Toxicology Reports 14 (2025) 101950

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Protective effects of *Allium sativum* essential oil against lead nitrate-induced cardiotoxicity: Modulation of lipid metabolism, nitric oxide dynamics, inflammatory mediators, and histological profiles in Swiss albino mice

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ARTICLE INFO

Keywords:
Allium sativum essential oil
Lipid dysregulation
Nitric oxide modulation
Cardiac inflammation

ABSTRACT

Background: Lead (Pb^{2+}) is a toxic metal known to induce oxidative stress and inflammation, contributing to cardiovascular diseases such as hypertension and atherosclerosis. Natural compounds like Allium sativum essential oil (ASEO) offer potential therapeutic benefits against lead-induced damage, but their cardioprotective effects remain underexplored. This study investigates the efficacy of ASEO in mitigating cardiovascular toxicity induced by lead nitrate in male Swiss albino mice.

Methods: Thirty-six male mice were divided into six groups: Control, Lead Nitrate (50 mg/kg), Lead Nitrate + Low-dose ASEO (50 mg/kg), Lead Nitrate + High-dose ASEO (80 mg/kg), Lead Nitrate + Silymarin (25 mg/kg), and Lead Nitrate + Olive Oil. After 12 days of lead exposure, treatments were administered for 30 days. Key cardiovascular parameters such as lipid profiles (total cholesterol, LDL, HDL), nitric oxide (NO), and inflammatory markers (TNF-α, IL-6, IFN-γ, IL-10, NF-κB) were evaluated alongside histological analysis of cardiac tissue.

Results: Lead nitrate exposure significantly increased total cholesterol (88.27 μg/mL) and LDL (93.78 μg/mL) while reducing HDL (17.51 μg/mL) compared to controls ($P < 0.001$). High-dose ASEO lowered total cholesterol (66.07 μg/mL) and LDL (49.62 μg/mL) while increased HDL (27.2 μg/mL) ($P < 0.001$). NO levels, reduced by lead exposure, were significantly restored by high-dose ASEO ($P < 0.001$). Inflammatory markers, including TNF-α, NF-kB, and IL-6, were elevated in the lead group but decreased significantly following ASEO treatment ($P < 0.001$). Histological analysis showed that ASEO markedly preserved myocardial architecture, reducing degeneration and inflammation.

Conclusion: High-dose ASEO demonstrated significant cardioprotective effects against lead-induced toxicity by improving lipid profiles, enhancing NO levels, and modulating inflammatory markers. Further studies are warranted to validate these results.

1. Introduction

Lead (Pb^{2+}) is a hazardous heavy metal that is pervasive in the human environment due to extensive anthropogenic activities, and it poses significant health risks to various biological systems, including the cardiovascular and immune systems. Chronic exposure to lead, particularly through occupational and environmental sources, is a serious public health concern, contributing to the development of cardiovascular diseases (CVDs) such as hypertension, atherosclerosis, and heart failure. The cardiovascular toxicity associated with lead is largely attributed to its ability to induce oxidative stress and inflammation—key mechanisms that drive cardiovascular dysfunction. Lead exposure generates reactive oxygen species (ROS), which overwhelm the body's antioxidant defenses, leading to oxidative damage to cells and tissues. This damage, in turn, activates inflammatory pathways, exacerbating cardiovascular injury and can also promote atherosclerosis, an immunoinflammatory disease [7,33]. The immune system is highly sensitive to lead exposure, and its dysregulation underlies many of the pathological conditions associated with lead toxicity. Cytokines, small bioactive proteins produced by immune cells, play a central role in mediating the inflammatory response and facilitating communication between immune cells [28]. Lead exposure has been shown to

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https://doi.org/10.1016/j.toxrep.2025.101950

Available online 9 February 2025

upregulate pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), while anti-inflammatory cytokines like IL-10 may be suppressed. These cytokines are pivotal in the development and progression of atherosclerosis, which is now recognized as an immunoinflammatory disease. Nuclear factor-kappa B (NF-κB), a transcription factor involved in immune regulation, is also activated in response to lead exposure, further amplifying the inflammatory response. Given the complex interplay between oxidative stress, inflammation, and lipid metabolism in lead-induced cardiovascular damage, there is an urgent need to develop therapeutic interventions that can target these pathways [28]. To model the cardiovascular toxicity associated with lead exposure, lead nitrate [$Pb(NO_3)_2$], a soluble form of lead, was chosen for this study. Lead nitrate effectively induces oxidative stress and inflammatory responses, providing a reliable model for studying lead's cardiovascular effects.

Medicinal plants, with their rich bioactive compounds, have demonstrated significant pharmacological potential in managing oxidative stress and inflammation, making them valuable therapeutic resources. In this context, *Allium sativum* (garlic), a well-characterized plant with a rich chemical profile, has garnered significant attention as a potent natural remedy, particularly for its antioxidant, anti-inflammatory, and cardioprotective properties [27]. It contains a variety of bioactive organosulfur compounds, including diallyl disulfide, methyl allyl disulfide, methyl allyl trisulfide, diallyl trisulfide, and diallyl tetrasulfide. The key bioactive compounds are outlined in Table 1 [8, 17,35]. These compounds have been shown to effectively scavenge free radicals, inhibit the synthesis of pro-inflammatory cytokines, and enhance lipid metabolism [42]. Garlic, widely used as a spice and flavoring agent, is not only rich in essential nutrients such as carbohydrates, proteins, fats, minerals, water, and vitamins, but it also holds significant medicinal value. According to Tesfaye [45], garlic has been found to possess a wide array of therapeutic properties, including anti-inflammatory, antimicrobial, analgesic, and antioxidant effects. Additionally, it has been traditionally used to treat various conditions such as asthma, arthritis, chronic fever, tuberculosis, indigestion, diabetes, and liver protection, as well as offering benefits for skin conditions, kidney stones, and even certain cancers. While the health benefits of Allium sativum are well-documented, particularly in areas such as cardiovascular health and oxidative stress, its therapeutic potential in counteracting lead-induced cardiovascular toxicity remains underexplored. This study addresses this gap by investigating the novel application of *Allium sativum* essential oil in mitigating the adverse cardiovascular effects induced by lead nitrate exposure [35].

2. Materials and experimental procedures

2.1. Study rationale

This study was conceived to thoroughly investigate the potential of Allium sativum essential oil (ASEO) in mitigating lead nitrate [$Pb(NO_3)_2$]-induced cardiotoxicity in male Swiss albino mice. By systematically analyzing lipid profile alterations, inflammatory biomarkers, nitric oxide availability, and conducting histological evaluations of cardiac tissues, this research sought to unravel the protective mechanisms through which ASEO may counteract the harmful cardiovascular effects instigated by lead exposure.

2.2. Reagents

The lead nitrate [$Pb(NO_3)_2$] used in the study was sourced from HiMedia Laboratories Private Limited, with CAS No. 10099–74–8. All other reagents used were of high analytical grade and obtained from various reputable commercial suppliers to ensure the precision and reproducibility of the experimental procedures.

Table 1
Chemical components in Allium sativum essential oil obtained by hydrodistillation.

