered a thorough evaluation of the oxidative stress levels and biochemical integrity of the cardiac tissues across various treatment regimens. The assays performed included:

2.9.1 Superoxide Dismutase Activity

SOD activity was determined as per the procedure of Dhindsa *et al.*, to evaluate its role in facilitating the catalytic conversion of superoxide radicals ($O_2\bullet^-$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), representing a crucial antioxidant defense mechanism within the tissue. The assay relied on the inhibition of Nitro Blue Tetrazolium (NBT) reduction through superoxide radicals, produced through the photochemical reaction of riboflavin in the presence of methionine and light. A higher SOD activity corresponds to greater inhibition of NBT reduction. For the assay, tissue homogenates were prepared and incubated in a reagent mixture comprising of 50mM phosphate buffer (pH 7.4), 0.1mM NBT, 0.1mM EDTA, 13mM methionine, 0.01M riboflavin, and 50mM sodium carbonate (Na_2CO_3). The chemical reaction was triggered by irradiating the mixture with fluorescent light for 10 minutes at ambient conditions. The optical absorbance of reduced NBT was observed at 560nm relative to a blank. A single unit of SOD activity was established as the amount of the enzyme essential to inhibit NBT reduction by 50%, and results were reported as units per minute per milligram of protein (U/min/mg protein)[22].

2.9.2 Glutathione Peroxidase Activity

The GPx assay assessed the enzyme's ability to reduce lipid hydroperoxides and hydrogen peroxide using glutathione, safeguarding cells against oxidative damage. GPx activity was measured indirectly by tracking the Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent conversion of Glutathione Disulfide (GSSG) back to Glutathione (GSH) via glutathione reductase, with a decline in absorbance at 340nm indicating GPx activity. Tissue homogenates were incubated with an assay mixture consisting of Tris-HCl buffer (2 mM, pH 7.4), GSH (4 mM), sodium azide (1 mM) to inhibit catalase, hydrogen peroxide (0.25 mM), and distilled water. After 15 minutes, the reaction was stopped with 10% Tricarboxylic Acid (TCA), and proteins were precipitated by centrifugation. The remaining GSH in the supernatant reacted with Ellman's reagent (DTNB) to form a yellow compound, quantified at 420nm. The activity of GPx was quantified as nM NADPH oxidised per minute per mg protein using a standard formula[23].

2.9.3 Glutathione-S-Transferase Activity

Glutathione-S-Transferase (GST) activity was analysed to evaluate the enzyme's role in conjugating reduced GSH to electrophilic compounds, aiding detoxification. The assay quantified GST activity by tracking the generation of a GSH-CDNB conjugate, catalysed by GST enzymes. The reaction mixture contained GSH (1 mM), CDNB (1mM), and phosphate buffer (50mM, pH 7.4) to maintain optimal

conditions. After adding tissue homogenate, the chemical reaction was activated, and the rise in absorbance at 340 nm was continuously observed. The absorbance change reflected GST activity, as the enzyme facilitated the formation of a coloured GSH-CDNB conjugate. GST activity was quantified according to the adduct formation rate, using its molar extinction coefficient and a standard formula. Results were presented as nmol CDNB-GSH conjugate formed per minute per mg protein[24].

2.9.4 Catalase Activity

CAT activity was evaluated to assess the enzyme's efficiency in decomposing hydrogen peroxide ($H_2O_{2)}$ into water (H_2O) and oxygen (O_2), a critical process for preventing oxidative damage to cells. The enzyme action was assessed through the rate of H_2O_2 depletion at its absorbance peak of 240nm. Tissue homogenates were incubated with a 3mM H_2O_2 solution in phosphate buffer (50 mM, pH 7.4). The decline in absorbance at 240nm over time, which directly reflects H_2O_2 consumption by CAT, was utilised to quantify enzyme bioactivity. The molar extinction value of H_2O_2 (43.6 $M^{-1}cm^{-1}$) and a standard equation were applied to calculate the catalase activity as micromoles of H_2O_2 consumed per minute per milligram of protein (µmol H_2O_2/min/mg protein)[25].

