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Original Article

In Vitro Antioxidant Activities and *In Vivo* Modulation of Testicular Oxidative Stress Markers by Verminoside and Specioside Isolated from *Spathodea campanulata*

Kingsley Chinedu Enyi[1], Adefunke Olukemi Opajobi[1], Alfred Ikechukwu Ajoh[1], Chukwudi Cyril Dunkwu[1], Osuvwe Clement Orororo[1], Kingsley Okwurinka[1], Joseph Mordi[1], Innocent Onyesom[1]

[1]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria*

*Correspondence: enyi-kingsley@delsu.edu.ng | 09035433618 | ORCID: 0009-0008-6861-3731

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Abstract:

Background: Lead exposure remains a major public health concern, with oxidative stress being a principal mechanism underlying its reproductive toxicity. Due to adverse effects associated with conventional chelation therapy, plant-derived antioxidants are increasingly explored as safer alternatives. Verminoside and specioside, iridoid glycosides isolated from Spathodea campanulata, possess antioxidant properties that may protect against lead-induced oxidative damage.

Methods: The in vitro antioxidant activities of verminoside and specioside were evaluated using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Oxidative stress markers including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), nitric oxide (NO), and total antioxidant capacity (TAC) were assessed in testicular tissue homogenates following lead exposure.

Results: Verminoside and specioside demonstrated significant concentration-dependent antioxidant activity in DPPH and FRAP assays. The FRAP EC_{50} values were 39.36±4.09 µg/ml, 48.50±8.98 µg/ml and 34.05±4.94 µg/ml for specioside, verminoside and ascorbic acid respectively, while the DPPH IC_{50} values were 59.67±0.58 µg/ml, 61.50±0.50 µg/ml and 50.00±0.00 µg/ml for specioside, verminoside and ascorbic acid respectively. Both compounds significantly ($p< 0.05$) reduced lipid peroxidation and nitrosative stress, while significantly ($p< 0.05$) restoring antioxidant enzyme activities and non-enzymatic antioxidant levels in testicular tissues.

Conclusion: Verminoside and specioside exhibit strongin vitro antioxidant activity and demonstrate protective effects against lead-induced oxidative stress in vivo.

Keywords: *Verminoside; Specioside; Oxidative stress; In vitro antioxidant activity; In vivo oxidative stress modulation; Lead toxicity; Spathodea campanulata*

1. Introduction

Lead is a pervasive environmental and occupational toxicant with well-documented adverse effects on male reproductive health. Oxidative stress, resulting from excessive generation of reactive oxygen species (ROS), is a major mechanism underlying lead-induced toxicity [1]. Testicular tissue is particularly vulnerable due to its high polyunsaturated fatty acid content and relatively low endogenous antioxidant defense capacity [2].

Excessive production of reactive oxygen species (ROS) leads to lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing spermatogenesis and steroidogenesis [3]. Although chelation therapy using agents such as dimercaptosuccinic acid (DMSA; succimer) effectively reduces the systemic burden of lead, its clinical use is associated with adverse effects, including gastrointestinal disturbances and nephrotoxicity [4]. Consequently, increasing attention has been directed toward plant-derived antioxidants as safer and potentially effective therapeutic alternatives.

Spathodea campanulata is a medicinal plant rich in bioactive iridoid glycosides, particularly verminoside and specioside, which have been reported to possess potent antioxidant and anti-inflammatory properties [5–7]. These compounds may confer cytoprotective effects by enhancing endogenous antioxidant defense mechanisms and attenuating oxidative damage. Therefore, the present study evaluated the in vitro antioxidant activities of verminoside and specioside, as well as their modulatory effects on oxidative stress biomarkers associated with lead-induced testicular toxicity.