Number	Compound Name	Chemical Formula
1	Prop−2-ene−1-thiol	C_3H_6S
2	Allyl(methyl)sulfane	C_4H_8S
3	(*E*)-but−2-enal	C_4H_6O
4	2-Methylpent−4-enal	$C_6H_{10}O$
5	1,2-Dimethyldisulfane	$C_2H_6S_2$
6	(*E*)−2-methylbut−2-enal	C_5H_8O
7	2-Methylenepentanal	$C_6H_{10}O$
8	(*E*)-pent−2-enal	C_5H_8O
9	1,2-Diallyldisulfane	$C_6H_{10}S_2$
10	Diallylsulfane	$C_6H_{10}S$
11	2-Methylfuran	C_5H_6O
12	2,4-Dimethylthiophene	C_6H_8S
13	3-Methylpyridine	C_6H_7N
14	2,5-Dimethylpyridine	C_7H_9N
15	(2*E*, 4*E*)-Hexa−2,4-dienal	C_6H_8O
16	O-Allyl ethanethioate	C_5H_8OS
17	2-Ethylidene−1,3-dithiane	$C_6H_{10}S_2$
18	2-Vinyl−1,3-dithiolane	$C_5H_8S_2$
19	Thiophene−2-carbaldehyde	C_5H_4OS
20	3,5-Diethyl−1,2,4-trithiolane	$C_6H_{12}S_3$
21	1,3,5-Trithiane	$C_3H_6S_3$
22	(*E*)−2-(Prop−1-en−1-ylthio)thiophene	$C_7H_8S_2$
23	Methyl−2-(thiophen−2-yl)acetate	$C_7H_6O_2S$
24	4,5-Dimethylisothiazole	C_5H_7NS
25	1,3-Phenylenedimethanethiol	$C_8H_{10}S_2$
26	Benzo[*b*]thiophene	C_8H_6S
27	S-Allyl prop−2-ene−1-sulfinothioate	$C_6H_{10}OS_2$
28	(*E*)−1-Allyl−2 (3-[allyl(methylene)-(λ^4-sulfaneyl) prop−1-en−1-yl]disulfane	$C_9H_{16}S_4$
29	Allyl(methyl)sulfane	C_4H_8S
30	1-Allyl−2-methyldisulfane	$C_5H_{10}S_2$
31	(*E*)−1-Allyl−2-[3-(allylsulfinyl)prop−1-en−1-yl] disulfane	$C_9H_{16}OS_2$
32	(*Z*)−1-Allyl−2-[3-(allylsulfinyl)prop−1-en−1-yl] disulfane	$C_9H_{16}OS_2$
33	3-Vinyl−3,4-dihydro−1,2-dithiine	$C_5H_6S_2$
34	1,3-Diallyltrisulfane	$C_6H_{10}S_3$
35	1-Allyl−3-methyltrisulfane	$C_4H_8S_3$
36	1,4-Diallyltetrasulfane	$C_6H_{10}S_4$
37	1-Allyl−4-methyltetrasulfane	$C_6H_{12}S_4$
38	2-(Allylthio)−2-aminoacetic acid	$C_5H_9NO_2S$
39	(*E*)−2-Allyl−1methylene−1-(prop−1-en−1-yl)−1λ^4-disulfane	$C_6H_{10}S_2$
40	(*E*)-S-(Prop−1-en−1-yl)prop−2-ene−1-sulfinothioate	$C_6H_{10}OS_2$
41	S-Methyl prop−2-ene−1-sulfinothioate	$C_4H_8OS_2$
42	S-Allyl methanesulfinothioate	$C_4H_6OS_2$
43	S-(Allylthio)cysteine	$C_6H_{11}NO_2S_2$
44	(*E*)-Prop−1-ene−1-thiol	C_3H_6S
45	2,2-Dimethylthiirane	C_4H_8S
46	Ethyl acetate	$C_4H_8O_2$
47	Propylidene-λ^4-sulfanone	C_3H_6OS
48	(*E*)−1-Methyl−2-(prop−1-en−1-yl)disulfane	$C_4H_8S_2$
49	1-Methyl−3-propyltrisulfane	$C_5H_{10}S_3$
50	Methyldimethylene-λ^6-sulfanol	C_4H_8OS
51	Isopropyl (methyl)sulfane	$C_4H_{10}S$
52	Tetrahydrothiophene	C_4H_8S
53	1,2-Dithiolane	$C_3H_6S_2$
54	3,4-Dimethylthiophene	C_6H_8S
55	S-Methyl methanesulfinothioate	$C_2H_6OS_2$
56	Tert-butyl(methyl)sulfane	$C_5H_{12}S$
57	Tetrahydro-2H-thiopyran−3-ol	$C_5H_{10}OS$
58	2-Methyl−1,3-oxathiane	C_4H_8OS
59	3-(Allylthio)propanoic acid	$C_6H_{10}O_2S$
60	3-Methylene−3,6-dihydro−1,2-dithiine	$C_5H_6S_2$
61	3-Methylene−3,4-dihydro−1,2-dithiine	$C_5H_6S_2$
62	(*Z*)−1-Methyl−3-(prop−1-en−1-yl)trisulfane	$C_4H_8S_3$
63	Isobutyl(2-methoxyallyl)sulfane	$C_8H_{16}OS$
64	S-Methyl methanesulfonothioate	$C_2H_6O_2S_2$
65	Tetrahydro-2H-thiopyran−4-ol	$C_5H_{10}OS$
66	Cyclopentyl(ethyl)sulfane	$C_7H_{14}S$
67	2-(Methyldisulfanyl)ethyl benzenesulfonate	$C_9H_{10}O_3S_3$
68	Benzyl(methyl)sulfane	$C_8H_{10}S$

2.3. Extraction and preparation of ASEO via hydrodistillation

Mature, disease-free bulbs of *Allium sativum* L. were procured from the medicinal plant garden at Banasthali Vidyapith, India. Rigorous selection criteria were applied to ensure the highest quality, focusing on bulbs with optimal physiological characteristics to enhance the yield and purity of the essential oil. The garlic bulbs were then carefully cleaned, peeled, and processed into a uniform paste with distilled water. This paste underwent hydrodistillation using a Clevenger apparatus, maintained at a controlled temperature of 65–75°C for 3–4 hours. The essential oil was extracted as volatile components carried by steam, which were subsequently condensed and separated from the aqueous phase. Sodium sulfate (Na_2SO_4) was employed to remove any residual moisture, and the oil was stored at 4°C in a light-proof environment to maintain its stability throughout the research period.

2.4. Experimental animals

Male Swiss albino mice (Mus musculus) aged 2–3 months, weighing between 20 and 30 g, were used as the study subjects. The mice were procured from Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana, India. They were housed in individually ventilated cages in a pathogen-free environment with controlled temperature (23–25°C), relative humidity (50 ± 15 %), and a 12-hour light/dark cycle. The mice were fed a nutritionally balanced pelleted diet and had unlimited access to fresh drinking water. Before the study began, the mice underwent a one-week acclimatization period to reduce stress, and were then randomly assigned to experimental groups.

2.5. Ethical clearance

All animal experiments conducted in this study were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines. The study adhered strictly to the guidelines set by the Institutional Animal Ethics Committee (IAEC) in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), governed by the Ministry of Environment, Forest, and Climate Change, Government of India. Approval for the experimental protocols was granted by IAEC under reference number BV/IAEC/January/2020/10 from the Department of Bioscience and Biotechnology at Banasthali Vidyapith.

2.6. Animal allocation and treatment regimen

In this study, 36 mice were systematically divided into six experimental cohorts, each comprising six subjects, over a 30-day investigative period. The administration of Allium sativum essential oil, silymarin, and the vehicle oil was initiated on the 12th day post-exposure and in conjunction to lead nitrate and persisted until the experiment's conclusion [2,21,9]. The dosages for both lead nitrate and Allium sativum essential oil were carefully calibrated in accordance with established guidelines from prior studies. The treatments are summarized in the table below (Table 2).