2.9.5 Lipid Peroxidation

Lipid Peroxidation (LPO) was assessed by quantifying Malondialdehyde (MDA), an indicator of oxidative damage, using the TBARS method. This detects MDA through its reaction with Thiobarbituric Acid (TBA), forming a pink chromogen. Briefly, tissue homogenates were subjected to incubation at 90°C for 1 hour with a reaction mixture composed of 0.67% w/v TBA, 20% v/v acetic acid, and 8.1% w/v Sodium Dodecyl Sulfate (SDS). After incubation, the reaction components were isolated using a solution of n-butanol and pyridine (15:1 v/v) to isolate the MDA-TBA chromogen. The optical absorbance value of the chromogen was quantified at 532nm, its peak wavelength. The LPO level was determined by calculating the tissue concentration of MDA, expressed as nanomoles of MDA formed per milligram of tissue (nmol MDA/mg tissue), using a standardised molar extinction coefficient[26].

2.9.6 Total Protein Content

TPC was measured to normalise enzyme activities and biochemical parameters, allowing for accurate comparisons between experimental groups. Protein concentration in heart tissue homogenates was determined using the Lowry method, a colorimetric assay. Briefly, the test solution was incubated with Solution A (4 mg/mL sodium hydroxide and 20 mg/mL sodium carbonate) and then with Solution B (10 mg/mL potassium sodium tartrate and 5 mg/mL copper sulfate). After incubation, the colour intensity was quantified at 750nm with a microplate spectrophotometer. Protein concentration was determined based on a standard curve prepared with bovine serum albumin[27].

2.9.7 Alanine Transferase Activity

ALT facilitates the biochemical conversion of alanine and α-ketoglutarate to pyruvate and glutamate. Elevated ALT levels in cardiac tissue or serum can indicate myocardial damage, often linked to conditions like myocardial infarction, cardiac ischemia, or exposure to cardiotoxic agents, reflecting cellular injury. ALT catalytic activity was measured employing the Reitman and Frankel method (1957). Briefly, ALT substrate (2mM α-ketoglutarate and 200mM dl-alanine) was preincubated at 40°C for 10 minutes, then a 0.2mL aliquot of tissue homogenate was added. The reaction was allowed to proceed for 30 minutes, then stopped with 1mL hydrazine reagent. Absorbance was measured at 505nm 30 minutes after adding 10 mL 0.4 N NaOH. ALT activity was determined using a pyruvate standard curve, expressed as micromoles of pyruvate produced per minute at 25°C[28].

2.9.8 Aspartate Aminotransferase Activity

AST catalyses the translocation of an amino group from aspartate to α-ketoglutarate, resulting in the formation of oxaloacetate and glutamate. AST is more abundant in cardiac tissue than ALT, and elevated levels are a sensitive marker of myocardial injury. AST enzymatic activity was measured using the Reitman and Frankel method. Briefly, AST substrate (2mM α-ketoglutarate and 200mM dl-aspartate) was subjected to incubation at 40°C for 10 minutes. Tissue homogenate was added, and the reaction proceeded for 1 hour. The reaction was stopped with 1 mL of 2,4-dinitrophenylhydrazine reagent (mM) and allowed to stabilise at ambient temperature for 20 minutes. Afterward, 10 mL of 0.4 N sodium hydroxide was added, and the absorbance was measured at 505nm. A serum control was prepared similarly, and the change in absorbance was measured to determine AST activity,

expressed as micromoles of oxaloacetate plus pyruvate produced per minute at 25°C, using a standard curve[28].

2.9.9 Alkaline Phosphatase Activity

ALP is an enzyme that mediates the hydrolytic cleavage of phosphate moieties from different substrates, such as nucleotides, proteins, and alkaloids. While it is not as specific to cardiac tissue as ALT and AST, elevated ALP levels can indicate abnormal cardiac function, especially in conditions involving calcification or fibrosis. ALP activity was assessed employing the protocol established by Tietz *et al*. ALP facilitates the hydrolysis of p-nitrophenyl phosphate (pNPP) to produce yellow-coloured p-nitrophenol and phosphate at pH 10.3. The resulting absorbance change at 405 nm is directly proportional to enzyme activity. The reagents used were AMP buffer (300mM AMP, 2mM magnesium acetate, 0.8mM zinc sulfate) and 10mM pNPP substrate. The sample was combined with the working ALP reagent, and absorbance was measured at 30-second intervals for up to 120 seconds. ALP activity was observed as a change in absorbance per minute using a kinetic factor[29].

2.10 Statistical Analysis

All the experiments were executed in three independent replicates to ensure reproducibility and accuracy of the obtained results. The data are expressed as mean ± SEM to reflect both the central tendency and the variability of the results. Statistical evaluation was conducted utilising One-Way ANOVA, followed by Tukey's Honest Significant Difference (HSD) post-hoc test. This approach allows for the identification of statistically significant differences among the experimental groups. SPSS version 20.0 was employed for all statistical computations. A p-value below the threshold of $0.05^{*/\#}$, $0.01^{**/\#\#}$, and $0.001^{***/\#\#\#}$ were deemed statistically significant.

