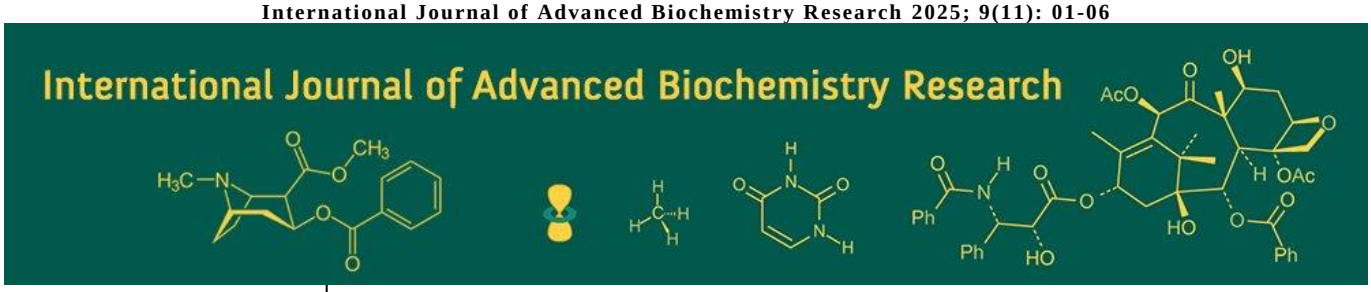

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Effect of *Diospyros mespiliformis* seed extract on lead toxicity induced changes in prefrontal cortex antioxidants and inflammatory cytokines level in mice

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Abstract
Heavy metals like lead (Pb) are persistent pollutants causing oxidative stress, inflammation, and organ damage. Lead (Pb), a neurotoxicant and persistent pollutant from industrial and domestic sources, impairs cognitive and behavioral functions causing oxidative stress, inflammation and organ damage. This study evaluated the effect of *Diospyros mespiliformis* seed extract on lead toxicity induced changes in prefrontal cortex antioxidants and inflammatory cytokines level in mice. Twenty-five mice were divided into five groups (n = 5). Group A is control administered distilled water only, group B mice were induced with lead (50 mg/kg b.wt), group C mice received 200 mg/kg b.wt of *D. mespiliformis* while group D were given 400 mg/kg b. wt and group E were administered 100 mg/kg b.wt of vitamin E. Results indicated that Pb exposure (50 mg/kg, 28 days) induced neurotoxicity in the prefrontal cortex, increasing total protein, malondialdehyde (MDA), IL-6, TNF-α, and myeloperoxidase (MPO), while reducing protein thiols, catalase (CAT), and acetylcholinesterase (AChE) activity, reflecting oxidative stress, inflammation, and cholinergic disruption. Treatment with *D. mespiliformis* extract (200 and 400 mg/kg) or vitamin E (100 mg/kg) mitigated these effects. The 400 mg/kg extract dose-dependently reduced MDA, normalized TNF-α, IL-6, and CAT, often outperforming vitamin E, likely due to its antioxidant flavonoids and polyphenols. However, neither fully restored AChE or thiol levels, indicating limited efficacy against Pb's direct binding. Vitamin E better reduced MPO. These findings highlighted *D. mespiliformis*'s potential as a neuroprotective agent against Pb-induced neurotoxicity, with efficacy rivaling vitamin E. Its low acute toxicity supports therapeutic promise, however, further studies on its active compounds, long-term safety, and optimization for thiol and cholinergic pathways are recommended.

Keywords: Lead, antioxidants, anti-inflammation, cytokines, prefrontal cortex, *Diospyros mespiliformis*

Introduction
Heavy metals, naturally occurring elements with high atomic mass and density, are persistent environmental pollutants that bioaccumulate and resist biodegradation [1, 2]. Human activities like mining, smelting, industrial processes, and agricultural use of metal-containing compounds drive environmental contamination and exposure [3]. Exposure occurs primarily through ingestion, inhalation, or dermal contact [4, 5]. Non-essential heavy metals, such as cadmium, lead, and mercury, are systemic toxicants, inducing organ damage and generating reactive oxygen species [6, 7]. These metals cause carcinogenic, mutagenic, neurotoxic, nephrotoxic, and immunotoxic effects, potentially leading to diseases like cancer or autoimmunity [3]. Lead, widely used in batteries, paints, ceramics, and other products, is a potent neurotoxicant, particularly affecting the developing brains of children [8]. It crosses the blood-brain barrier by mimicking calcium ions, disrupting cellular processes and causing intellectual and behavioral deficits [9]. Lead's systemic toxicity impacts multiple organs, notably the central nervous system, leading to functional impairments [8].
The brain, the central hub of the nervous system in vertebrates and most invertebrates, relies on the prefrontal cortex, located in the frontal lobe, for higher-order cognitive, emotional, and behavioral functions. Lead (Pb) exposure disrupts the blood-brain barrier, primarily damaging astrocytes and secondarily affecting endothelial microvasculature [10].