2. Materials and Methods

2.1 Plant Collection and Identification

Fresh leaves of *Spathodea campanulata* were collected from Abraka, Ethiope East Local Government Area, Delta State, Nigeria (Latitude: 5°46′48.6″ N; Longitude: 6°08′03″ E). Preliminary field identification was performed using the Leaf Snap mobile application, after which the plant material was authenticated by Mr. Michael Ozioma Emmanuel at the Herbarium, Delta State University, Abraka. A voucher specimen (Voucher No. DELSUH 42) was prepared and deposited in the herbarium for future reference.

2.2 Experimental Animals and Husbandry

Fifty healthy adult male Wistar albino rats weighing between 150 and 200 g were obtained and housed in standard metallic cages under conventional laboratory conditions (28 ± 3°C; 12 h light/12 h dark photoperiod). The animals were acclimatized for two weeks before the commencement of the experiment and were provided with standard laboratory chow and clean drinking water *ad libitum* throughout the study.

2.3 Experimental Design and Treatment Protocol

The animals were randomly assigned into ten experimental groups (n = 5 rats per group) as follows:

Group 1 (Normal Control): Received 0.1% dimethyl sulfoxide (DMSO).

Group 2 (Negative Control): Received lead acetate (50 mg/kg body weight).

Group 3 (Positive Control): Received lead acetate (50 mg/kg body weight) followed by vitamin E (100 mg/kg body weight).

Group 4: Received lead acetate (50 mg/kg body weight) followed by verminoside (100 mg/kg body weight).

Group 5: Received lead acetate (50 mg/kg body weight) followed by specioside (100 mg/kg body weight).

Group 6: Received lead acetate (50 mg/kg body weight) followed by verminoside (100 mg/kg body weight) and specioside (100 mg/kg body weight).

Group 7: Received lead acetate (50 mg/kg body weight) followed by dimercaptosuccinic acid (DMSA; 50 mg/kg body weight).

Group 8: Received lead acetate (50 mg/kg body weight), DMSA (50 mg/kg body weight), and verminoside (100 mg/kg body weight).

Group 9: Received lead acetate (50 mg/kg body weight), DMSA (50 mg/kg body weight), and specioside (100 mg/kg body weight).

Group 10: Received lead acetate (50 mg/kg body weight), DMSA (50 mg/kg body weight), verminoside (100 mg/kg body weight), and specioside (100 mg/kg body weight).

Lead acetate was administered orally by gavage once daily for 10 consecutive days to induce testicular toxicity. Following lead exposure, treatment with verminoside, specioside, DMSA, and vitamin E was administered orally for 42 consecutive days according to previously established protocols [8–12].

2.4 Ethical Approval

All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th Edition, 2011) and the ARRIVE Guidelines 2.0 for reporting animal research. Ethical approval was obtained from the Faculty of Basic Medical Sciences Research and Ethics Committee, Delta State University, Abraka, Nigeria (Approval No. RBC/FBMC/DELSU/25/663).

2.5 Preparation of Plant Extract

Fresh leaves of *Spathodea campanulata* were air-dried under shade at room temperature and pulverized into fine powder using a mechanical grinder. The powdered material was extracted with methanol using standard phytochemical extraction procedures. The resulting crude extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further analysis [13].

2.6 Isolation and Identification of Verminoside and Specioside

Isolation and quantification of verminoside and specioside were performed using high-performance liquid chromatography (HPLC). Separation was achieved on a

reversed-phase C18 analytical column (250 mm × 4.6 mm, 5 μm particle size) using a binary mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). Gradient elution was programmed from 10% to 90% solvent B over 30 minutes at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C, and chromatographic detection was carried out at 254 nm using a diode-array detector (DAD).

The crude methanolic extract was dissolved in 70% methanol and filtered through a 0.45 μm nylon membrane before analysis. Approximately 20 μL of the filtered extract was injected into the HPLC system. Standard solutions of verminoside and specioside (1–10 mg/mL) were prepared in methanol. Compound identification was based on comparison of retention times and UV absorption spectra with authentic reference standards. Quantification was achieved using calibration curves generated from the standards.