3. Results

3.1 Assessment of Oxidative-Reductive Balance Indices

Exposure to lead nitrate significantly reduced SOD levels to 0.014 U/min/mg protein, relative to 0.028 U/min/mg protein in the control group ($P < 0.01$). Administration of low-dose ASEO and olive oil led to minor increases, reaching 0.020 and 0.015 U/min/mg protein, respectively, although these changes were statistically insignificant ($P > 0.05$). Conversely, high-dose ASEO significantly elevated SOD levels to 0.025 U/min/mg protein ($P < 0.05$), while silymarin showed a similar effect, increasing SOD levels to 0.026 U/min/mg protein ($P < 0.05$).

Lead nitrate exposure significantly reduced GPx levels to 0.020 nM NADPH oxidized/min/mg protein, compared to 0.043 in the control group ($P < 0.01$). Treatment with low-dose ASEO and olive oil resulted in slight increases to 0.024 and 0.027, respectively, nonetheless, these changes did not reach statistical significance ($P > 0.05$). In contrast, high-dose ASEO significantly elevated GPx levels to 0.039 ($P < 0.05$), while silymarin showed a comparable effect, increasing GPx levels to 0.041 ($P < 0.05$).

Lead nitrate exposure caused a significant decline in GST levels, dropping to 0.016 nM CDNB-GSH conjugate formed/min/mg protein, compared to 0.060 in the control group ($P < 0.001$). Treatment with low-dose ASEO and olive oil led to modest increases, reaching 0.026 and 0.029, respectively, though these changes were statistically insignificant ($P > 0.05$). In contrast, high-dose ASEO markedly elevated GST levels to 0.050 ($P < 0.001$), while silymarin also produced a significant increase, raising GST levels to 0.043 ($P < 0.01$).

Exposure to lead nitrate resulted in a significant decrease in CAT levels, dropping to 2.32 µmol H_2O_2 consumed/min/mg protein, compared to 5.76 in the control group ($P < 0.01$). Low-dose ASEO and olive oil treatments led to modest increases in CAT levels, reaching 3.25 and 3.53, respectively, but these changes were not statistically significant ($P > 0.05$). On the other hand, high-dose ASEO significantly restored CAT levels to 4.95 ($P < 0.01$), while silymarin demonstrated a more pronounced effect, elevating levels to 5.55 ($P < 0.01$).

Exposure to lead nitrate induced a significant elevation in LPO levels, rising to 2.83 nmol MDA formed/mg tissue, compared to 0.90 in the control group ($P < 0.001$). Treatment with low-dose ASEO led to a slight reduction in LPO levels, decreasing to 2.79, though this change did not exhibit statistical significance ($P > 0.05$). However, high-dose ASEO significantly lowered LPO levels to 1.87 ($P < 0.01$), demonstrating a more substantial effect. Both silymarin and olive oil also produced significant decreases in LPO levels, reducing them to 2.13 and 2.26, respectively, compared to the lead nitrate-induced increase ($P < 0.05$).

Figure 2 displays bar graphs that depict these changes, with the corresponding numerical data provided in Table 1 for the oxidative parameters.