This leads to altered oxidative stress biomarkers, enzymes, and inflammatory markers, causing significant biochemical changes. Cytokines, small proteins critical for immune responses, coordinate immune cell actions, promote inflammation, and combat infections. Key cytokines include interleukins, interferons, tumor necrosis factors (TNF-α), and colony-stimulating factors. Lead-induced brain lesions primarily affect the prefrontal cortex, cerebellum, and hippocampus, triggering oxidative stress and inflammatory responses [10].

Medicinal plants, rich in phytochemicals, offer diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective effects [11, 12, 13]. These plants produce secondary metabolites like alkaloids, phenolic acids, polyphenols, terpenoids, and saponins [14, 15]. *Diospyros mespiliformis*, known as African ebony, belongs to the Ebenaceae family [16]. The plant's seed have been reported to offer nutraceutical benefits for cholesterol, diabetes and weight control [17, 18]. *D. mespiliformis* leaves *a*ccording to Von Maydell, and Ruffo *et al.* [19, 20] treats fevers, leprosy and syphilis. However, there is dearth of information concerning the effect of *D. mespiliformis* on brain tissue of lead-induced mice. Hence, this research evaluates the changes in antioxidant and inflammatory cytokines in the prefrontal cortex of mice brain after exposure to lead and subsequent treatment with aqueous seed extracts of *D. mespiliformis.*

Materials and Methods

Collection of Plant (*Diospyros mespiliformis*) Seeds

The seeds of *D. mespiliformis* were purchased from a local market in Mararaban Guruku, Karu Local Government Area of Nasarawa State Nigeria. The seeds were identified at the Herbarium Unit of Ekiti State University, Ado-Ekiti Nigeria, as *Diospyros mespiliformis* (*Ebenaceae*) with voucher number UHAE 2025045.

Preparation of Plant (*Diospyros mespiliformis*) Extract

The *Diospyros mespiliformis* seeds were sun-dried to obtain a constant weight for two weeks, then grounded into powder using Warren blender. Twenty-five grams (25 g) of the grounded seeds was soaked in 100 ml distilled water boiled for 5 minutes and shaken for 10 minutes and allowed to cool then filtered. The extract was concentrated using a rotary evaporator at 40 to 50 ^{o}C under reduced pressure. The extracts were then stored frozen until required.

Ethical Approvals

The ethical approval was obtained from the Directorate of Research and Quality Assurance of the Federal University Otuoke, Bayelsa State, Nigeria. To ensure international conformity, the research protocol adhered strictly to the Animal Welfare Act of 1985 of the United States of America, as well as the guidelines of the Research and Institutional Animal Care and Use Committee (IACUC).

Experimental Design

Toxicity Testing

Eighteen mice, divided into 6 groups were used for the determination of LD_{50} of the extract (*D. mespiliformis*). The six groups were exposed to 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight of extract respectively. The LD_{50} was subsequently determined by the method of Lorke [21].

$$LD_{50} = \sqrt{a * b}$$

Where; a = highest dose that gave no mortality, b = lowest dose that produced mortality

Treatment of Animals

Another set of twenty-five mice were divided into five groups and administered the following: Group A is control and were given distilled water. Group B mice received 50 mg/kg body weight of Lead (Pb) while animals in Group C were administered Pb and 200 mg/kg body weight of extract. Group D received Pb and 400 mg/kg body weight and Group E mice were administered Pb and vitamin E (100 mg/kg body weight). The mice were given this treatment daily for 28 days. At the end of the exposure time, mice were made to fast overnight, anaesthetized with chloroform and prefrontal cortex section of brain tissue was obtained.

