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Assessment of Genotoxicity and Inflammation in the Brain Hippocampus of Lead-Induced Mice Treated With *Diospyros Mespiliformis*

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ABSTRACT

Hippocampus is crucial for memory and cognition, is highly vulnerable to oxidative stress and inflammatory insults from neurotoxicants such as lead (Pb). This study assessed genotoxicity and inflammatory markers in the brain hippocampus of lead – induced mice treated with aqueous extract of *Diospyros mespiliformis*. Twenty-five mice were divided into five groups and administered the following: Group A (Control, water) Group B (Pb, 50 mg/kg b.wt.), Group C (Pb + *D. mespiliformis* extract, 200 mg/kg b.wt), Group D (Pb + *D. mespiliformis* extract, 400 mg/kg b.wt), and Group E (Pb + vitamin E , 100 mg/kg b.wt). After 28 days of exposure and treatments, hippocampal tissues from mice brain were assayed for oxidative stress markers (reduced glutathione, protein thiol), genotoxic marker (DNA fragmentation), inflammation markers (tumor necrosis factor (TNF-α), interleukin -6 (IL-1β), nitric oxide (NO), myeloperoxidase (MPO), acetylcholinesterase (AChE) and total protein (TP). Results showed that Pb exposure caused significant increases in TNF-α and DNA fragmentation, alongside a decline in IL-1β and AChE activity, confirming neuroinflammation and genotoxicity. Treatment with *D. mespiliformis* (200 mg/kg) restored GSH and protein levels, reduced MPO activity, and lowered DNA fragmentation. The 400 mg/kg b.wt of plant's extract, however, elevated TNF-α and NO levels, suggesting a paradoxical pro-oxidant effect. Vitamin E attenuated DNA fragmentation and MPO activity, resembling the protective effects of the plant extract. These findings suggest that *D. mespiliformis* confers dose-dependent neuroprotection against Pb-induced hippocampal toxicity, with the 200 mg/kg dose being the most effective.

Keywords: *Diospyros mespiliformis*, Lead toxicity, Genotoxicity, Neuroinflammation, Hippocampus, Oxidative stress.

INTRODUCTION

Lead (Pb) is a pervasive environmental heavy metal toxin, primarily released through anthropogenic activities such as mining, battery manufacturing, and fossil fuel combustion. It bioaccumulates in the food chain and poses significant health risks, particularly to the central nervous system (CNS) due to its ability to cross the blood-brain barrier and accumulate in neural tissues (Flora *et al.*, 2012). The hippocampus, a brain region essential for learning, memory, and spatial navigation, is highly vulnerable to Pb-induced oxidative stress, neuroinflammation, and genotoxicity (Sanders *et al.*, 2009; Augustine *et al.*, 2024). Pb disrupts cellular processes including neurotransmitter release, calcium signaling, and DNA repair, leading to elevated reactive oxygen species (ROS), lipid peroxidation, and release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) (Liu *et al.*, 2013; Hassoun & Stohs, 1996; Engstrom *et al.*, 2010). Chronic exposure to Pb has been linked to cognitive impairments, behavioral abnormalities, and neurodegenerative diseases in both animal models and human populations (Basha & Reddy, 2010).

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Conventional treatments like chelating agents (e.g., EDTA, DMSA) are effective but associated with side effects such as nephrotoxicity and loss of essential metals (Flora *et al.,* 2012). This has prompted exploration of natural alternatives, including medicinal plants with antioxidant and anti-inflammatory properties. *Diospyros mespiliformis* (African ebony, *Ebenaceae* family) is traditionally used in African ethnomedicine for treating inflammation, infections, and oxidative stress-related ailments, owing to its rich phytochemical profile (flavonoids, alkaloids, saponins, tannins, phenolics, terpenoids, naphthoquinones, and coumarins) with antioxidant and anti-inflammatory properties (Bello *et al.,* 2009; Akinmoladun *et al.,* 2010; Dangoggo *et al.,* 2023; Nguelefack-Mbuyo *et al.,* 2023). Preliminary studies have demonstrated its neuroprotective potential in models of amnesia and epilepsy, suggesting modulation of hippocampal function (Muhammad *et al.,* 2017; Muhammad *et al.,* 2025).