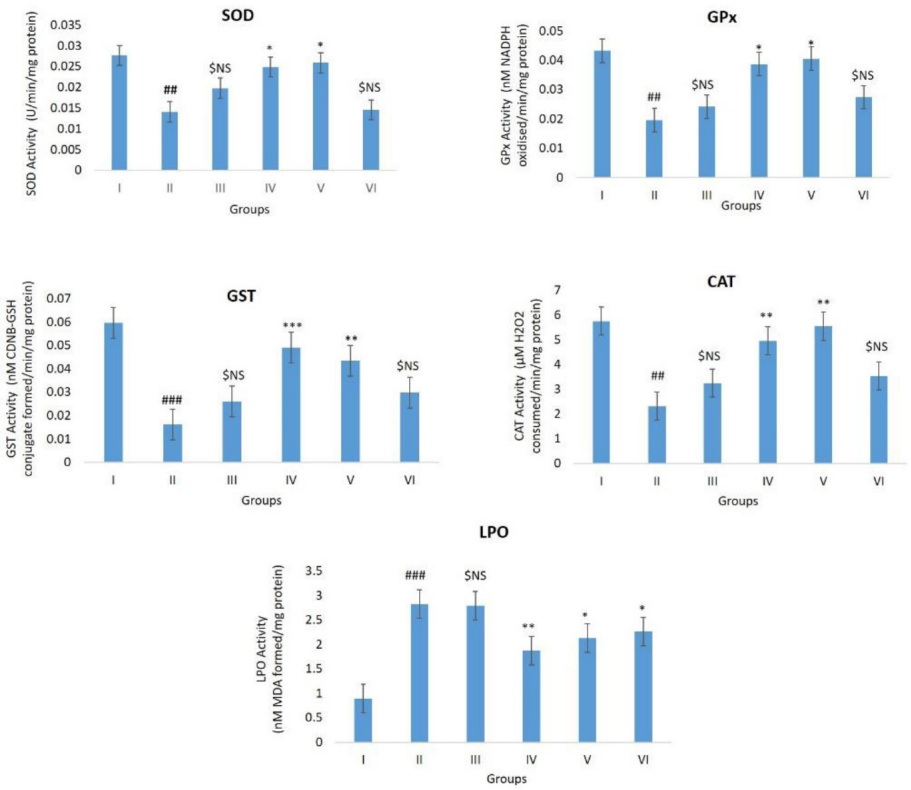


Figure 2. Bar graphs depicting changes in oxidative stress parameters (SOD, GPx, GST, CAT, and LPO) across study groups.

Table 1. Changes observed in oxidative stress parameters (SOD, GPx, GST, CAT, and LPO) across study groups.

Groups	SOD (U/min/mg protein)	GPX (nM NADPH oxidised/min/mg protein	GST (nmol CDNB-GSH conjugate formed/min/ mg protein)	CAT (µmol H_2O_2 consumed /min/ mg protein)	LPO (nmol MDA formed/mg tissue)
Group I	0.028 ± 0.001084	0.043 ± 0.001	0.060 ± 0.003	5.76 ± 0.25	0.90 ± 0.03
Group II	0.014 ± 0.001076$^{\#\#}$	0.020 ± 0.001$^{\#\#}$	0.016 ± 0.003$^{\#\#\#}$	2.32 ± 0.09$^{\#\#}$	2.83 ± 0.06$^{\#\#\#}$
Group III	0.02 ± 0.000939$^{\$NS}$	0.024 ± 0.001$^{\$NS}$	0.026 ± 0.003$^{\$NS}$	3.25 ± 0.12$^{\$NS}$	2.79 ± 0.11$^{\$NS}$
Group IV	0.025 ± 0.000899*	0.039 ± 0.001*	0.050 ± 0.004***	4.95 ± 0.15**	1.87 ± 0.05**
Group V	0.026 ± 0.000718*	0.041 ± 0.002*	0.043 ± 0.003**	5.55 ± 0.17**	2.13 ± 0.06*
Group VI	0.015 ± 0.001304$^{\$NS}$	0.027 ± 0.001$^{\$NS}$	0.029 ± 0.002$^{\$NS}$	3.53 ± 0.09$^{\$NS}$	2.26 ± 0.02*

All data are represented as mean ± SEM of six mice per group. Statistically significant variations in data are indicated as $p<0.001^{\#\#\#/***}$, $p<0.01^{\#\#/**}$, $p<0.05^{\#/*}$ compared to the control group. Hash signs (#) denote differences between the lead nitrate-treated group (group II) and the control group (group I), while asterisks (*) indicate comparisons between the lead nitrate-treated group (group II) and the remaining four treatment groups (group III to group VI). $NS stands for non-significant changes.

3.2 Quantification of Biochemical Markers

ALT levels increased significantly following lead nitrate exposure, reaching 1.79 IU/L compared to 0.20 in the control group ($P < 0.001$). Low-dose ASEO led to a slight, non-significant reduction to 1.72 IU/L ($P > 0.05$). Conversely, high-dose ASEO and silymarin markedly lowered ALT levels to 0.50 and 0.46 IU/L, respectively ($P < 0.001$). Olive oil also significantly reduced ALT levels to 1.20 IU/L ($P < 0.01$), though its effect was less pronounced.

Lead nitrate exposure significantly elevated AST levels to 2.00 IU/L, compared to 0.43 IU/L in the control group ($P < 0.001$). Intervention with low-dose ASEO resulted in a slight, non-significant reduction to 1.78 IU/L ($P > 0.05$). In contrast, high-dose ASEO and silymarin significantly lowered AST levels to 0.73 and 0.49 IU/L, respectively ($P < 0.01$), with silymarin exhibiting a more pronounced effect. Olive oil also reduced AST levels to 1.22 IU/L, though this decline was statistically insignificant ($P > 0.05$).