Preparation of Brain Tissue Supernatant for Biochemical Assay

The mice were dissected, the brain tissue was quickly removed and the prefrontal cortex obtained. (0.025 g) of wet prefrontal cortex brain tissues was homogenized in 2.25 mL of the physiological solution (phosphate buffer, pH 7.4). The resulting homogenates were centrifuged at x5000 g for 20 minutes. The supernatants were decanted and used for further biochemical analysis.

Biochemical Analyses

The concentrations of total protein, protein thiol and tissue reduced glutathione were determined by the methods of Doumas *et al.* [22], Sedlack & Lindsey [23] and Ellman [24]. The assays for the activities of superoxide dismutase, catalase and acetylcholinesterase were estimated in brain prefrontal cortex of mice employing the methods of Misra & Fridovich [26], Kaplan & Groves [27] and Ellman *et al.* [25] respectively. The methods of Buege & Aust [28] and Green *et al.* [29] were used to evaluate malondialdehyde and nitric oxide concentrations. The levels of interleukin-6 and tumor necrosis factor-α was measured in prefrontal cortex of mice brain according to the methods of March *et al.* [30] and Engelmann *et al.* [31]. While the activity of myeloperoxidase activity in prefrontal tissue supernatant was assayed for, as described by Bradley *et al.* [32].

Statistical analysis

The study data were analyzed using IBM SPSS Statistics (version 23.0). The data were presented as the means±standard deviations (SD) of the replicates in each group. The data from the groups were compared using a one-way analysis of variance (ANOVA). Tukey test was utilized as the descriptive statistical tool. The statistical significance level was set at $p<0.05$.

Results

According to the Globally Harmonized System (GHS) for classification of chemical substances, a substance with an LD_{50} greater than 5000 mg/kg is considered to have low acute toxicity. Therefore, the LD_{50} of *D. mespiliformis* is likely >5000 mg/kg, indicating that it is relatively non-toxic in acute exposure. However, we observed the presence of sublethal effects (reduced activity, fatigue, and writhing) at 2900 mg/kg and 5000 mg/kg indicating that the compound may cause physiological or neurological effects at higher doses.

Table 1: Toxicity Test Results of *D. mespiliformis* aqueous seed extract

Groups	Number of Mice	Death	Behavioral Change	Fatigue	Writhing Effect
10 mg/kg	3	0	Nil	Nil	Nil
100 mg/kg	3	0	Nil	Nil	Nil
1000 mg/kg	3	0	Nil	Nil	Nil
1600 mg/kg	3	0	Nil	Nil	Nil
2900 mg/kg	3	0	Reduced activity	Yes	Nil
5000 mg/kg	3	0	Reduced activity	Yes	Yes

Table 2: Concentrations of Total Protein and Protein Thiol in Prefrontal Cortex of Lead-Induced Mice Treated with Aqueous Extract of *D. Mespiliformis*

Groups	Total Protein (mg/dl)	Protein Thiol (%)
A	9.027 ± 1.07^{a}	24.068 ± 4.55^{a}
B	14.820 ± 0.86^{b}	16.767 ± 2.57^{b}
C	14.448 ± 0.79^{b}	8.933 ± 1.36^{c}
D	13.653 ± 0.80^{b}	11.212 ± 2.36^{c}
E	10.124 ± 1.21^{a}	10.419 ± 0.51^{c}

Values are expressed as mean±standard deviation; n = 5. Means not sharing the same superscript alphabets in a column differ significantly at $p<0.05$

Group A = Control; Group B = Pb (50 mg/kg b.wt.); Group C = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group D = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group E = (Pb + 100 mg/kg b. wt of vitamin E).

Results in Table 2 showed that the levels of total protein in the prefrontal cortex of lead-induced mice were significantly higher ($p<0.05$) than the control (group A) but comparable ($p>0.05$) to that treated mice in groups C and D respectively. The administration of vitamin C reduced total protein concentration as compared with the lead-induced mice. The results also indicated lead exposure significantly reduced ($p<0.05$) protein thiol levels in group B mice as compared with group A (control). Treatment with *D. mespiliformis* aqueous seed extract reduces ($p<0.05$) protein thiol levels.