2.7. Animal dissection and tissue homogenate preparation

On the 31st day, the mice were sacrificed via cervical dislocation. Hearts were excised, carefully removing any adhering adipose tissue with sterilized instruments. The cardiac tissues were rinsed in ice-cold saline (0.9 % sodium chloride), blotted dry, and weighed for further analysis. The tissues were then minced to increase surface area and homogenized in 0.1 M sodium phosphate buffer (pH 7.4) to create a 10 % (w/v) homogenate. This suspension was centrifuged at 10,000 rpm for 15–20 minutes at 4°C, yielding a supernatant that was collected for inflammatory marker, lipid, and histological analyses.

Table 2
Treatment groups for assessing cardioprotective effects in lead nitrate-exposed mice.

Groups	Treatment Description	Dose	Reference
Group I	Distilled water	N/A	N/A
Group II	Lead nitrate treatment	50 mg/kg body weight	[39]
Group III	Lead nitrate + Low dose Allium sativum essential oil	Lead nitrate: 50 mg/kg body weight ASEO: 50 mg/kg body weight	[15,38, 55]
Group IV	Lead nitrate + High dose Allium sativum essential oil	Lead nitrate: 50 mg/kg body weight ASEO: 80 mg/kg body weight	[55]
Group V	Lead nitrate + Silymarin	Lead nitrate: 50 mg/kg body weight Silymarin: 25 mg/kg body weight	[35]
Group VI	Lead nitrate + Vehicle control (Olive oil)	Lead nitrate: 50 mg/kg body weight Olive oil: N/A	[35]

2.8. Assessment of lipid profile in cardiac tissue

This evaluation involved the measurement of total cholesterol (TC) [54], while low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were quantified using the Elabscience kits (Catalog No: E-EL-M1363) and (Catalog No: E-EL-M1402), respectively.

2.9. Assessment of nitric oxide levels

Nitric oxide (NO) levels were indirectly quantified by measuring nitrite (NO_2^-) concentrations using the Nitric Oxide (NO) Colorimetric Assay Kit (Elabscience, Catalog No: E-BC-K035-M). Following the manufacturer's instructions, absorbance at 550 nm was measured via spectrophotometry, and nitrite levels were calculated based on a standard curve.

2.10. Cardiac inflammatory biomarkers

The assessment of inflammatory biomarkers in cardiac tissue was conducted using ELISA kits obtained from Invitrogen by Life Technologies (Thermo Fisher). The analysis followed the manufacturer's instructions for accurate measurement of the following parameters: NF-κB p65 (Total) (Catalogue Number: 85–86081), TNF-α (Catalogue Number: KMC3011), IFN-γ (Catalogue Number: BMS228), IL-6 (Catalogue Number: A35573), and IL-10 (Catalogue Number: BMS614INST). Each assay involved the preparation of cardiac tissue samples, which were then incubated with specific antibodies provided in the kits. Following incubation, a detection system that produces a measurable signal was applied, enabling the quantification of each biomarker. The optical density (OD) was measured using a microplate reader, and concentrations were calculated based on standard curves generated from known concentrations of each biomarker. This methodology facilitated a comprehensive evaluation of the inflammatory response in cardiac tissue, providing valuable insights into the pathophysiological mechanisms involved in cardiovascular disease.

2.11. Histological analysis of cardiac tissue

Cardiac tissues from all experimental groups were promptly collected and fixed in 10 % neutral buffered formalin for 24–48 hours to maintain structural integrity. After fixation, the tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 μm thickness were cut with a rotary microtome and mounted on glass slides. These sections were

deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (H&E) for histological evaluation of myocardial integrity, inflammation, and fibrosis. Morphological alterations, including necrosis, inflammatory cell infiltration, and fibrosis, were assessed under a light microscope at 10x magnification, and photomicrographs were captured using a digital imaging system.

3. Results

3.1. Lipid profile alterations and treatment effects

In this study, the lead nitrate-exposed group showed a substantial increase in total cholesterol levels, reaching 88.27 μg/mL compared to 54.09 μg/mL in the control group ($P < 0.001$). Treatment with low-dose Allium sativum essential oil (ASEO) resulted in cholesterol levels of 86.8 μg/mL, showing no statistically significant change. High-dose ASEO, however, significantly reduced cholesterol levels to 66.07 μg/mL ($P < 0.001$). Silymarin and olive oil also caused significant reductions in cholesterol, with levels of 85.13 μg/mL ($P < 0.05$) and 83.76 μg/mL ($P < 0.01$), though the effects were less pronounced (Table 3).

LDL levels in the lead nitrate-exposed group were markedly elevated at 93.78 μg/mL, compared to 20.03 μg/mL in the control group ($P < 0.001$). Low-dose ASEO led to a reduction in LDL levels to 90.24 μg/mL, but without statistical significance. High-dose ASEO significantly decreased LDL levels to 49.62 μg/mL ($P < 0.001$). Silymarin and olive oil treatments also lowered LDL levels significantly, with values of 86.57 μg/mL ($P < 0.05$) and 85.75 μg/mL ($P < 0.01$), though these reductions were not as strong as with high-dose ASEO (Table 3).

HDL levels, significantly reduced in the lead nitrate-exposed group (17.51 μg/mL vs. 35.96 μg/mL in controls, $P < 0.001$), showed no significant improvement with low-dose ASEO (17.11 μg/mL). However, high-dose ASEO significantly elevated HDL to 27.2 μg/mL ($P < 0.01$). Silymarin and olive oil treatments also improved HDL levels, raising them to 22.14 μg/mL and 22.36 μg/mL, respectively. These increases, although statistically significant ($P < 0.05$), were less pronounced than the effect seen with high-dose ASEO (Table 3).

Table 3
Effects of lead nitrate exposure and treatment with Allium sativum essential oil (ASEO), silymarin, and olive oil on lipid profiles (Cholesterol, LDL, HDL) in male Swiss albino mice.

Group	Total Cholesterol (μg/mL)	LDL-C (μg/mL)	HDL-C (μg/mL)
Group I (Control)	54.09 ± 0.33	20.03 ± 0.82	35.96 ± 0.16
Group II (Lead Nitrate)	88.27 ± 0.27 * **	93.78 ± 1.54 * **	17.51 ± 0.76 * **
Group III (Lead Nitrate + Low dose of ASEO)	86.8 ± 0.56$^{\#NS}$	90.24 ± 1.83$^{\#NS}$	17.11 ± 1.41$^{\#NS}$
Group IV (Lead Nitrate + High dose of ASEO)	66.07 ± 0.91$^{\#\#\#}$	49.62 ± 0.45$^{\#\#\#}$	27.2 ± 0.70$^{\#\#\#}$
Group V (Lead Nitrate + Silymarin)	85.13 ± 0.33$^{\#}$	85.43 ± 0.51$^{\#}$	22.14 ± 0.62$^{\#}$
Group VI (Lead Nitrate + Olive Oil)	83.76 ± 0.49$^{\#\#}$	81.62 ± 0.87$^{\#\#}$	22.36 ± 1.07$^{\#}$

All data are represented as mean ± SEM of 6 mice. Significant differences in data are expressed as $p < 0.001^{\#\#\#/***}$, $p < 0.01^{\#\#/**}$, $p < 0.05^{\#/*}$ compared to the control group. Asterisks (*) denote comparisons between the lead nitrate-treated group (group II) and the control group (group I), while hash signs (#) indicate comparisons between the lead nitrate-treated group (group II) and the remaining four treatment groups. NS stands for non-significant changes.

3.2. Modulation of nitric oxide levels

In the lead nitrate-exposed group, nitric oxide (NO) levels significantly decreased to 1.38 nmol/mL, compared to 10.43 nmol/mL in the control group ($P < 0.001$). The administration of low-dose ASEO resulted in NO levels of 1.82 nmol/mL; however, this increase was not statistically significant. In contrast, high-dose ASEO significantly raised NO levels to 6.29 nmol/mL ($P < 0.001$). Both silymarin and olive oil also contributed to increases in NO levels, with values of 2.00 nmol/mL ($P < 0.05$) and 2.24 nmol/mL ($P < 0.01$), respectively, indicating statistically significant but modest elevations (Table 4).