Fractions corresponding to verminoside and specioside peaks were collected separately, pooled, and re-chromatographed to confirm chromatographic purity [14]. Purified fractions were concentrated under reduced pressure at 40°C to dryness and stored at 4°C until subsequent analyses.

2.7 In Vitro Antioxidant Assays

2.7.1 DPPH Radical Scavenging Assay

Free radical scavenging activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as previously described [15].

2.7.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant capacity was determined using the Ferric Reducing Antioxidant Power (FRAP) assay according to the method of Benzie and Strain [16].

2.8 Evaluation of Oxidative Stress Biomarkers

2.8.1 Lipid Peroxidation

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) concentrations using the thiobarbituric acid reactive substances (TBARS) assay [17].

2.8.2 Antioxidant Enzymes and Non-Enzymatic Antioxidants

The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and the level of reduced glutathione (GSH) were determined using standard spectrophotometric methods [18–21].

2.8.3 Nitric Oxide and Total Antioxidant Capacity

Nitric oxide (NO) concentration was determined using the Griess reagent method, while total antioxidant capacity (TAC) was evaluated using the ABTS radical cation decolorization assay [22,23].

2.9 Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) for five animals per group (n = 5). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1 HPLC analysis of Verminoside and Specioside

Figure 3.1 below presents a representative HPLC chromatogram in which the y-axis denotes detector response (mAU, 200 - 375 nm) and the x-axis represents retention time (0 - 4.5 min). Distinct peaks at 2.45 min and 3.17 min correspond to verminoside and specioside, respectively, with purity >95%. This result confirms the presence and relative abundance of these compounds in the analyzed sample, which is crucial for quantitative and qualitative phytochemical studies.