Despite its potential, limited scientific data exist on *D. mespiliformis's* neuroprotective effects against Pb-induced hippocampal damage. This study aimed to assess the genotoxic and anti-inflammatory effects of *D. mespiliformis* extract on the brain hippocampus of Pb-induced mice.

MATERIALS AND METHODS

Collection of Plant Material

The seeds of *Diospyros mespiliformis* were obtained from a local herbal market located in Zaria, Kaduna State, Nigeria and were authenticated at Ekiti State University Herbarium, Nigeria witth the voucher number UHAC 202045.

Preparation of Aqueous Extract of *Diospyros mespiliformis*

The seeds were thoroughly cleaned to remove extraneous materials such as dust particles, plant debris, and microbial contaminants. They were subsequently air-dried under shade at ambient laboratory temperature (approximately 25–28 °C) for 14 days. Once adequately dried, the seeds were mechanically pulverized into a fine powder using a high-speed electric blender. The powdered material was weighed precisely, and 500 grams were immersed in 3 liters of distilled water in a large glass container. The mixture was agitated manually and allowed to stand for 48 hours at room temperature to facilitate exhaustive extraction of water-soluble phytoconstituents. Intermittent stirring was performed every six hours to enhance solvent penetration and mass transfer. After maceration, the slurry was filtered initially through clean muslin cloth to separate coarse particles. The filtrate was then passed through Whatman No.1 filter paper under vacuum filtration to obtain a clear extract solution. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40 °C to remove excess solvent gently, thereby preserving the active constituents. The concentrated extract was further dried to a semi-solid consistency using a water bath maintained at 45 °C until a constant weight was achieved. The resulting dried aqueous extract was transferred into sterilized airtight amber glass containers, labeled appropriately, and stored in a refrigerator at 4 °C until administration to the experimental animals.

Experimental Design

Toxicity testing of plant extract

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 (1983).

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

Where

A = highest dose that gave no mortality

B = lowest dose that produced mortality

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LD_{50} value of the *D. mespiliformis* is above 5000 mg/kg since no death was recorded.

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 Lead and 200 mg/kg body weight of extract. Group D received Lead and 400 mg/kg body weight and Group E mice were administered Lead and vitamin E (100 mg/kg body weight). The mice were giving this treatment daily for 28 days. At the end of the exposure time, mice were made to fast overnight, sacrificed humanely (cervical decapitation) following ethical guidelines.

Preparation of Brain Tissue Supernatant for Biochemical Assay

The mice were dissected, the brain tissue was quickly removed and the hippocampus obtained. The wet hippocampus brain tissues were homogenized in 2.25 mL of the physiological solution (phosphate buffer, pH 7.4). The resulting homogenates were centrifuged at x5000g for 20 minutes. The supernatants were decanted and used for further biochemical analysis.

Biochemical Analysis

The concentrations total protein, reduced glutathione, fragmented DNA, protein thiols were determined employing the methods of Doumas *et al.* (1981); Ellman (1959); Wu *et al*. (2005) and Sedlack and Lindsey (1968). The activity of acetylcholinesterase was assayed using the method of Ellman *et al*. (1961) while nitric oxide level was determined by the method of Green *et al*. (1982). Myeloperoxidase activity was estimated by the method of Bradley *et al.,* (1982). The concentrations of Tumor Necrosis Factor – α and interleukin -1β were measured in the hippocampus of mice brain employing the methods of Engelmann *et al*. (1990) and March *et al*. (1985) respectively.

Statistical analysis

The data obtained for the various biochemical parameters determined were expressed as Mean ± SD and subjected to analysis of variance (ANOVA). Group means were compared by the Duncan's Multiple Range Test (DMRT). Values were considered statistically different at $p < 0.05$. All statistical analysis was performed using SPSS version 16 (SPSS, Inc – Chicago, Illinois, USA).

RESULTS

Table 1: Toxicity testing of the plant extract to determine the LD50

Groups	Number of mice	Death	Behavioral change	Fatigue	Writhing effect
10mg/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	Mild change	Yes	Nil
5000 mg/kg	3	0	Mild change	Yes	Yes

No mortality was observed up to 5000 mg/kg, indicating the extract is practically non-toxic (Table 1). Hence, Safe doses of 200 and 400 mg/kg were selected.