Table 3: Activities of Oxidative Stress Enzymes and Lipid Peroxidation Indices in Prefrontal Cortex of Lead-Induced Mice Treated with Aqueous Extract of *D. Mespiliformis*

Groups	Reduced glutathione (Unit/mg protein)	Superoxide dismutase (Unit/mg protein)	Catalase (Unit/mg protein)	Malondialdehyde (nmol/g wet tissue)
A	38.379 ± 3.84^{a}	18.082 ± 2.24^{a}	46.801 ± 2.56^{a}	0.842 ± 0.13^{a}
B	43.753 ± 4.99^{b}	18.098 ± 1.25^{a}	37.359 ± 3.03^{b}	1.320 ± 0.38^{b}
C	39.014 ± 4.50^{a}	23.562 ± 4.18^{b}	48.723 ± 2.74^{a}	0.740 ± 0.18^{a}
D	33.932 ± 3.85^{a}	19.637 ± 2.08^{a}	49.987 ± 1.43^{a}	0.870 ± 0.23^{a}
E	26.067 ± 3.90^{d}	19.465 ± 0.91^{a}	44.984 ± 4.24^{a}	0.649 ± 0.13^{a}

Values are expressed as mean±standard deviation; n = 5. Means not sharing the same superscript alphabets in a column differ significantly at $p<0.05$

Group A = Control; Group B = Pb (50 mg/kg b.wt.); Group C = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group D = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group E = (Pb + 100 mg/kg b. wt of vitamin E).

Results in table 3 indicated that lead exposure causes an increase ($p<0.05$) in the levels of reduced glutathione (GSH) and malondialdehyde (MDA) in group B animals as compared with the control (group A) mice. Treatment with 200 mg/kg b. wt.; 400 mg/kg b.wt. of *D. mesipiliformis extract* and 100 mg/kg b.wt of vitamin E resulted in decreased ($p<0.05$) concentrations of GSH and MDA respectively. The activity of superoxide dismutase in prefrontal cortex of mice exposed to lead remain unaltered ($p>0.05$) while catalase activity was significantly reduced ($p<0.05$) as compared with the control animals (group A). Treatments with plant's extract and vitamin E increases ($p<0.05$) catalase activity but such increase in superoxide dismutase activity was only observed in mice treated with 200 mg/kg b.wt. of *D. mespiliformis* extract.

Table 4: Concentration of Nitric Oxide and Activity of Acetylcholinesterase in Prefrontal Cortex of Lead-Induced Mice Treated with Aqueous Extract of *D. Mespiliformis*

Groups	Nitric oxide (%)	Acetylcholinesterase (Unit/mg protein)
A	20.204 ± 2.69^{a}	9.468 ± 0.89^{a}
B	20.482 ± 2.59^{a}	3.739 ± 0.82^{b}
C	21.590 ± 2.14^{a}	3.240 ± 0.66^{b}
D	16.186 ± 0.96^{c}	3.797 ± 0.43^{b}
E	19.588 ± 2.08^{a}	4.551 ± 0.38^{b}

Values are expressed as mean±standard deviation; n = 5. Means not sharing the same superscript alphabets in a column differ significantly at $p<0.05$

Group A = Control; Group B = Pb (50 mg/kg b.wt.); Group C = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group D = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group E = (Pb + 100 mg/kg b. wt of vitamin E).

It is observed from the results in table 4 that lead exposed mice had comparable (p>0.05) nitric oxide level with control mice. However, treatment with 400 mg/kg b. wt. of *D. mesipiliformis extract* reduces (p<0.05) the nitric oxide concentration. The results in table 4 also revealed that the activity of acetylcholinesterase was significantly (p<0.05) inhibited in lead induced mice (group B) as compared with the control mice. Treatment with *D. mespiliformis* extract at 200 mg/kg (C) and 400 mg/kg (D), as well as vitamin E (E), did not restore AChE activity.

Table 5: Concentrations of Inflammatory Markers in Prefrontal Cortex of Lead-Induced Mice Treated with Aqueous Extract of *D. Mespiliformis*

Groups	Interleukin-6 (IL-6) (ng/ml)	Tumor Necrosis Factor-α (TNF-α) (pg/ml)	Myeloperoxidase (Unit/mg protein)
A	29.000±0.99^{a}	11.524±0.71^{a}	3.004±0.56^{a}
B	43.494±0.59^{b}	57.788±1.59^{b}	5.310±0.13^{b}
C	35.019±1.088^{c}	26.617±1.84^{c}	5.237±0.42^{b}
D	38.329±0.56^{c}	15.403±0.45^{a}	4.688±0.32^{b}
E	39.332±0.49^{c}	11.916±0.67^{a}	3.502±0.84^{a}

Values are expressed as mean±standard deviation; n = 5. Means not sharing the same superscript alphabets in a column differ significantly at p<0.05.