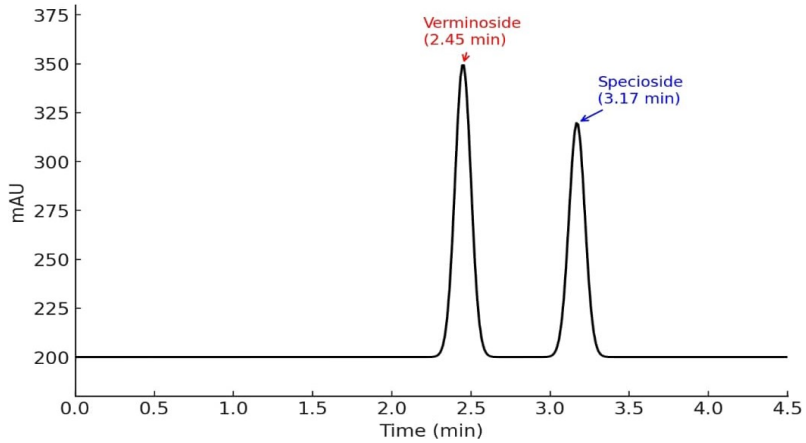

Figure 3.1: HPLC Chromatograms of Verminoside and Specioside

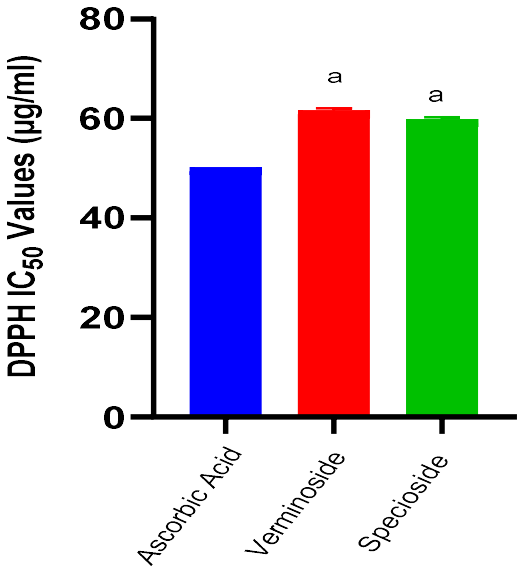

Figure 3.2: DPPH radical scavenging activity of Verminoside and Specioside

3.2 *In Vitro* Antioxidant Activities

3.2.1 DPPH radical scavenging activity of Verminoside and Specioside

DPPH scavenging activity (IC_{50}) of specioside and verminoside. Data is presented as Mean ± SEM, n = 5. $^{a}p<0.05$ is significant when compared with ascorbic acid. Blue colour: Ascorbic Acid (Standard), red colour: Verminoside and green colour: Specioside. Verminoside and specioside exhibited concentration-dependent DPPH radical scavenging activity. IC_{50} values were 61.50±0.50 µg/ml for verminoside, 59.67±0.58 µg/ml for specioside, compared with 50.00±0.00 µg/ml for ascorbic acid. This indicates moderate radical scavenging ability, confirming their role as antioxidants capable of donating electrons to neutralize free radicals.

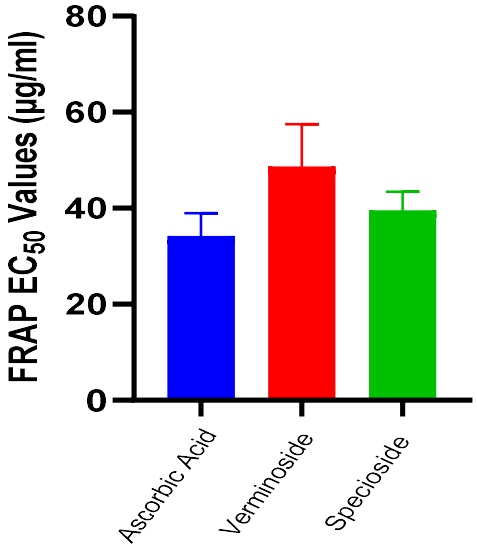

Figure 3.3: Ferric reducing antioxidant power (FRAP) of Verminoside and Specioside

3.2.2 Ferric reducing antioxidant power (FRAP) of verminoside and specioside

Ferric reducing antioxidant power (EC_{50}) of specioside and verminoside. Data is presented as Mean ± SEM. Blue colour: Ascorbic Acid (Standard), red colour: Verminoside and green colour: Specioside. Both compounds also demonstrated ferric reducing capacity. EC_{50} values were 48.50±8.98 µg/ml (verminoside) and 39.36±4.09 µg/ml (specioside), relative to 34.05±4.94 µg/ml for ascorbic acid (Figure 3). The results suggest that both iridoid glycosides are capable of reducing Fe^{3+} to Fe^{2+}, supporting their electron-donating antioxidant potential.

3.3 Oxidative stress markers

3.3.1 Lipid Peroxidation

Lead exposure significantly increased malondialdehyde (MDA) levels in testicular tissue compared with the control group. Treatment with verminoside and specioside markedly reduced MDA levels, comparable to the succimer-treated group.