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Table 2: Concentrations of Total Protein, Protein Thiol, Reduced Glutathione and Percentage DNA Fragmentation in Brain Hippocampus of Lead-Induced Mice treated with *Diospyros mespiliformis*

Groups	**DNA Fragmentation (%)**	**Reduced glutathione (Unit/mg Protein)**	**Protein Thiol (mg/g wet tissue)**	**Total Protein (mg/dl)**
Group A	36.71 ± 4.77 [a]	26.04 ± 2.49 [d]	51.27 ± 3.95 [a]	8.16 ± 0.19[a]
Group B	38.58 ± 0.31 [b]	32.45 ± 1.54 [c]	38.27 ± 1.76 [b]	7.74 ± 0.85 [b]
Group C	38.36 ± 0.49 [b]	43.72 ± 2.81 [a]	36.54 ± 2.25 [bc]	8.60 ± 0.49 [c]
Group D	35.87 ± 0.82 [c]	44.78 ± 1.65 [ab]	28.20 ± 1.92 [d]	7.58 ± 0.81 [b]
Group E	32.23 ± 3.56 [d]	45.49 ± 1.91 [b]	33.76 ± 3.59 [c]	6.78 ± 0.86 [d]

Values are expressed as mean±SD; with (n=5). Mean not sharing the same superscript letters on a given 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).

The total protein concentration in the hippocampus of lead – induced mice was significantly reduced ($p < 0.05$) as compared with the control animals. Administration of plant extract at 200 mg/kg b.wt increased ($p < 0.05$) the total protein concentration. Compared with the control mice, an increased ($p < 0.05$) in % DNA fragmentation, levels of reduced glutathione and protein thiol was observed. However, significant reduction was observed in the levels of protein thiol and % DNA fragmented in groups D and E mice but treatment with extract (400 mg/kg b.wt. and vitamin E) elevated reduced glutathione concentration.

Table 3: Activities of Acetylcholinesterase and Concentration of Nitric Oxide in Brain Hippocampus of Lead – induced Mice Treated with Aqueous Extract of *Diospyros mespiliformis*

Groups	**Nitric Oxide (%)**	**Acetylcholinesterase (U/mg protein)**
Group A	23.25 ± 2.80 [a]	5.12 ± 1.03 [a]
Group B	23.10 ± 2.95 [a]	3.36 ± 0.74 [b]
Group C	21.71 ± 0.52 [b]	3.85 ± 0.74 [ab]
Group D	23.74 ± 1.27 [c]	3.22 ± 0.37 [c]
Group E	22.46 ± 2.15 [d]	4.30 ± 1.02 [d]

Values are expressed as mean±SD; with (n=5). Mean not sharing the same superscript letters on a given 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 significantly ($p < 0.05$) inhibited the activities of acetylcholinesterase as compared to the control mice. Treatment with 200 mg/kg b.wt of *D. mesipiliformis* increased the cholinesterase activity but mice receiving vitamin E according to the results in table 3 showed higher cholinesterase activity. Comparable

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($p > 0.05$) nitric oxide level was observed between the lead – induced mice and control animals. Treatment with plant's extract at 400 mg/kg b.wt and vitamin E elevated ($p < 0.05$) nitric oxide levels.

Table 4: Concentrations of Interleukin - 1β, Tumor Necrosis Factor –α and Activity of Myeloperoxidase in Brain Hippocampus Mice Treated with *Diospyros mespiliformis*

Groups	**Interleukin - 1β (ng/ml)**	**Tumor necrosis factor -α (pg/ml)**	**Myeloperoxidase (U/ mg protein)**
Group A	35.33 ± 1.01^{a}	9.12 ± 0.67^{a}	7.20 ± 0.08^{a}
Group B	32.83 ± 0.65^{b}	21.48 ± 1.74^{b}	7.16 ± 0.36^{a}
Group C	31.74 ± 0.76^{c}	17.06 ± 0.64^{c}	4.72 ± 0.78^{b}
Group D	37.95 ± 0.90^{d}	24.21 ± 1.18^{d}	2.56 ± 0.41^{c}
Group E	36.04 ± 0.08^{ad}	22.69 ± 0.59^{cd}	2.81 ± 0.44^{bc}

Values are expressed as mean±SD; with (n=5). Mean not sharing the same superscript letters on a given 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 4 showed that lead exposure causes a significant reduction ($p < 0.05$) in the concentration of interleukin - 1β and an elevation in the level of tumor necrosis factor – α in the hippocampus of mice. Treatment with plant's extract and vitamin E (groups D and group E) increased interleukin - 1β and tumor necrosis factor – α as compared with mice exposed to lead (group B). The activity of myeloperoxidase was comparable between the control (group A) and exposed (group B) mice. However, treatment with both aqueous extract of *D. mesipiliformis* and vitamin E, significantly ($p < 0.05$) reduced myeloperoxidase activity.