3.3. Impact of treatments on inflammatory markers

In the group exposed to lead nitrate, IFN-Gamma levels decreased significantly to 1.9 pg/mL, compared to 12.5 pg/mL in the control group ($P < 0.001$). Treatment with low doses of ASEO and silymarin resulted in IFN-Gamma levels of 2.25 pg/mL and 2.43 pg/mL, respectively, though these differences were not statistically significant. In contrast, high-dose ASEO significantly increased IFN-Gamma levels to 7.36 pg/mL ($P < 0.001$), while olive oil also contributed to an increase, reaching 2.57 pg/mL ($P < 0.05$) (Table 4).

In the lead nitrate-exposed group, IL-10 levels dropped significantly to 4.35 pg/mL, down from 16.26 pg/mL in the control group ($P < 0.001$). Low doses of ASEO and silymarin resulted in IL-10 levels of 4.88 pg/mL and 5.38 pg/mL, respectively, but these changes were not statistically significant. Conversely, high-dose ASEO significantly elevated IL-10 levels to 11.75 pg/mL ($P < 0.001$), while olive oil increased IL-10–5.68 pg/mL ($P < 0.05$) (Table 5).

NF-kB levels in the lead nitrate-treated group increased significantly to 17.3 pg/mL, compared to 5.17 pg/mL in the control group ($P < 0.001$). Low-dose ASEO resulted in NF-kB levels of 18.13 pg/mL, which did not yield a statistically significant result. However, high-dose ASEO significantly lowered NF-kB levels to 8.42 pg/mL ($P < 0.001$). Silymarin and olive oil also led to modest reductions in NF-kB levels, measuring 15.88 pg/mL and 15.7 pg/mL, respectively, with statistically significant but less pronounced effects ($P < 0.05$) (Table 5).

In the lead nitrate-exposed group, TNF-alpha levels rose significantly to 16.87 pg/mL, compared to 6.86 pg/mL in the control group ($P < 0.001$). Treatment with low-dose ASEO reduced TNF-alpha levels to 14.82 pg/mL, although this change was not statistically significant. In contrast, high-dose ASEO significantly lowered TNF-alpha levels to 9.66 pg/mL ($P < 0.001$). Both silymarin and olive oil also caused moderate reductions in TNF-alpha, recorded at 14.42 pg/mL ($P < 0.05$) and 13.07 pg/mL ($P < 0.01$), respectively, demonstrating statistically significant, albeit less pronounced, effects (Table 5).

Finally, in the lead nitrate-exposed group, IL-6 levels significantly increased to 36.36 pg/mL compared to 14.65 pg/mL in the control group ($P < 0.001$). Treatment with low-dose ASEO and silymarin

Table 4
Effects of lead nitrate exposure and treatment with Allium sativum essential oil (ASEO), silymarin, and olive oil on nitric oxide (NO) levels in male Swiss albino mice.

Groups	NO nmol/mL
Group I (Control)	10.43 ± 0.14
Group II (Lead Nitrate)	1.38 ± 0.08***
Group III (Lead Nitrate + Low dose of ASEO)	1.82 ± 0.1$^{\#NS}$
Group IV (Lead Nitrate + High dose of ASEO)	6.29 ± 0.24$^{\#\#\#}$
Group V (Lead Nitrate + Silymarin)	2 ± 0.04$^{\#}$
Group VI (Lead Nitrate + Olive Oil)	2.24 ± 0.04$^{\#\#}$

All data are represented as mean ± SEM of 6 mice. Significant differences in data are expressed as $p < 0.001^{\#\#\#/***}$, $p < 0.01^{\#\#/**}$, $p < 0.05^{\#/*}$ compared to the control group. Asterisks (*) denote comparisons between the lead nitrate-treated group (group II) and the control group (group I), while hash signs (#) indicate comparisons between the lead nitrate-treated group (group II) and the remaining four treatment groups. NS stands for non-significant changes.

Table 5
Effects of lead nitrate exposure and treatment with Allium sativum essential Oil (ASEO), silymarin, and olive oil on inflammatory markers (IFN-Gamma, IL-10, NF-kB, TNF-alpha, IL-6) in male Swiss albino mice.

Group	IFN-ɣ (pg/mL)	IL- 10 (pg/mL)	NFk-B (pg/mL)	TNF-α (pg/mL)	IL−6 (pg/mL)
Group I (Control)	12.5 ± 0.1	16.26 ± 0.44	5.17 ± 0.24	6.86 ± 0.4	14.65 ± 0.36
Group II (Lead Nitrate)	1.95 ± 0.08 * **	4.35 ± 0.2 * **	17.3 ± 0.31 * **	16.87 ± 0.52 * **	36.36 ± 0.2 * **
Group III (Lead Nitrate + Low dose of ASEO)	2.25 ± 0.11$^{\#NS}$	4.88 ± 0.15$^{\#NS}$	18.13 ± 0.4$^{\#NS}$	14.82 ± 0.95$^{\#NS}$	35.44 ± 0.46$^{\#NS}$
Group IV (Lead Nitrate + High dose of ASEO)	7.36 ± 0.17$^{\#\#\#}$	11.75 ± 0.35$^{\#\#\#}$	8.42 ± 0.1$^{\#\#\#}$	9.66 ± 0.22$^{\#\#\#}$	22.13 ± 0.25$^{\#\#\#}$
Group V (Lead Nitrate + Silymarin)	2.43 ± 0.05$^{\#NS}$	5.38 ± 0.07$^{\#NS}$	15.88 ± 0.29$^{\#}$	14.42 ± 0.17$^{\#}$	34.95 ± 0.29$^{\#}$
Group VI (Lead Nitrate + Olive Oil)	2.57 ± 0.02$^{\#}$	5.68 ± 0.15$^{\#}$	15.7 ± 0.14$^{\#}$	13.07 ± 0.38$^{\#\#}$	34.37 ± 0.14$^{\#\#}$

All data are represented as mean ± SEM of 6 mice. Significant differences in data are expressed as $p < 0.001^{\#\#\#/***}$, $p < 0.01^{\#\#/**}$, $p < 0.05^{\#/*}$ compared to the control group. Asterisks (*) denote comparisons between the lead nitrate-treated group (group II) and the control group (group I), while hash signs (#) indicate comparisons between the lead nitrate-treated group (group II) and the remaining four treatment groups. NS stands for non-significant changes.

resulted in IL-6 levels of 35.44 pg/mL and 34.95 pg/mL, respectively, but these reductions were not statistically significant. However, high-dose ASEO significantly lowered IL-6 levels to 22.13 pg/mL ($P < 0.001$), while olive oil also caused a moderate reduction to 34.37 pg/mL, achieving a statistically significant effect ($P < 0.01$) (Table 5).

3.4. Histological examination

Control (Group I): Histological analysis of heart sections from the control group treated with distilled water shows normal cardiac structure. The myocardium is well-organized, with cardiac muscle fibers displaying clear cross-striations and centrally located nuclei. Myocardial cells are consistent in size and shape, without any signs of degeneration or necrosis (arrow). No inflammatory cell infiltration, fibrosis, or other pathological alterations are observed. The minimal interstitial space suggests the absence of edema or tissue injury (Fig. 1).

Lead Nitrate-Treated (Group II): In the lead nitrate-treated group, significant histological alterations are observed. The cardiac muscle fibers of the myocardium display significant distortion, with loss of organization and typical striations. Several focal cells exhibit cytoplasmic vacuolation (arrow), indicating cellular damage and degeneration. Mild enlargement of the interstitial space is observed, along with occasional inflammatory cell infiltration (circle). These findings suggest early signs of tissue injury and stress resulting from lead nitrate exposure (Fig. 1).