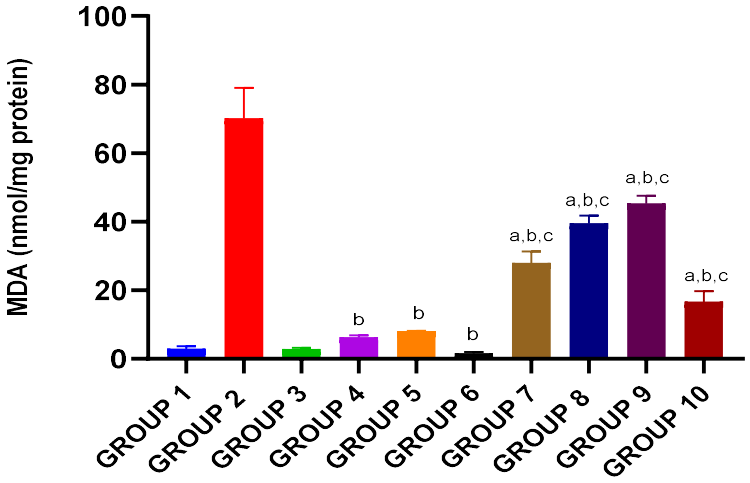

Figure 3.4: MDA levels in testicular tissue across experimental groups

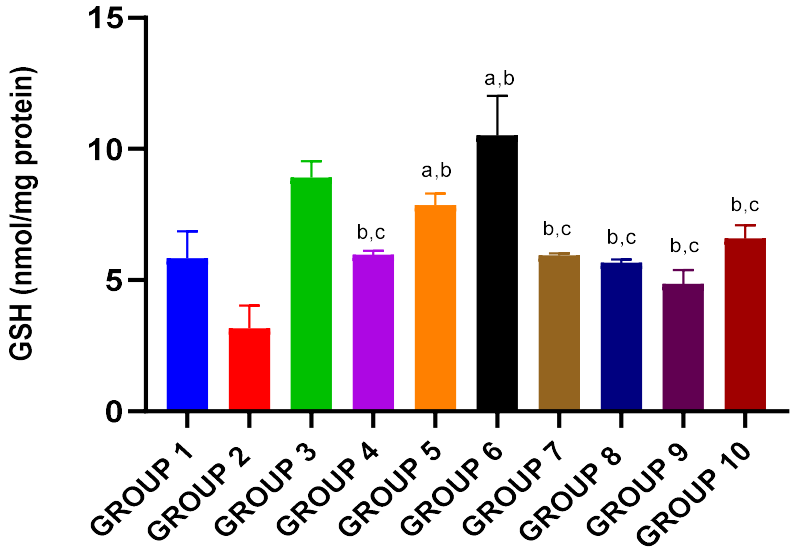

Figure 3.5: Testicular reduced glutathione (GSH) levels

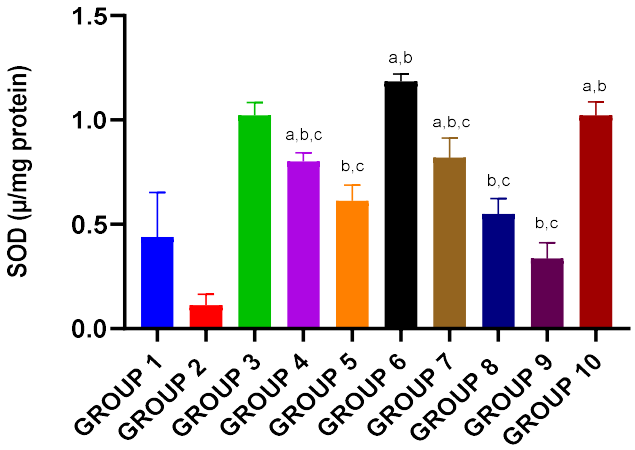

Figure 3.6: Superoxide dismutase (SOD) activity in testicular tissue

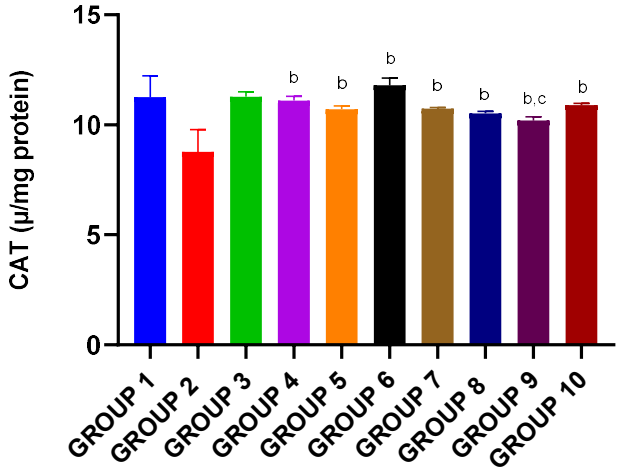

Figure 3.7: Catalase (CAT) activity in testicular tissue

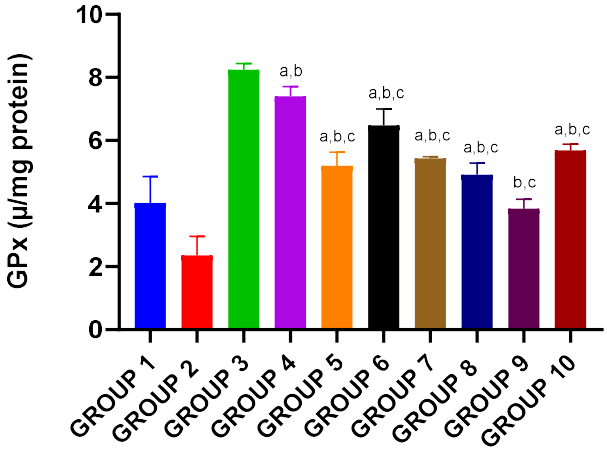

Figure 3.8: Glutathione peroxidase (GPx) activity in testicular tissue

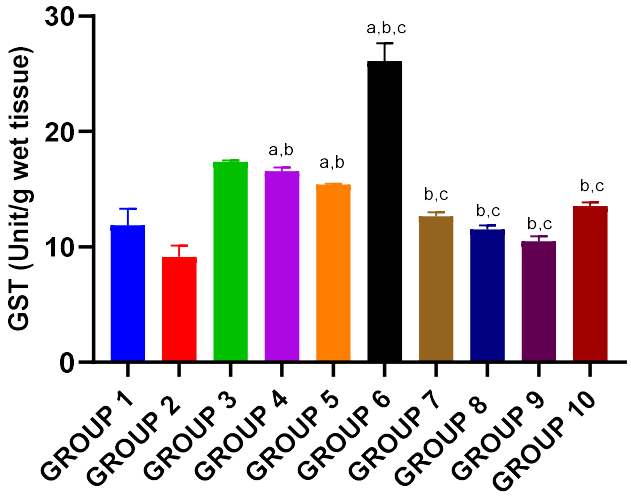

Figure 3.9: Glutathione S-transferase (GST) activity in testicular tissue

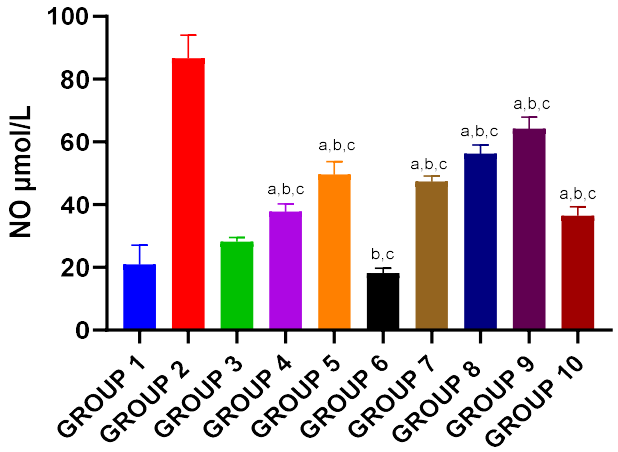

Figure 3.10: Nitric oxide (NO) levels in testicular tissue

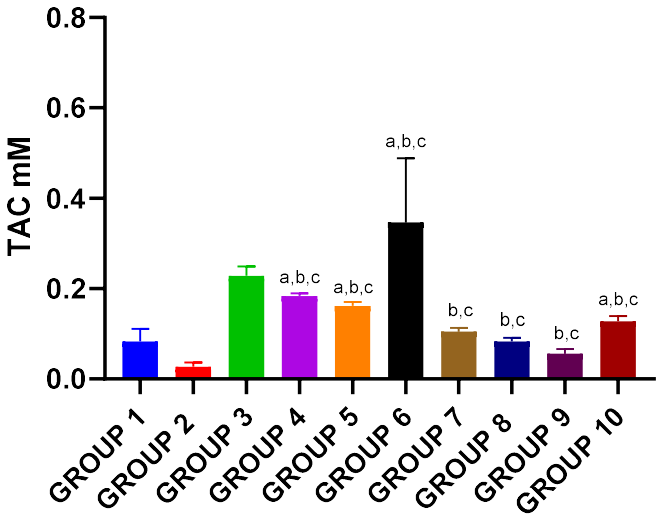

Figure 3.11: Total antioxidant capacity (TAC) in testicular tissue

3.2.2 Reduced Glutathione (GSH)

Lead acetate exposure caused a significant reduction in testicular reduced glutathione (GSH) levels compared with the normal control group. Treatment with verminoside and specioside, either individually or in combination, significantly restored GSH levels toward normal values, indicating enhanced endogenous antioxidant defense.

3.2.3 Superoxide Dismutase (SOD) Activity