A substantial rise in ALP levels was noted, reaching 351.10 IU/L in the lead nitrate-exposed group, compared to 90.45 IU/L in the control group ($P < 0.001$). Treatment with low-dose ASEO led to a mild, non-significant reduction to 272.76 IU/L ($P > 0.05$). In contrast, high-dose ASEO and silymarin significantly decreased ALP levels to 130.01 and 103.25 IU/L, respectively ($P < 0.01$), with silymarin showing a stronger effect. Olive oil also produced a significant reduction, lowering ALP levels to 162.66 IU/L ($P < 0.05$).

A marked reduction in total protein levels was observed in the lead nitrate-exposed group, decreasing to 7.45 µg/ml compared to 17.9 µg/ml in the control group ($P < 0.001$). Treatment with low-dose ASEO led to a modest but non-significant increase to 12.93 µg/ml ($P > 0.05$). In contrast, high-dose ASEO significantly restored total protein levels to 15.99 µg/ml ($P < 0.001$). Silymarin and olive oil also contributed to a significant increase, elevating total protein levels to 16.47 µg/ml and 9.29 µg/ml, respectively ($P < 0.01$).

Figure 3 presents bar graphs illustrating these observed changes. The detailed numerical data are compiled in Table 2.

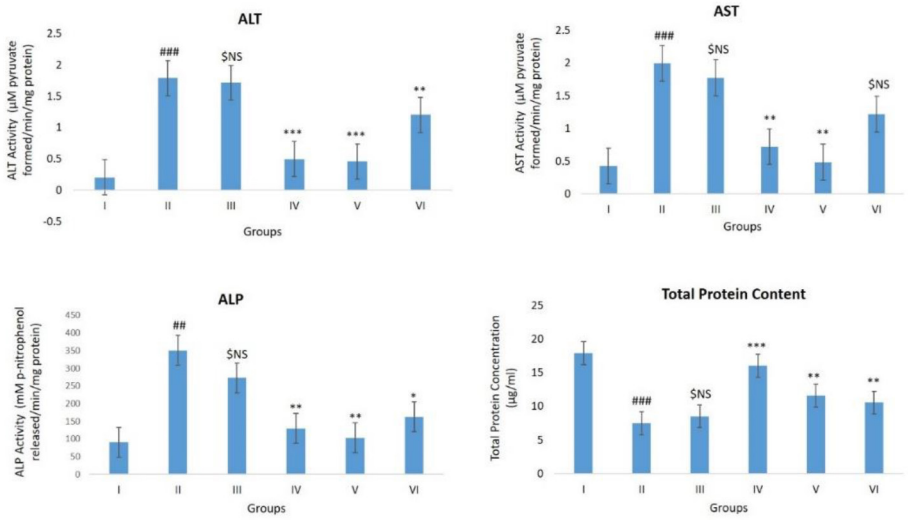


Figure 3. Bar graphs illustrating changes in biochemical parameters (ALT, AST, ALP, and TPC) across different experimental groups.

Table 2. Changes observed in biochemical parameters (ALT, AST, ALP, and TPC) across study groups.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Protein (µg/ml)
Group I	0.20 ± 0.01	0.43 ± 0.12	90.45 ± 7	17.9 ± 0.01
Group II	$1.79 \pm 0.05^{\#\#\#}$	$2.00 \pm 0.49^{\#\#\#}$	$351.10 \pm 31^{\#\#}$	$7.45 \pm 0.078^{\#\#\#}$
Group III	$1.72 \pm 0.02^{\$NS}$	$1.78 \pm 0.46^{\$NS}$	$272.76 \pm 52.8^{\$NS}$	$12.93 \pm 0.026^{\$NS}$
Group IV	$0.50 \pm 0.07^{***}$	$0.73 \pm 0.22^{**}$	$130.01 \pm 0.76^{**}$	$15.99 \pm 0.029^{***}$
Group V	$0.46 \pm 0.08^{***}$	$0.49 \pm 0.15^{**}$	$103.25 \pm 2.06^{**}$	$16.47 \pm 0.016^{**}$
Group VI	$1.20 \pm 0.04^{**}$	$1.22 \pm 0.21^{\$NS}$	$162.66 \pm 10.76^{*}$	$9.29 \pm 0.083^{**}$

All data are represented as mean ± SEM of six mice per group. Statistically significant variations in data are indicated as $p<0.001^{\#\#\#/***}$, $p<0.01^{\#\#/**}$, $p<0.05^{\#/*}$ compared to the control group. Hash signs (#) denote differences between the lead nitrate-treated group (group II) and the control group (group I), while asterisks (*) indicate comparisons between the lead nitrate-treated group (group II) and the remaining four treatment groups (group III to group VI). $NS stands for non-significant changes.