Group A = Control; Group B = Pb (50 mg/kg b.wt.); Group C = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group D = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group E = (Pb + 100 mg/kg b. wt of vitamin E).

Lead exposure (Group B) significantly increased (p<0.05) all inflammatory markers compared to the control (Group A). Treatment with *D. mespiliformis* extract at 200 mg/kg (Group C) and 400 mg/kg (Group D), as well as vitamin E (Group E), significantly reduced TNF-α and IL-6 levels compared to lead alone, though IL-6 remained elevated relative to control. Myeloperoxidase activity was significantly higher in lead-exposed and extract-treated groups compared to control, except in the vitamin E group, which showed MPO levels similar to control.

Discussion

The acute toxicity study of *D. mespiliformis* aqueous seed extract revealed no mortality across all tested doses, including 5000 mg/kg, indicating an LD_{50} >5000 mg/kg. According to the Globally Harmonized System (GHS), this classifies the extract as having low acute toxicity, suggesting a wide safety margin for acute exposure [33]. However, sublethal effects (e.g., reduced activity, fatigue, writhing) at 2900 mg/kg and 5000 mg/kg indicate potential physiological or neurological impacts at higher doses, necessitating further subacute and chronic toxicity studies. The low toxicity aligns with other medicinal plants, supporting its potential safety for traditional or clinical use, though the observed sublethal effects require comparison with similar plant extracts to elucidate mechanisms and organ-specific toxicity.

According to the results, toxicity induction with Pb elicited significant perturbations in biomarkers of protein dysfunction, oxidative stress, neurotoxicity, and inflammation in the prefrontal cortex of mice and consistent with prior reports of Pb's ability to generate reactive oxygen species (ROS) and impair antioxidant defenses [34]. Treatment with aqueous seed extract of *D. mespiliformis* at 200 mg/kg (Group C) and 400 mg/kg (Group D), or vitamin E (100 mg/kg, Group E), demonstrated varying degrees of amelioration, highlighting the potential neuroprotective role of *D. mespiliformis* which may be attributable to its rich phytochemical profile including flavonoids and polyphenols with antioxidant and anti-inflammatory properties [35]. The total protein concentration in the prefrontal cortex reflects overall protein content that can be altered by lead-induced neurotoxicity or mitigated by treatments, while protein thiol levels indicate the availability of reduced sulfhydryl groups (-SH) essential for protein function and protection against oxidative stress, with decreases in thiol levels signaling oxidative damage caused by lead-induced ROS oxidation. Significant total protein observed in Pb-exposed mice may reflect compensatory protein synthesis or cellular hypertrophy in response to Pb-induced stress. . Concurrently, protein thiol levels, indicative of sulfhydryl (-SH) groups essential for protein stability and antioxidant function, were markedly reduced in lead induced and treated. This depletion may be caused by Pb's affinity for thiol groups, leading to protein oxidation and conformational changes that exacerbate neurotoxicity. Similar thiol reductions have been documented in Pb-exposed models, where oxidative damage contributes to elevated protein carbonyls and impaired cellular redox status [36].

Elevated malondialdehyde (MDA), a lipid peroxidation byproduct observed in Group B mice could be related to Pb's induction of ROS-mediated membrane damage in the brain. *D. mespiliformis* treatment dose-dependently and vitamin E reduced MDA which suggest its antioxidant capacity to scavenge free radicals. Levels of reduced glutathione (GSH) increased in Group B compared to Group A, potentially as an adaptive response to initial oxidative insult, before depletion in chronic scenarios; this compensatory rise has been noted in early Pb exposure phases. The extract at 400 mg/kg and vitamin E normalized or lowered GSH (groups D & E), indicating reduced demand due to mitigated stress. Superoxide dismutase (SOD) activity remained unchanged in Group B but increased in group C mice, implying enhanced dismutation of superoxide radicals by the extract. Catalase (CAT) activity decreased in Pb-exposed mice relative to control, reflecting impaired hydrogen peroxide detoxification, a common Pb effect in cortical regions. Both doses of the extract restored CAT (groups C & D) maybe likely due to polyphenolic compounds present in the plant enhancing enzymatic antioxidants, as reported in studies on Diospyros species [37].