A significant decrease in testicular superoxide dismutase (SOD) activity was observed in the lead acetate-treated group compared with the normal control. Administration of verminoside and specioside significantly increased SOD activity relative to the lead-only group, suggesting improved scavenging of superoxide radicals and attenuation of oxidative stress.

3.2.4 Catalase (CAT) Activity

Lead acetate exposure significantly suppressed catalase (CAT) activity in the testes when compared with the normal control group. Treatment with verminoside and specioside significantly restored CAT activity relative to the lead-only group, indicating enhanced decomposition of hydrogen peroxide and improved antioxidant defense.

3.2.5 Glutathione Peroxidase (GPx) Activity

Testicular glutathione peroxidase (GPx) activity was significantly reduced following lead acetate exposure. Treatment with verminoside and specioside significantly increased GPx activity compared with the lead-only group, suggesting enhanced detoxification of hydrogen peroxide and lipid hydroperoxides.

3.2.6 Glutathione S-Transferase (GST) Activity

Lead acetate administration resulted in a significant reduction in testicular glutathione S-transferase (GST) activity compared with the normal control group. Treatment with verminoside and specioside significantly enhanced GST activity relative to the lead-only group, reflecting improved cellular detoxification and antioxidant capacity.

3.2.7 Nitric Oxide (NO) Levels

Testicular nitric oxide (NO) levels were significantly elevated in the lead acetate-treated group, indicating increased nitrosative stress. Treatment with verminoside and specioside significantly reduced NO levels compared with the lead-only group, suggesting attenuation of nitrosative damage.

3.2.8 Total Antioxidant Capacity (TAC)

Lead acetate exposure significantly decreased total antioxidant capacity (TAC) in testicular tissue compared with the normal control group. Treatment with verminoside and specioside significantly increased TAC relative to the lead-only group, indicating restoration of the overall antioxidant defense system.

4. Discussion

Lead (Pb) is one of the most pervasive and persistent environmental pollutants and poses a significant threat to human and animal health. Exposure to lead has been implicated in male reproductive dysfunction, primarily through the induction of oxidative and nitrosative stress, which disrupts cellular redox homeostasis, impairs spermatogenesis, and compromises steroidogenesis [1,4,10]. The present study demonstrates that lead acetate-induced testicular toxicity is characterized by increased lipid peroxidation and nitrosative stress, evidenced by elevated malondialdehyde (MDA) and nitric oxide (NO) levels, together with marked depletion of endogenous antioxidant defenses. These findings corroborate previous reports that oxidative stress is a major mechanism underlying lead-induced reproductive toxicity [1,4].