Low Dose ASEO (Group III): The group treated with a low dose of ASEO shows histological changes similar to the lead nitrate-treated group. Distortion of cardiac muscle fibers and focal cytoplasmic vacuolation are present, indicating that the low dose of ASEO was insufficient in mitigating the toxic effects of lead nitrate. The tissue structure remains disorganized, reflecting ongoing cellular stress and damage (Fig. 2).

High Dose ASEO (Group IV): In the group treated with a high dose of ASEO, a moderate improvement in tissue organization is observed. While cardiac muscle fibers are more aligned than in the lead nitrate-treated group, some degree of disorganization remains. Signs of cellular damage are reduced, though not entirely absent. There is a noticeable increase in interstitial space compared to the control group (arrow). Despite these remaining structural irregularities, the high dose of ASEO demonstrates significant therapeutic potential in reducing the severity of lead nitrate-induced cardiac toxicity (Fig. 2).

Silymarin-Treated (Group V): Histological sections from the silymarin-treated group reveal minimal improvement in tissue organization, similar to the effects seen with the low dose of ASEO. Cardiac muscle fibers show some disorganization, and the presence of blood clots is observed (arrow). While there is a slight reduction in tissue damage compared to the lead nitrate-treated group, silymarin provides only partial protection against lead-induced cardiac toxicity. The myocardium remains distorted in uneven areas, reflecting incomplete recovery. (Fig. 3).

Olive Oil-Treated (Group VI): In the olive oil-treated group, histological analysis shows better organization of cardiac muscle fibers compared to the silymarin-treated group. The muscle fibers are more aligned with less disarray, although fiber distortion and increased

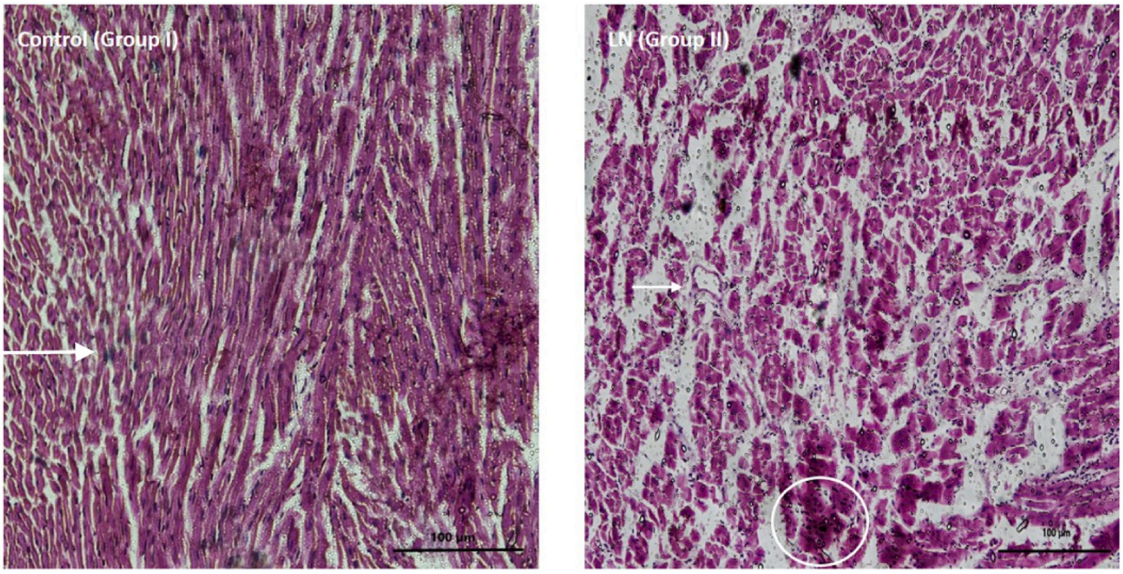

Fig. 1. Comparative Histological Analysis of Cardiac Tissue: Control Group with Normal Myocardial Structure vs. Lead Nitrate-Induced Cardiac Damage Exhibiting Cellular Degeneration, Cytoplasmic Vacuolation, and Inflammatory Infiltration.

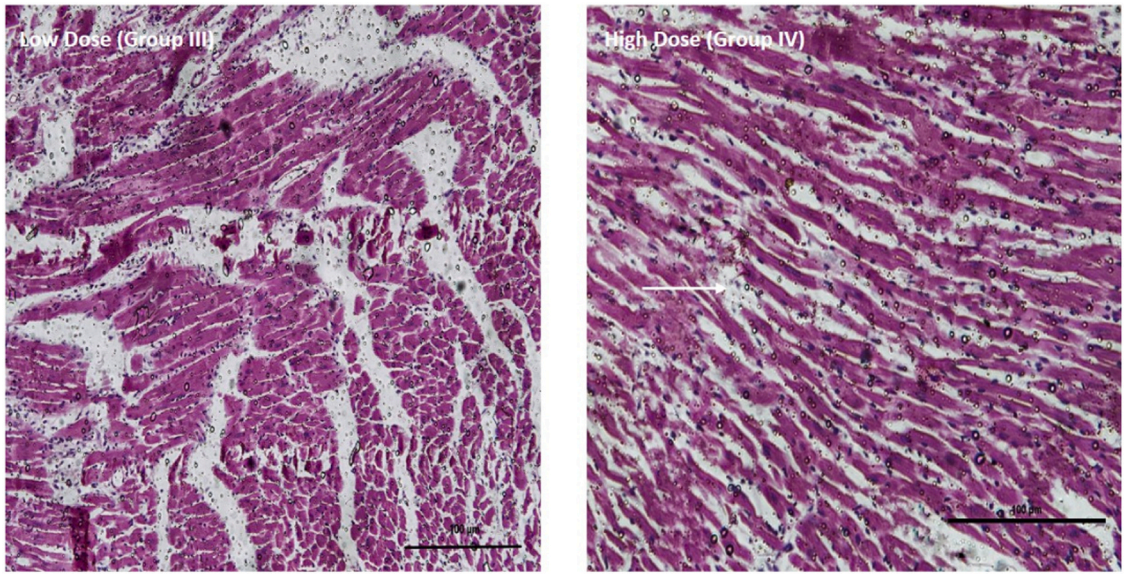

Fig. 2. Histological Evaluation of Cardiac Tissue Post-ASEO Treatment: Low Dose Showing Insufficient Protection Against Lead Nitrate Toxicity vs. High Dose Demonstrating Moderation of Tissue Damage and Improved Myocardial Structure.