Acetylcholinesterase (AChE) is an enzyme essential for hydrolyzing acetylcholine in the cholinergic system, with its inhibition serving as a hallmark of neurotoxicity that can impair neurotransmission and cognitive function. At the same time, nitric oxide (NO), a signaling molecule involved in neuronal activity, can contribute to nitrosative stress and neurotoxicity when elevated, particularly during lead exposure. Acetylcholinesterase (AChE) activity was significantly inhibited in prefrontal cortex of lead exposed mice and this could be due to Pb's interference in neurotransmitter systems, leading to cognitive deficits in mice [38, 39]. Treatment with plant' extract and vitamin E fail to restore the activity of acetylcholinesterase suggesting limited efficacy against Pb's direct enzyme binding. Nitric oxide (NO) levels did not show a significant increase following Pb and this could mean that NO-mediated

nitrosative stress may not be a major pathway in the prefrontal cortex within this model. IL-6 and TNF-α are pro-inflammatory cytokines involved in neuroinflammatory responses, with elevated levels of both being associated with neuronal damage, cognitive impairment, and neuronal dysfunction, whereas MPO is an enzyme associated with neutrophil activity and oxidative stress, serving as a marker of inflammatory and oxidative damage in tissues. Inflammatory markers (Table 5) demonstrated Pb's pro-inflammatory action, with significant elevations in IL-6 & TNF in Pb-induced mice as compared with the control, partially attenuated. Treatment with plant's extract reduces the concentrations of these inflammatory markers. Myeloperoxidase (MPO) activity, a neutrophil infiltration marker, increased in Group B. Administration of *D. mespiliformis* did not appreciably reduced the activity of MPO. However, vitamin E, a potent antioxidant significantly decreases MPO activity.as against that of group B mice.

Conclusion

The acute toxicity study of *D. mespiliformis* aqueous seed extract showed no mortality up to 5000 mg/kg, indicating an LD50 >5000 mg/kg and low acute toxicity per GHS standards, suggesting a wide safety margin. However, sublethal effects at higher doses (2900 and 5000 mg/kg) necessitate further subacute and chronic toxicity studies. Lead (Pb) exposure (50 mg/kg, 28 days) caused neurotoxicity in mice prefrontal cortex, increasing total protein and malondialdehyde (MDA), reducing protein thiols and catalase (CAT) activity, and elevating IL-6, TNF-α, and myeloperoxidase (MPO), indicating oxidative stress and inflammation. Glutathione (GSH) unexpectedly increased, possibly as a compensatory response, while superoxide dismutase (SOD) remained stable. Acetylcholinesterase (AChE) inhibition confirmed cholinergic disruption. *D. mespiliformis* extract (200 and 400 mg/kg) and vitamin E (100 mg/kg) mitigated these effects. The 400 mg/kg extract dose-dependently reduced MDA, normalized TNF-α, IL-6, and CAT, often surpassing vitamin E, likely due to its antioxidant flavonoids and polyphenols. Neither fully restored AChE or thiol levels, suggesting limited counteraction of Pb's direct binding. Vitamin E better reduced MPO, indicating broader antioxidant effects. *D. mespiliformis* shows promise as a neuroprotective agent against Pb-induced neurotoxicity, with low acute toxicity and efficacy rivaling vitamin E. Further studies are needed to clarify its active compounds' mechanisms, long-term safety, and optimization for thiol and cholinergic pathways.