Spathodea campanulata is a medicinal plant rich in bioactive iridoid glycosides, particularly verminoside and specioside, which possess potent antioxidant and anti-inflammatory properties. The present study evaluated the antioxidant potential of these compounds and their ability to ameliorate lead-induced oxidative damage in testicular tissue. The in vitro antioxidant activities of verminoside and specioside were assessed using the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. Both compounds exhibited appreciable reducing power and free radical scavenging activities. Specioside demonstrated greater antioxidant potency, with an EC_{50} value of 39.36 ± 4.09 μg/mL in the FRAP assay and an IC_{50} value of 59.67 ± 0.58 μg/mL in the DPPH assay, whereas verminoside exhibited EC_{50} and IC_{50} values of 48.50 ± 8.98 μg/mL and 61.50 ± 0.50 μg/mL, respectively. The antioxidant activities of both compounds were comparable with those of ascorbic acid, supporting previous reports that iridoid glycosides possess potent antioxidant properties attributable to their electron-donating and free radical-neutralizing capacities [5–7].

Consistent with previous studies, lead acetate exposure significantly increased testicular MDA and NO levels while markedly reducing the concentrations or activities of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and total antioxidant capacity (TAC). These alterations indicate severe oxidative and nitrosative stress, leading to compromised cellular antioxidant defense mechanisms. Elevated MDA reflects enhanced membrane lipid peroxidation, whereas increased NO production is indicative of excessive

reactive nitrogen species generation, both of which contribute to testicular cellular injury and impaired reproductive function [1,4,10].

Treatment with verminoside and specioside significantly attenuated these biochemical alterations by reducing MDA and NO levels while restoring GSH, SOD, CAT, GPx, GST, and TAC toward normal values. These findings suggest that the protective effects of both compounds are mediated through multiple complementary mechanisms, including direct scavenging of reactive oxygen and nitrogen species, inhibition of lipid peroxidation, and enhancement of endogenous antioxidant defense systems. Restoration of antioxidant enzyme activities further indicates preservation of cellular redox balance and protection against oxidative injury. The combined administration of verminoside and specioside appeared to provide greater protection than either compound administered alone, suggesting possible synergistic antioxidant interactions.

The observed protective effects are consistent with earlier reports demonstrating that iridoid glycosides possess potent antioxidant, anti-inflammatory, and cytoprotective activities capable of mitigating chemically induced oxidative tissue injury [5–8]. Unlike conventional chelating agents such as dimercaptosuccinic acid (DMSA), which primarily reduce systemic lead burden but may be associated with adverse effects, verminoside and specioside appear to exert protective effects by strengthening endogenous antioxidant defenses while minimizing oxidative damage. Consequently, these naturally occurring phytochemicals may represent promising adjunctive or alternative therapeutic agents for the management of lead-induced reproductive toxicity.

The antioxidant-mediated protective effects observed in the present study are also consistent with findings reported for other medicinal plants. For example, *Polyalthia longifolia* extract has been shown to exhibit significant antioxidant, spleen-protective, and antiplasmodial activities in *Plasmodium berghei*-infected mice, highlighting the therapeutic potential of plant-derived phytochemicals in the management of oxidative stress-related disorders [13].

5. Conclusion

The findings of the present study demonstrate that verminoside and specioside possess potent in vitro antioxidant activities and exert significant antioxidant-mediated protective effects against lead acetate-induced testicular oxidative damage. Both compounds effectively attenuated lipid peroxidation and nitrosative stress while restoring endogenous antioxidant defenses, thereby preserving testicular redox homeostasis. These findings suggest that verminoside and specioside are promising naturally occurring antioxidant compounds with potential therapeutic value in the prevention or management of lead-induced reproductive toxicity. Nevertheless, further studies are warranted to elucidate their molecular mechanisms of action, pharmacokinetic properties, safety profile, and clinical applicability before their potential therapeutic use can be established.

Declarations

Consent for publication: All authors have read and approved the final version of the manuscript and consent it to publication.

Data availability statement: All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Competing Interests: The authors declare no conflict of interest.

Ethical Compliance: All experimental procedures complied with institutional ethical guidelines.

Authors' Contributions: OI and EKC conceptualized the study. DCC contributed to biochemical analysis. OAA and AAI contributed to the literature review and drafting of manuscript. OOC contributed to the statistical analysis. OI and MJ supervised the study. OK contributed to data collection and manuscript editing.

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