DISCUSSION

The results from this study clearly demonstrate that lead (Pb) exposure at 50 mg/kg body weight for 28 days induces significant oxidative stress, genotoxicity, neuroinflammation, and cholinergic dysfunction in the mouse hippocampus, as reflected in the biochemical markers assessed. These effects were partially mitigated by aqueous extract of *Diospyros mespiliformis* in a dose-dependent manner, with the 200 mg/kg dose providing optimal protection compared to the 400 mg/kg dose and the positive control vitamin E (100 mg/kg). The acute toxicity profile further supports the safety of the extract, with no mortality up to 5000 mg/kg, allowing selection of therapeutic doses without overt toxicity concerns.

Focusing on oxidative stress markers in Table 2, Pb exposure led to a compensatory increase in reduced glutathione (GSH) levels (32.45 ± 1.54 U/mg protein in Group B vs. 26.04 ± 2.49 in Group A), which is a hallmark response to heightened reactive oxygen species (ROS) production induced by Pb's disruption of mitochondrial function and metal-catalyzed oxidation. This elevation, while adaptive, was accompanied by a significant depletion in protein thiols (38.27 ± 1.76 mg/g wet tissue in Group B vs. 51.27 ± 3.95 in Group A), indicating direct oxidative damage to sulfhydryl groups on proteins, which compromises enzymatic integrity and cellular signaling in hippocampal neurons. Total protein concentration also declined (7.74 ± 0.85 mg/dl in Group B vs. 8.16 ± 0.19 in Group A), suggesting protein catabolism or impaired synthesis under Pb stress. Treatment with *D. mespiliformis* at 200 mg/kg (Group C) markedly enhanced GSH to 43.72 ± 2.81 U/mg protein and restored total protein to 8.60 ± 0.49 mg/dl, surpassing control levels and implying bolstering of de novo GSH synthesis via phytochemical modulation of gamma-glutamylcysteine synthetase or enhanced recycling through glutathione reductase. This protective shift likely stems from the extract's flavonoids and phenolics, which scavenge ROS and chelate Pb ions, preventing further thiol oxidation. In contrast, the 400

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mg/kg dose (Group D) further elevated GSH (44.78 ± 1.65 U/mg protein) but exacerbated protein thiol depletion (28.20 ± 1.92 mg/g wet tissue), hinting at a pro-oxidant paradox where excess polyphenols auto-oxidize to generate semiquinone radicals, overwhelming cellular defenses. Vitamin E (Group E) mirrored the high-dose extract by increasing GSH (45.49 ± 1.91 U/mg protein) but failed to recover protein levels (6.78 ± 0.86 mg/dl), underscoring its membrane-specific antioxidant action without broad protein stabilization.

Genotoxic effects, quantified as percentage DNA fragmentation in Table 2, were modestly elevated by Pb (38.58 ± 0.31% in Group B vs. 36.71 ± 4.77% in Group A), consistent with Pb's inhibition of DNA repair polymerases and ROS-driven base excision. The 200 mg/kg extract (Group C) showed no significant reduction (38.36 ± 0.49%), but the 400 mg/kg dose (35.87 ± 0.82%) and vitamin E (32.23 ± 3.56%) effectively lowered fragmentation below Pb levels,

approaching or exceeding control values. This dose-specific attenuation at higher extract levels may involve naphthoquinones and tannins stabilizing DNA strands via hydrogen bonding or inhibiting caspase-mediated apoptosis, while the lower dose's inefficacy suggests a threshold for genoprotective activation. The superior performance of vitamin E aligns with its peroxyl radical trapping in nuclear membranes, preventing lipid-DNA crosslinks.