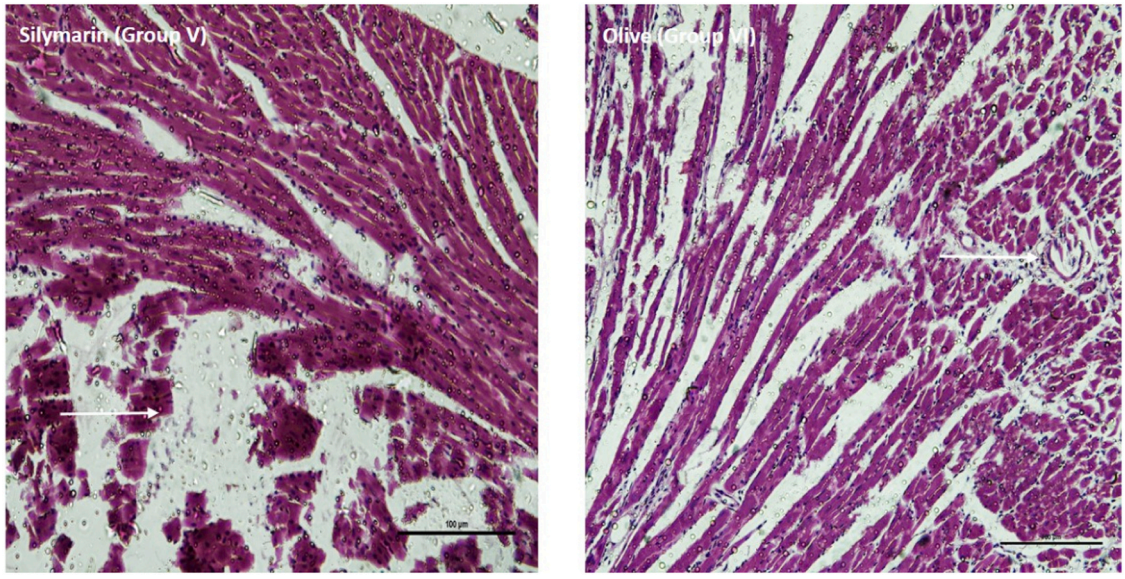

Fig. 3. Comparative Histological Analysis of Silymarin- and Olive Oil-Treated Groups: Partial Protection by Silymarin with Blood Clots and Tissue Disorganization vs. Olive Oil Showing Enhanced Cardiac Fiber Alignment but Persistent Cellular Stress Post-Lead Nitrate Exposure.

interstitial space remain noticeable. Focal cytoplasmic vacuolation persists (arrow), suggesting some ongoing cellular stress. While olive oil demonstrates a more pronounced protective effect than silymarin, it does not fully prevent the histological abnormalities induced by lead nitrate exposure (Fig. 3).

4. Discussion

Lead exposure remains a critical environmental and occupational concern due to its established links with cardiovascular abnormalities, including coronary artery disease, atherosclerosis, stroke, peripheral arterial disease, and hypertension. Studies have consistently shown that lead nitrate administration leads to elevated levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C), alongside a significant reduction in high-density lipoprotein cholesterol (HDL-C). These lipid profile disturbances are known to increase the risk of cardiovascular diseases (CVD) such as atherosclerosis and coronary artery disease, primarily through the disruption of lipid metabolism and the promotion of oxidative stress. Atherosclerosis, an inflammatory condition characterized by lipid accumulation within the arterial wall, is closely tied to lead exposure through the dysregulation of lipid metabolism. Lead nitrate exposure has been shown to promote cholesterol accumulation in β-lipoprotein (LDL) and pre-β lipoprotein fractions, both of which are prone to deposition in blood vessels, thus accelerating the formation of atherosclerotic plaques. Atherosclerosis is now recognized as an inflammatory condition, with oxidized LDL-C playing a key role in triggering inflammatory responses that perpetuate plaque formation and destabilization [32]. The cardioprotective role of HDL, known for reverse cholesterol transport, is impaired by lead, which further amplifies cardiovascular risks [42,29]. In this study, the reduction in HDL levels under lead exposure, observed specifically in the cardiac tissue, aligns with this mechanism, contributing to enhanced cardiovascular

risk.

While HDL-C is primarily synthesized in the liver and small intestine, as well as in other tissues like adipose and macrophages, its role in cardiac tissue is more functional than directly related to its synthesis. In our study, HDL-C levels were assessed in heart tissue to investigate its cardioprotective properties and its role in mitigating lead-induced oxidative damage. Although HDL-C is not synthesized in cardiac myocytes, it is taken up by cardiac tissue and exerts significant effects, including antioxidant, anti-inflammatory, and endothelial-protective roles. The assessment of HDL-C levels in heart tissue reflects its functional impact on cardiovascular health [3]. Emerging evidence suggests that HDL particles, although not directly expressed in cardiac myocytes, interact with heart cells and provide cardioprotective effects. For instance, studies have demonstrated that HDL can reduce apoptosis in cardiomyocytes induced by ischemia-reperfusion injury, further highlighting its protective role in heart cells. The cardioprotective effects are likely due to HDL particles synthesized in other tissues interacting with heart cells [44].

Treatment with ASEO significantly restored lipid balance in the cardiac tissue, reducing total cholesterol and LDL-C levels while markedly elevating HDL-C concentrations. This highlights its protective role in mitigating lead-induced dyslipidemia, corroborating findings from previous research on garlic oil's therapeutic potential [16,53]. The elevation of HDL-C after garlic oil treatment suggests enhanced cholesterol clearance and protection against oxidative stress-induced lipid peroxidation. These findings are further supported by the lipid-lowering effects of ASEO, which significantly reduced serum total cholesterol, LDL-C, and triglycerides (TG), while moderately increasing HDL-C levels [8]. The mechanisms through which garlic oil exerts its lipid-regulating effects are multi-faceted. ASEO is known to inhibit key enzymes in cholesterol biosynthesis, such as HMG-CoA reductase and Coenzyme A (CoASH), reducing endogenous cholesterol production in the liver. Additionally, garlic prevents the oxidative modification of LDL-C, a critical event in atherogenesis, by maintaining the structural integrity of LDL and preventing its uptake by macrophages. This action potentially mitigates plaque formation and subsequent cardiovascular complications [20]. The findings align with previous studies demonstrating garlic's ability to reduce serum total cholesterol, triglycerides, and LDL-C, while increasing HDL-C through modulation of key enzymes involved in lipid metabolism, such as fatty acid synthase and malic dehydrogenase. The dyslipidemia observed in lead-exposed animals reinforces the causal role of cholesterol, particularly LDL-C, in the pathogenesis of atherosclerosis. Elevated LDL-C has been consistently associated with increased cardiovascular risk, particularly myocardial infarction, while HDL-C is inversely correlated with coronary heart disease mortality [12,23]. The cholesterol hypothesis, which posits LDL-C as atherogenic and its reduction as key to lowering cardiovascular events, remains central to understanding atherogenesis [43]. Emerging evidence, however, suggests that cardiovascular risk is determined not only by total cholesterol but by its distribution among lipoproteins. LDL-C acts as the primary carrier of cholesterol to tissues, promoting plaque formation, whereas HDL-C facilitates cholesterol excretion to the liver, exerting protective effects [18].

In addition to ASEO, olive oil also demonstrated cardioprotective effects by promoting improvements in lipid profiles in the cardiac tissue. Rich in monounsaturated fatty acids and antioxidants, olive oil is known for its anti-inflammatory properties and ability to improve endothelial function, which may contribute to its lipid-modulating effects. Its beneficial role in reducing total cholesterol and LDL-C levels, while moderately increasing HDL-C, aligns with findings from prior studies that highlight olive oil's ability to reduce the risk of atherosclerosis and coronary artery disease. The antioxidant components of olive oil, particularly phenolic compounds, play a key role in reducing oxidative stress, thus supporting cardiovascular health by preventing lipid peroxidation and LDL-C oxidation [6]. Silymarin, another treatment investigated in this study, is well-known for its lipid-modulating properties, largely due to its antioxidant and anti-inflammatory actions. It helps reduce oxidative damage and lipid peroxidation in liver cells, which can indirectly lead to improvements in lipid profiles. Although its effects on total cholesterol, LDL-C, and HDL were significant, they were less pronounced in this study. However, silymarin's capacity to mitigate lead-induced oxidative stress remains crucial, especially in relation to hepatic lipid metabolism. Its modest impact on cholesterol regulation is likely due to its protective role in shielding the liver from toxin-induced damage, helping to maintain cholesterol balance and thereby offering cardiovascular protection [11].