4. Discussion

Cellular membranes are primary targets of oxidative damage induced by toxic compounds such as heavy metals. Lead (Pb) exposure disrupts potassium (K^+) and calcium (Ca^{2+}) channels, leading to cellular rupture. This disruption, along with cytoskeletal alterations, increases susceptibility to lysis and negatively impacts intracellular calcium signalling, endoplasmic reticulum function and protein synthesis, further exacerbating cellular damage[30].

Lead nitrate exposure induces severe oxidative damage by impairing the enzymatic antioxidant protection and exacerbating LPO. The pronounced reductions SOD, GPx, GST and CAT, alongside a significant increase in LPO, highlight the extensive oxidative damage inflicted on cardiac tissue[31]. Lead toxicity further exacerbates this damage by interfering with iron absorption and inhibiting heme biosynthesis, thereby impairing the function of these critical antioxidant enzymes. Since enzymes like GPx, CAT, and SOD rely on vital trace elements such as selenium, iron, and copper for optimal activity, they become primary targets of lead nitrate toxicity, further amplifying oxidative stress and cardiac dysfunction[32].

SOD plays a pivotal role in the primary phase of defence against reactive oxygen species (ROS), catalysing the dismutation of superoxide radicals ($O_2^-\bullet$) into hydrogen peroxide (H_2O_2)[33]. The observed decrease in SOD activity following lead exposure suggests an overwhelming ROS burden that surpasses the enzyme's detoxification capacity. This decline may be due to the direct association of lead with essential metal cofactors, such as zinc and copper, within the enzyme's active site, thereby impairing its structural integrity and catalytic function[34]. A decline in SOD activity suggests an accumulation of superoxide radicals, which can directly impair mitochondrial function and promote further oxidative damage. H_2O_2 must be promptly detoxified to prevent cellular damage, a role primarily fulfilled by CAT and GPx[35]. The significant restoration of SOD levels upon high-dose ASEO treatment indicates the potent antioxidant potential of its organosulfur compounds, particularly S-allyl cysteine and diallyl trisulfide, which may enhance the bioavailability of essential cofactors and upregulate SOD gene expression.

GPx and CAT work synergistically to neutralise the effects of hydrogen peroxide (H_2O_2), converting it into water and oxygen. This process prevents H_2O_2 from generating highly reactive hydroxyl radicals ($OH\bullet$), which can cause severe oxidative damage. While CAT rapidly catalyses the decomposition of H_2O_2 into water and oxygen, GPx utilises glutathione (GSH) to neutralise both H_2O_2 and lipid hydroperoxides, thereby protecting cellular membranes from oxidative injury[35]. The pronounced reduction in GPx levels following lead exposure highlights the depletion of reduced GSH, a key cofactor required for GPx activity. Lead has been shown to form covalent bonds with sulfhydryl groups on GSH, thereby reducing its availability for GPx-mediated detoxification[36]. High-dose ASEO significantly restored GPx levels, likely by replenishing intracellular GSH pools through the induction of γ-glutamylcysteine synthetase, a rate-limiting enzyme in GSH synthesis[37]. Similarly, the restoration of CAT activity upon ASEO administration suggests that its sulfur-containing metabolites may enhance CAT expression and stability, facilitating more efficient degradation of H_2O_2.

GST is another crucial antioxidant enzyme that plays a pivotal role in phase II detoxification, catalysing the conjugation of xenobiotics and lipid peroxidation byproducts with GSH to facilitate their excretion. The significant depletion of GST levels in lead-exposed mice indicates a compromised detoxification system, likely due to excessive consumption of GSH and direct inhibition of GST activity by lead ions[38]. The high-dose ASEO-mediated restoration of GST levels suggests that its bioactive compounds may upregulate the expression of GST isoforms, enhance the regeneration of GSH, and mitigate oxidative damage.