References

1. Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L. A review of toxicity and mechanisms of heavy metals in the environment. Environ Sci Pollut Res. 2016;23(9):8249-8262.
2. Cesur H, Akkaya Y, Akkaya B. Heavy metal pollution in the environment: sources, impacts, and mitigation strategies. Environ Chem Lett. 2021;19(2):1237-1252.
3. Anka IZ, Bala SA, Dalhat MH. Heavy metal contamination and health risk assessment: a review. Environ Monit Assess. 2022;194(6):420-436.
4. Pizzol M, Thomsen M, Andersen MS. Long-term human exposure to heavy metals: a review. Environ Sci Technol. 2010;44(24):9437-9443.
5. Cheng Z, Wang X, Li J. Human exposure pathways to heavy metals: a review. Environ Geochem Health. 2018;40(5):1823-1837.
6. Masindi V, Muedi KL. Environmental contamination by heavy metals: a global perspective. Environ Monit Assess. 2018;190(10):585-602.
7. Abolhassani M, Asadikaram G, Karimi G. Mechanisms of heavy metal-induced toxicity: a review. Environ Sci Pollut Res. 2020;27(1):123-145.
8. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: a review. Rev Environ Health. 2009;24(1):15-45.
9. Rana SVS. Metals and neurotoxicity: a review. Indian J Exp Biol. 2008;46(10):671-678.
10. Finkelstein Y, Markowitz ME, Rosen JF. Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. Brain Res Rev. 1998;27(2):168-176.
11. Kalyniukova A, Holuša J, Musiolek M. Bioactive compounds in medicinal plants: a review of their therapeutic potential. Phytochem Rev. 2021;20(4):789-814.
12. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: a move towards nature. Biotechnol Mol Biol Rev. 2009;2(4):97-104.
13. Lesellier E, Lefebvre T, Destandau E. Recent advances in the analysis of bioactive compounds in medicinal plants. J Chromatogr A. 2021;1654:462458-462467.
14. Agidew MG. Phytochemical analysis and pharmacological activities of medicinal plants: a review. Evid Based Complement Alternat Med. 2022;2022:6725341-6725355.
15. Pawase PA, Kamboj A, Pawase VA. Secondary metabolites in medicinal plants: biosynthesis and therapeutic potential. Phytochem Lett. 2024;57:192-204.
16. USDA, NRCS. The PLANTS Database. Greensboro (NC): National Plant Data Team; 2017. p. 1-400.
17. Chivandi E, Erlwanger KH. Nutritional and health benefits of *Diospyros mespiliformis* seeds: a review. J Food Sci Technol. 2011;48(6):651-657.
18. El-Kamali HH. Ethnopharmacological uses of *Diospyros mespiliformis* in Sudan. J Plant Sci. 2011;6(4):146-152.
19. Von Maydell HJ. *Trees and shrubs of the Sahel: their characteristics and uses.* Eschborn: Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ); 1990. p. 1-500.
20. Ruffo CK, Birnie A, Tengnäs B. *Edible wild plants of Tanzania*. Nairobi: Regional Land Management Unit (RELMA); 2002. p. 1-800.
21. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54(4):275-287.
22. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1981;31(1):87-96.
23. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.
24. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-77.
25. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of

acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95.
26. Misra HP, Fridovich I. The role of superoxide ion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-3175.
27. Kaplan JH, Groves J. Liver and blood cell catalase activity in tumor-bearing mice. Cancer Res. 1972;32:1190-1194.
28. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-310.
29. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982;126(1):131-138.
30. March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G, Price V, *et al.* Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature. 1985;315(6021):641-647.
31. Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine. J Biol Chem. 1990;265(3):1531-1536.
32. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982;78(3):206-209.
33. OSHA. *Globally harmonized system of classification and labelling of chemicals (GHS).* Washington (DC): Occupational Safety and Health Administration; 2005. p. 1-300.
34. Harshitha S, Kumar A, Sharma V. Lead-induced oxidative stress and neurotoxicity: mechanisms and mitigation. Neurotoxicology. 2024;100:45-56.
35. Ebbo AA, Sani D, Suleiman MM. Phytochemical and pharmacological properties of *Diospyros mespiliformis.* J Nat Prod Plant Resour. 2014;4(3):22-28.
36. Kasperczyk S, Dobrakowski M, Birkner E. Oxidative stress in workers exposed to lead: protein carbonyls and thiol levels. J Occup Health. 2013;55(4):261-267.
37. Hong L, Li J, Wei X. Antioxidant and neuroprotective effects of *Diospyros* species: a comprehensive review. Antioxidants. 2023;12(4):789-804.
38. Reddy GR, Devi BC, Chetty CS. Developmental lead exposure and cholinergic system: a review. Neurotoxicology. 2003;24(4-5):735-743.
39. Phyu MP, Tangpong J. Neuroprotective effects of antioxidants against lead-induced neurotoxicity in rats. J Toxicol Sci. 2013;38(5):769-777.