Lead nitrate exposure is well-documented to disrupt nitric oxide (NO) synthesis, which plays a pivotal role in vascular homeostasis and blood pressure regulation. The decline in NO levels following exposure to lead nitrate underscores the mechanism by which lead induces endothelial dysfunction and contributes to cardiovascular complications, such as hypertension [31]. NO is synthesized from L-arginine by nitric oxide synthase (NOS), a process that is essential for maintaining vasodilation through its downstream signaling pathways, particularly by activating soluble guanylate cyclase (sGC). Once activated, sGC catalyzes the conversion of GTP to cyclic guanosine monophosphate (cGMP), which serves as a secondary messenger to promote vasodilation by reducing intracellular calcium levels in vascular smooth muscle cells (VSMCs). Disruption in this NO-sGC-cGMP axis is a hallmark of lead toxicity [24,30,46]. The observed reduction in NO levels in the lead nitrate-exposed group is a direct consequence of lead's interference with this pathway. Lead exposure has been shown to downregulate sGC expression, likely driven by oxidative stress mechanisms, including an upregulation of reactive oxygen species (ROS) and increased expression of cyclooxygenase-2 (COX-2) [10]. This oxidative stress not only reduces NO bioavailability but also impairs its signaling through sGC, as observed in studies where Pb-exposed rats demonstrated a significant reduction in sGC expression and heightened oxidative damage. Furthermore, the elevated ROS levels react with NO, producing peroxynitrite, a potent pro-oxidant that exacerbates endothelial damage and vascular dysfunction [50].

Phytotherapeutic interventions such as ASEO, silymarin, and olive oil appear to counteract some of these detrimental effects. High-dose ASEO, in particular, demonstrated a significant improvement in NO levels, suggesting that its protective effects may be mediated through both antioxidant and anti-inflammatory mechanisms. ASEO is rich in organosulfur compounds that have been shown to upregulate NOS activity and increase NO production, likely by mitigating ROS-induced damage and restoring sGC function [30]. This restoration of NO bioavailability is crucial for reversing endothelial dysfunction and promoting vasodilation, which helps normalize blood pressure and alleviate cardiovascular stress. The modest improvements observed with silymarin and olive oil also indicate their capacity to modulate NO signaling, albeit to a lesser extent than high-dose ASEO. Silymarin, known for its antioxidant properties, likely exerts its effects by scavenging ROS and reducing oxidative stress, thereby preserving NO levels. Olive oil, rich in polyphenols, has been associated with improved endothelial function through similar antioxidant pathways, contributing to its protective role in mitigating lead-induced vascular damage [41].

In correlation with these oxidative stress-induced mechanisms, this study observed a significant elevation in pro-inflammatory markers such as nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in lead-exposed mice. Concurrently, there was a reduction in anti-inflammatory cytokines, including interferon-gamma (IFN-γ) and interleukin-10 (IL-10), contributing to a cytokine imbalance that promotes chronic inflammation, oxidative stress, and cardiovascular damage. This pattern aligns with lead toxicity's known effects, where ROS-induced lipid peroxidation amplifies inflammatory responses, exacerbating cardiovascular dysfunction.

NF-κB, a redox-sensitive transcription factor, serves as a master regulator of inflammation and cell survival [47]. Its upregulation in lead-exposed mice, as demonstrated in this study, aligns with previous

reports of its role in inflammatory diseases, including atherosclerosis [49]. The activation of NF-κB is driven by various stimuli, including ROS, cytokines, and PKC activators, which are abundantly produced during lead exposure. NF-κB activation triggers the transcription of genes encoding pro-inflammatory cytokines such as TNF-α and IL-6, adhesion molecules, and inducible nitric oxide synthase (iNOS). These factors collectively promote endothelial dysfunction, plaque formation, and instability, exacerbating cardiovascular damage. Lead-induced oxidative stress further activates NF-κB by phosphorylating inhibitory IκB proteins targeting them for ubiquitination and degradation., allowing NF-κB to translocate to the nucleus and drive inflammation. In cardiovascular tissues, NF-κB activation has been detected in macrophages, endothelial cells, and smooth muscle cells, contributing to the formation of atherosclerotic plaques and promoting plaque instability, which is a precursor to coronary events.

TNF-α, a pivotal pro-inflammatory cytokine produced predominantly by activated macrophages, is significantly overexpressed in the lead-exposed group. It promotes leukocyte recruitment to vascular lesions and contributes to plaque instability, thus accelerating atherosclerosis progression [19]. The heightened production of TNF-α likely results from lead-induced oxidative stress activating protein kinase C (PKC) and the mitogen-activated protein kinase (MAPK) pathway, which modulates TNF-α at the post-transcriptional level [26]. Phosphorylation of proteins binding to the AUUUA sequence in the 3′-untranslated region (UTR) of TNF-mRNA elements via mitogen-activated protein kinase (MAPK)–CSBP (cytokinin specific binding protein) kinase pathway may abolish the normal repression of TNF-α translation, further contributing to its overproduction [25].

In this study, high-dose ASEO effectively downregulated NF-κB and TNF-α expression, likely through its bioactive sulfur-containing compounds such as allicin, ajoene, and diallyl sulfides. ASEO inhibits NF-κB activation by suppressing IκB kinase (IKK) activation, where its components, particularly DATS and ajoene, prevent the phosphorylation and degradation of IκBα, ensuring NF-κB remains inactive in the cytoplasm and thereby reducing inflammatory gene expression (Batiha et al., 2020). Additionally, allicin and DADS block NF-κB nuclear translocation by interfering with the PI3K/Akt and MAPK pathways, both of which are integral to NF-κB activation. ASEO also suppresses ROS-mediated NF-κB activation by acting as a redox modulator, reducing oxidative stress and reactive oxygen species (ROS) production, thereby indirectly inhibiting NF-κB-driven inflammation [34]. Furthermore, ASEO effectively downregulates TNF-α expression, a key pro-inflammatory cytokine implicated in cardiac inflammation and oxidative stress. This inhibition occurs through direct suppression of TNF-α gene transcription, where ajoene and DADS modulate epigenetic mechanisms such as histone modifications and DNA methylation, leading to reduced TNF-α mRNA expression (Rodríguez et al., 2022). Additionally, DATS interferes with TNF-α receptor (TNFR) signaling by disrupting the TNFR1-associated signaling complex (TNFR1-TRADD-TRAF2), thereby preventing downstream activation of inflammatory cascades, including NF-κB. Moreover, ASEO enhances Nrf2 activation, promoting its nuclear translocation and upregulating antioxidant genes such as HO-1, NQO1, and SOD, which collectively counteract TNF-α-induced oxidative stress and inflammation [17].

IL-6, another critical pro-inflammatory cytokine, is notably elevated in lead-exposed mice. It plays a dual role in immune activation and cardiovascular pathology by stimulating the acute-phase response, promoting T-cell proliferation, and enhancing antibody production [28]. Uncontrolled IL-6 production disrupts immune balance, leading to autoimmune conditions and cardiovascular dysfunction. IL-6 also fosters the growth of non-immune cells, such as vascular smooth muscle cells, compounding cardiovascular damage in lead toxicity by promoting unregulated T-cell activity. Moreover, elevated IL-6 stimulates the production of C-reactive protein (CRP), a known marker of cardiovascular risk, further linking chronic lead exposure to cardiovascular disease [26].

In contrast, the observed reduction in IFN-γ and IL-10 highlights the impaired anti-inflammatory response following lead exposure. IFN-γ is essential for cellular immunity, macrophage activation, and antigen presentation of MHC class II molecules. Its suppression, as seen in our study, compromises both innate and adaptive immune responses, increasing susceptibility to infections and promoting chronic inflammation [22,48]. This inhibition may result from lead's interference with intracellular signaling pathways that regulate IFN-γ synthesis [52].