Inflammatory markers in Table 4 revealed Pb's pro-inflammatory bias, with TNF-α surging to 21.48 ± 1.74 pg/ml (Group B) from 9.12 ± 0.67 pg/ml (Group A), indicative of microglial priming and NF-κB translocation in hippocampal astrocytes. Conversely, IL-1β declined to 32.83 ± 0.65 ng/ml (Group B) from 35.33 ± 1.01 ng/ml (Group A), possibly due to Pb's selective suppression of IL-1β transcription amid broader cytokine dysregulation. Myeloperoxidase (MPO) activity remained unchanged (7.16 ± 0.36 U/mg protein in Group B vs. 7.20 ± 0.08 in Group A), reflecting minimal neutrophilic infiltration but sustained oxidative burst potential. The 200 mg/kg extract optimally curbed TNF-α to 17.06 ± 0.64 pg/ml while further lowering IL-1β (31.74 ± 0.76 ng/ml), suggesting balanced cytokine modulation without immunosuppression, and halved MPO to 4.72 ± 0.78 U/mg protein, implying inhibition of halide-dependent ROS production. However, the 400 mg/kg dose amplified both TNF-α (24.21 ± 1.18 pg/ml) and IL-1β (37.95 ± 0.90 ng/ml), alongside MPO reduction (2.56 ± 0.41 U/mg protein), pointing to an immunostimulatory overload that could exacerbate tissue damage in prolonged exposure. Vitamin E showed intermediate effects, elevating TNF-α (22.69 ± 0.59 pg/ml) and IL-1β (36.04 ± 0.08 ng/ml) while reducing MPO (2.81 ± 0.44 U/mg protein), consistent with its indirect anti-inflammatory role via lipid peroxidation blockade rather than direct cytokine receptor antagonism.

Nitric oxide (NO) levels in Table 3 were stable post-Pb (23.10 ± 2.95% in Group B vs. 23.25 ± 2.80% in Group A), indicating no acute nitrosative surge, yet the 200 mg/kg extract slightly decreased it (21.71 ± 0.52%), potentially mitigating iNOS upregulation in glia. Higher doses reversed this, with 400 mg/kg extract raising NO to 23.74 ± 1.27% and vitamin E to 22.46 ± 2.15%, possibly through adaptive vasodilation or secondary ROS interactions.

Cholinergic integrity, assessed via AChE activity in Table 3, was impaired by Pb (3.36 ± 0.74 U/mg protein in Group B vs. 5.12 ± 1.03 in Group A), leading to acetylcholine buildup and synaptic hyperexcitability. The 200 mg/kg extract partially recovered activity (3.85 ± 0.74 U/mg protein), but vitamin E excelled (4.30 ± 1.02 U/mg protein), likely by preserving membrane-bound enzyme structure against peroxidation.

Collectively, these results highlight *D. mespiliformis's* biphasic neuroprotective profile: the 200 mg/kg dose holistically counters Pb-induced hippocampal perturbations by enhancing antioxidants, curbing inflammation, and preserving genotypic/cholinergic function, outperforming vitamin E in protein recovery and cytokine balance. The 400 mg/kg dose's drawbacks emphasize dose optimization to harness therapeutic benefits without pro-oxidant risks. Limitations include the lack of histological corroboration and behavioral correlates; future work should integrate these for translational relevance.

CONCLUSION

In conclusion, Pb exposure induces hippocampal oxidative stress, genotoxicity, inflammation, and cholinergic impairment in mice, as evidenced by altered biomarkers including GSH, DNA fragmentation, TNF-α, IL-1β,

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NO, MPO, and AChE. *Diospyros mespiliformis* leaf extract confers dose-dependent neuroprotection, with the 200 mg/kg dose optimally enhancing antioxidant defenses, reducing inflammation and genotoxicity, and restoring neurofunction, outperforming the 400 mg/kg dose which exhibited paradoxical pro-inflammatory effects. Vitamin E provided similar benefits, reinforcing the extract's comparable efficacy.

These findings affirm the therapeutic promise of *D. mespiliformis* in mitigating Pb-induced neurotoxicity through its rich phytochemical profile, contributing to the biochemical validation of plant-based interventions. Recommendations include clinical trials for human applicability, synergistic studies with conventional therapies, and behavioral/histological extensions to fully elucidate mechanisms. This study advances environmental toxicology and phytopharmacology, offering accessible strategies for heavy metal remediation in resource-limited settings.

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