LPO marked by a significant rise in MDA levels in $Pb(NO_3)_2$-treated heart tissue homogenates underscores severe oxidative damage and compromised membrane integrity. This peroxidation cascade begins with reactive oxygen species (ROS)-mediated hydrogen abstraction from polyunsaturated fatty acids, leading to a chain reaction that propagates membrane damage and disrupts fluidity, further exacerbating cellular dysfunction[39]. Increased LPO in heart tissues reflects disrupted lipid homeostasis, leading to cardiovascular complications such as endothelial damage, impaired vasomotor activity, hypertension, and atherosclerosis. The remarkable reduction in LPO levels upon high-dose ASEO treatment highlights its role in stabilizing membrane integrity, likely through direct scavenging of peroxyl radicals ($ROO\bullet$) and inhibition of iron-mediated Fenton reactions[40]. Moreover, ASEO-derived organosulfur compounds may chelate lead ions, reducing their bioavailability and subsequent

participation in oxidative reactions, thereby enhancing tissue viability.

AST and ALT are crucial enzymes involved in biochemical metabolism, particularly in amino acid transamination and energy homeostasis. Their significant elevation following lead nitrate exposure suggests widespread cardiomyocyte damage, oxidative stress-driven mitochondrial dysfunction, and apoptotic signalling activation[35]. Lead exposure induces excessive ROS generation, which impairs the stability of the mitochondrial membrane, causing the release of cytochrome c and caspase-dependent apoptosis[4]. Additionally, the increased AST: ALT ratio further indicates myocardial stress, aligning with lead-induced calcium dysregulation, which disrupts excitation-contraction coupling and promotes arrhythmic and ischemic events. Biochemical analyses revealed that lead nitrate exposure markedly elevated ALT, AST, and ALP levels, signalling extensive myocardial damage and endothelial dysfunction[39]. ALP elevation suggests vascular endothelial stress and extracellular matrix remodelling, potentially contributing to increased arterial stiffness and atherogenesis[7]. On treatment with high-dose ASEO, the levels of ALT, AST, and ALP were notably restored, suggesting that ASEO not only mitigates oxidative stress but also protects against cellular damage in the heart. Similarly, the reduction in ALP levels, an indicator of cell membrane integrity, further underscores the stabilizing effect of ASEO on myocardial cells[9].

Lead nitrate exposure also led to a significant decrease in total protein content (TPC) in cardiac tissues, likely due to impaired protein synthesis, increased proteolysis, and endoplasmic reticulum (ER) stress[40]. Lead-induced calcium dyshomeostasis may disrupt protein translation by stimulating the Unfolded Protein Response (UPR), increasing eIF2α phosphorylation and inhibiting global protein synthesis[41]. Additionally, lead-mediated oxidative stress can also alter the function of proteasomal degradation pathways, exacerbating myocardial atrophy and fibrosis[42]. High-dose ASEO treatment significantly restored TPC, indicating its role in stimulating myocardial regeneration and cellular repair mechanisms. This is potentially mediated by initiating IGF-1/Akt/mTOR pathway, which stimulates protein synthesis, suppresses autophagy-mediated degradation, and promotes cardiomyocyte survival[43]. Furthermore, allicin's ability to enhance GSH synthesis supports intracellular redox balance, protecting structural proteins and contractile elements from oxidative damage[11].

In the present study, the observed restoration of antioxidant and biochemical enzyme activity, along with a reduction in lipid peroxidation and an increase in total protein levels following high-dose ASEO administration, underscores its promising therapeutic potential in counteracting lead-induced oxidative and biochemical damage. The bioactive constituents in ASEO, specifically allicin, play a crucial role in this protective effect by scavenging ROS, preserving mitochondrial function, and inhibiting NF-κB-mediated inflammatory cascades[12]. Additionally, ASEO modulates the Nrf2/Keap1 regulatory pathway, enhancing the level of expression of endogenous antioxidant defence genes like HO-1 and NQO1, which help mitigate oxidative stress-induced cardiac injury[44]. Its sulfur-rich compounds, such as diallyl disulfide and S-allyl cysteine, stabilise myocardial cell membranes, reduce LPO and suppress calcium overload, thus preventing cardiomyocyte apoptosis and necrosis. The metal-chelating properties of ASEO further reduce lead accumulation in cardiac tissues, alleviating oxidative stress and reinforcing its cardioprotective effect[10].