IL-10, a critical anti-inflammatory cytokine, regulates immune responses by repressing the expression of pro-inflammatory cytokines such as TNF-α and IL-6, thereby limiting excessive inflammation and tissue damage. Its downregulation in lead-exposed mice disrupts the body's natural anti-inflammatory mechanisms, allowing pro-inflammatory signals to dominate, thereby perpetuating tissue damage and chronic inflammation [51]. IL-10's pleiotropic effects on various immune cells are essential for maintaining immune homeostasis, and its reduction correlates with previous studies on lead toxicity [4,14]. The observed decrease in IL-10 in our study suggests that lead disrupts the natural anti-inflammatory mechanisms of the body, allowing pro-inflammatory signals to dominate and perpetuate tissue damage and inflammation. ASEO treatment significantly restored IFN-γ and IL-10 levels, pointing to its immunomodulatory effects. Garlic likely achieves this by inhibiting NF-κB activation and reducing oxidative stress, thus dampening the pro-inflammatory cytokine cascade triggered by lead exposure. This restoration of cytokine balance underscores garlic's potential in mitigating the immune dysregulation and inflammatory damage induced by lead.

The histological findings highlight the detrimental effects of lead nitrate on cardiac structure. The control group shows normal myocardial architecture, while the lead nitrate-treated group exhibits significant structural alterations, including disorganized cardiac fibers and cytoplasmic vacuolation, indicating oxidative stress and cellular damage. The low dose of ASEO provides limited protection, as it fails to sufficiently mitigate these toxic effects, resulting in ongoing cellular stress and disorganization. In contrast, the high dose of ASEO demonstrates a notable improvement in myocardial organization, with reduced cellular damage. This protective effect may be attributed to garlic's potent antioxidant properties, which help alleviate oxidative stress and restore cellular integrity [29].

Both silymarin and olive oil exhibit potent anti-inflammatory mechanisms that enhance their antioxidant activities. Silymarin scavenges free radicals and boosts antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), mitigating reactive oxygen species (ROS)-induced damage. This finding correlates with previous research indicating that silymarin suppresses the NF-κB signaling pathway, reducing the production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, thus protecting against cardiovascular damage associated with oxidative stress, particularly in lead-exposed conditions [11]. Similarly, olive oil's polyphenolic compounds, such as oleuropein and hydroxytyrosol, provide anti-inflammatory effects by inhibiting NF-κB and downregulating pro-inflammatory mediators. Earlier studies report that olive oil improves endothelial function through enhanced nitric oxide bioavailability, which reduces vascular inflammation [5]. Its ability to suppress oxidative stress and maintain lipid balance underscores its importance in mitigating cardiovascular complications arising from lead toxicity [40]. While silymarin treatment shows minimal improvement in cardiac tissue, indicating limited efficacy despite its hepatoprotective effects, the olive oil-treated group exhibits better organization than the silymarin group. However, both groups still present signs of distortion and cellular stress, suggesting that while olive oil's polyphenolic compounds provide some protection, they do not fully reverse lead-induced damage.

In addition to its cardioprotective effects, Allium sativum essential oil (ASEO) has also shown significant protective potential across other body systems. Previous studies report its ability to mitigate oxidative stress, inflammation, and histopathological alterations in the lungs [13],

testes [1], kidneys [37], and liver [36] under lead nitrate-induced toxicity, highlighting its multi-systemic therapeutic properties.

5. Conclusion

In summary, lead exposure poses significant cardiovascular risks through mechanisms involving dyslipidemia, oxidative stress, and inflammation. The findings of this study underscore the link between lead-induced disturbances in lipid metabolism and the development of cardiovascular diseases, particularly through the elevation of total cholesterol and LDL-C levels alongside the reduction of protective HDL-C. This dyslipidemic state accelerates atherosclerosis, driven by the inflammatory response mediated by oxidized LDL-C and increased pro-inflammatory cytokines such as TNF-α and IL-6. The marked activation of the NF-κB pathway highlights the role of oxidative stress in perpetuating inflammation, thereby exacerbating cardiovascular damage. The therapeutic interventions tested, particularly high-dose ASEO, demonstrated significant cardioprotective effects by restoring lipid balance, improving nitric oxide availability, and modulating inflammatory responses. These interventions also illustrate the potential of phytotherapeutics in counteracting the adverse effects of lead exposure. Olive oil and silymarin, while effective to varying degrees, also contribute to lipid regulation and inflammation reduction, indicating their potential as adjunct therapies in managing lead-induced cardiovascular toxicity. Chronic inflammation remains a pivotal factor in the pathogenesis of cardiovascular diseases associated with lead exposure. The imbalance between pro-inflammatory and anti-inflammatory cytokines observed in this study not only exacerbates cardiovascular dysfunction but also suggests a broader impact on immune regulation and tissue health. The findings highlight the need for continued research into the intricate pathways linking lead exposure, inflammation, and cardiovascular disease to reveal targeted therapeutic opportunities that can mitigate these effects.

6. Limitations and future directions

Despite the promising findings regarding the cardioprotective effects of ASEO, a few limitations warrant consideration for future research. First, optimizing the dosage of ASEO is crucial, as varying concentrations may yield different outcomes in terms of efficacy. Further investigations into the molecular mechanisms underlying ASEO's protective effects, including gene expression studies related to oxidative stress and inflammation, are needed to provide deeper insights into its action. Future studies should also focus on elucidating the intricate pathways linking lead exposure, inflammation, and cardiovascular disease, particularly by investigating the role of specific cytokines and inflammatory mediators that may reveal targeted therapeutic opportunities.

Additionally, while our study focused on a 12-day lead pretreatment followed by a 30-day investigative period, future studies should evaluate the effects of treatment duration across different parameters to ensure adequate therapeutic windows for oxidative stress, inflammation, and lipid profile modulation. Short-term versus long-term intervention impacts on cardiovascular health also need to be explored to clarify ASEO's efficacy in different exposure scenarios.

Additionally, exploring the synergistic effects of combined phytotherapeutic agents, such as ASEO, olive oil, and silymarin, could enhance the observed cardioprotective benefits; thus, investigating various doses and combinations may yield more effective strategies for managing lead-induced cardiovascular risks. Assessing the long-term impacts of these interventions on cardiovascular health and their potential to prevent lead-induced atherosclerosis will be essential, particularly in chronic exposure scenarios. Expanding the study to include a broader range of biomarkers, including inflammatory and oxidative stress markers, could enhance the robustness of the findings and clarify ASEO's role in cardiovascular protection. While animal models are indispensable for understanding the biological effects of lead exposure and testing therapeutic interventions, limitations exist in directly extrapolating these findings to humans due to species-specific differences in metabolism, physiology, and response to toxins and treatments. Finally, including human clinical trials will be critical in translating these findings into practical applications for preventing and treating cardiovascular diseases associated with lead exposure. Together, these future research directions will help elucidate the full therapeutic potential of ASEO and its applicability in clinical settings.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution

Anjali Rajpoot conceptualized and designed the study, conducted the experiments, analyzed the data, and wrote the manuscript. Veena Sharma provided guidance, supervised the research, and critically reviewed the manuscript for intellectual content.

Generative AI declaration

The author confirms that no generative AI tools were used to create the content or writing of this manuscript, apart from the editorial assistance provided by language models for grammar and style correction. All interpretations and scientific content are solely the work of the author.

CRediT authorship contribution statement

Sharma Veena: Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Rajpoot Anjali:** Writing – review & editing, Writing – original draft, Validation, Software, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors express their sincere gratitude to the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, for providing the necessary research facilities and support.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Exclusion criteria and data analysis

There were no exclusions of animals, experimental units, or data points in this study. All data collected were included in the final analysis.

Data availability

Data will be made available on request.

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