Silymarin exhibited strong antioxidant and biochemical properties, effectively restoring enzymatic activity and reducing LPO. The notable increase in SOD levels suggests its ability to neutralise superoxide radicals, thereby preventing further oxidative damage. Its positive impact on GPx and GST highlights its role in glutathione metabolism and detoxification, reducing the accumulation of harmful peroxides and electrophilic compounds[45]. Additionally, the significant improvement in CAT activity underscores its ability to break down hydrogen peroxide, preventing the formation of highly reactive hydroxyl radicals that contribute to oxidative stress in cardiac tissue. The reduction in LPO levels further suggests that silymarin stabilises cardiac cell membranes by inhibiting oxidative damage and lipid peroxidation. The observed decline in AST, ALT, and ALP levels following silymarin treatment supports its efficacy in preventing lead-induced cardiac apoptosis, mitochondrial dysfunction, and endothelial injury. Additionally, silymarin helps maintain cell integrity by modulating mitochondrial permeability to prevent apoptosis and chelating metal ions to reduce oxidative burden[46]. Its protective effects are primarily attributed to its bioactive flavonolignans, particularly silybin, which stimulates the Nrf2 signalling pathway to improve endogenous antioxidant defenses[47]. Moreover, silymarin mitigates cardiotoxicity by modulating NF-κB and MAPK regulatory pathways, leading to reduced

TNF-α and IL-6 expression. By downregulating NF-κB signalling, silymarin further suppresses the secretion of pro-inflammatory cytokines, providing significant protection against lead-induced cardiac injury[5].

Olive oil, while beneficial, exhibited antioxidant effects to a lesser extent than silymarin and ASEO. Its impact on antioxidant enzyme levels was relatively modest, yet it contributed to restoring LPO, ALT, ALP, and total protein levels, indicating its role in enzymatic detoxification and free radical neutralisation. Additionally, olive oil helped reduce ALT and ALP levels, though its effect on AST was comparatively limited[48]. Olive oil's cardioprotective effects stem from its rich composition of phenolic compounds, such as hydroxytyrosol and oleuropein, which are recognized to modulate oxidative stress pathways and enhance mitochondrial function[49]. Moreover, its rich content of Monounsaturated Fatty Acids (MUFAs) likely contributes to its ability to prevent lipid oxidation and mitigate oxidative damage. These protective effects are further supported by its ability to suppress NADPH oxidase activity, attenuate endothelial dysfunction, and upregulate nitric oxide bioavailability, collectively improving vascular integrity and cardiac health[50].

While silymarin and olive oil also demonstrated protective effects against lead-induced oxidative stress, ASEO emerged as the most effective intervention. In this study, ASEO exhibited remarkable therapeutic potential in mitigating lead nitrate-induced cardiotoxicity, surpassing both silymarin and olive oil in antioxidant capacity, membrane stabilisation, and oxidative stress attenuation. These findings highlight ASEO as a promising cardioprotective agent against lead-induced toxicity, offering a novel therapeutic strategy for combating heavy metal-induced cardiovascular damage. Future studies should focus on optimising ASEO dosing regimens, exploring its molecular mechanisms further, and conducting long-term assessments to assess its cumulative benefits and potential side effects. The encouraging findings from this research study lay the foundation for further trials to explore ASEO's role in mitigating lead toxicity and other forms of metal-induced oxidative stress across different biological systems.

5. Conclusion

This study underscores the significant oxidative stress and cardiovascular toxicity caused by $Pb(NO_3)_2$ exposure, emphasising the need for antioxidant-rich dietary supplements like ASEO in therapeutic strategies aimed at mitigating heavy metal-induced oxidative damage. Our findings indicate that lead nitrate exposure significantly impaired various oxidative stress indices, including reduced levels of SOD, GPx, GST, and CAT, along with an increase in LPO levels. The high-dose ASEO demonstrated the most robust protective effects by significantly restoring SOD, GPx, GST, and CAT activities, while reducing LPO levels in a more pronounced manner than silymarin and olive oil. While both silymarin and olive oil provided protective effects, ASEO demonstrated superior efficacy, especially in enhancing GST activity and significantly reducing LPO levels, highlighting its potent antioxidant properties. Significant improvements in ALT, AST, ALP, and total protein levels further corroborate the efficacy of high-dose ASEO in mitigating lead nitrate-induced biochemical dysfunction. These findings suggest that ASEO not only mitigates oxidative stress but also restores biochemical homeostasis more effectively than olive oil or silymarin. Future research should prioritise optimising ASEO dosing regimens and further elucidating its molecular mechanistic pathways. Long-term assessments are also needed to assess the potential cumulative benefits and any side effects of high-dose ASEO across different biological systems. The promising outcomes from this study set the stage for more comprehensive trials, potentially leading to the development of effective interventions against lead toxicity and other forms of metal-induced oxidative stress.

6. Author Contributions

A.R.: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Software, Visualisation, Writing – original draft. T.A.: Writing – review and editing. V.S.: Project Administration, Supervision, Review and Editing. All authors have read and approved the final version of the manuscript for publication.

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8. Data Availability Statement

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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