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Original Reserach Article

Evaluation of Apoptotic, Inflammatory and Oxidative Markers in the Brain Striatum of Mice Induced by Lead Toxicity and Treated with *Diospyros mespiliformis*

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Abstract: Lead (Pb) is a pervasive environmental neurotoxicant that causes neurodegeneration through oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis. This study evaluated apoptotic, inflammatory and oxidative biomarkers in the brain striatum of mice exposed to Pb and treated with aqueous extract of *Diospyros mespiliformis*. Twenty-five male mice were divided into five groups. All groups, except the control (Group 1) received Pb (50 mg/kg) and were subsequently treated with *D. mespiliformis* extract (200 mg/kg b.wt (Group 3), 400 mg/kg (Group 4) or vitamin E (100 mg/kg, Group 5), while Group 2 mice received 50mg/kg b.wt of Pb only. At the end of 28 days exposure and treatment, brain striatum was obtained, homogenized and used for biochemical assays which includes Caspase -3, DNA fragmentation, Tumor necrosis factor – α, myeloperoxidase, total protein, reduced glutathione and protein thiol in the laboratory following standard operating methods. Results indicated that exposure to Pb causes significant decreased ($p < 0.05$) in levels of caspase – 3, nitric oxide and percentage DNA fragmentation and an elevation ($p < 0.05$) in levels of TNF – α, reduced glutathione, protein thiol and activity of myeloperoxidase. Treatment with aqueous extract of *D. mesipiliformis* elevated the levels of caspase – 3 and reduced the levels of protein thiol reduced glutathione and myeloperoxidase activity. Together, these findings suggest that *D. mespiliformis* exerts neuroprotective effects which is comparable to vitamin E a potent antioxidant through anti-oxidant, anti-inflammatory and anti-apoptotic effects. *D. mesipiliformis* may also have potential as a natural intervention for heavy metal–induced neurodegeneration.

Keywords: Lead, *Diospyros Mespiliformis*, Caspase - 3, Striatum, Tumor Necrosis Factor - α.

INTRODUCTION

Lead (Pb) is a heavy metal with no known physiological function in biological systems, yet it remains one of the most pervasive environmental neurotoxicants due to its widespread industrial use and persistence in the environment. Human exposure occurs through contaminated air, soil, water, and food, particularly in developing countries where regulation is often inadequate. Lead readily crosses the blood-brain barrier, accumulating preferentially in the brain regions such as the striatum, hippocampus, and cerebral cortex, causing detrimental effects on neuronal integrity and cognitive function (Flora *et al.*, 2012; Sanders *et al.*, 2009).

The striatum, a subcortical part of the forebrain, plays a critical role in motor coordination and various aspects of cognitive function. Lead-induced neurotoxicity in this region is often characterized by elevated oxidative stress, inflammation, and apoptosis (Lidsky & Schneider, 2003). Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, leading to lipid peroxidation, DNA damage, and protein oxidation (Patra *et al*., 2011). Key oxidative biomarkers,

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such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), have been used to assess the extent of oxidative damage and the protective capacity of therapeutic agents (Patrick, 2006).

In addition to oxidative damage, lead exposure can induce apoptotic pathways in neurons, primarily via mitochondrial dysfunction and caspase activation. The upregulation of apoptotic markers such as caspase-3, Bax, and the downregulation of anti-apoptotic protein Bcl-2 further confirm neuronal death and neurodegeneration (Sanders *et al*., 2009). Therefore, evaluating both oxidative and apoptotic markers offers a holistic understanding of the pathophysiological processes involved in lead-induced striatal neurotoxicity.

In recent years, the use of medicinal plants as therapeutic agents against heavy metal-induced toxicity has gained significant attention due to their antioxidant, anti-inflammatory, and neuroprotective properties. *Diospyros mespiliformis*, a medicinal plant widely distributed in Africa, is traditionally used to treat various ailments including fever, infections, and inflammatory conditions. Phytochemical analyses of *Diospyros mespiliformis* have revealed the presence of flavonoids, tannins, alkaloids, and saponins, which are known for their potent antioxidant and anti-apoptotic properties (Akinmoladun *et al*., 2010; Adebayo-Tayo *et al*., 2017). These constituents are known to scavenge free radicals, stabilize membranes, and modulate apoptotic signaling pathways, thereby protecting neural tissues from oxidative and apoptotic damage. Therefore, this study aimed to evaluate the apoptotic, inflammatory and oxidative biomarkers in the brain striatum of mice induced by lead toxicity and treated with aqueous extract of *Diospyros mespiliformis.*

MATERIALS AND METHODS

Collection of Plant Material

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

Preparation of *Diospyros mespiliformis Extract*

The dried seeds of *D. mespiliformis* were thoroughly cleaned to remove debris such as dust particles, plant debris, and microbial contaminants. 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 then concentrated using a rotary evaporator under reduced pressure at 40 °C. 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

Twenty-five mice were divided into five groups (n = 5) per group.
Group 1: Control - received distilled water only
Group 2: Pb only - received Pb (50 mg/kg b.wt)
Group 3: Pb +200 mg/kg b.wt of *D. mespiliformis*
Group 4: Pb + 400 mg/kg b. wt of *D. mespiliformis*
Group 5: Pb + Vitamin E (100 mg/kg)

Preparation of Brain Tissue Supernatant for Biochemical Assay

Exposure and administration of plant extract lasted for 28 days. At the end of the exposure time, mice were made to fast over-night, anaesthetized with chloroform and brain striatum was removed, 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 Analyses

The total protein concentration in the supernatant stratum was determined by the method of Doumas *et al.* (1981). Tissue reduced glutathione by the procedure of Ellman (1959). The protein thiols in the supernatant stratum were determined using the method of Sedlack and Lindsey (1968). The method of Bradley *et al.,* (1982) was employed for the determination of myeloperoxidase and Wu *et al*., (2005) for DNA fragmentation assay. Tumor necrosis factor – α and Caspase – 3 were evaluated by the methods of Engelmann *et al.,* (1990) and Liu *et al*., (2002) respectively.

Statistical Analysis

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

RESULTS

Table 1: Acute oral toxicity of Aqueous Extract of *Diospyros mespiliformis*

Groups	Number of rats	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

Acute oral toxicity testing of *Diospyros mespiliformis* seed extract showed no mortality at doses up to 5000 mg/kg; mild behavioral changes and fatigue were observed at the highest doses. Acute toxicity (LD_{50}) no deaths recorded at any dose (10–5000 mg/kg); LD_{50} > 5000 mg/kg.

Table 2: Percentage DNA Fragmentation, Nitric Oxide and Concentration of Caspase -3 in Brain Striatum of Lead – induced Mice Treated with Aqueous Extract of *Diospyros mespiliformis*

Groups	DNA Fragmentation (%)	Nitric oxide (%)	Caspase-3 (mM/g protein)
Group 1	37.63±1.20^{a}	24.72±2.12a	231.22±2.15^{a}
Group 2	35.37±2.93^{b}	22.61±2.94ab	106.01±2.46^{d}
Group 3	37.37±1.73^{a}	21.89±2.51b	178.15±3.55^{b}
Group 4	35.46±2.17^{b}	22.93±3.64ab	172.87±1.43^{b}
Group 5	36.34±1.64^{ab}	21.90±1.04b	129.26±1.34^{c}

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 1 = Control; Group 2 = Pb (50 mg/kg b.wt.); Group 3 = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group 4 = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group 5 = (Pb + 100 mg/kg b. wt of vitamin E).

In table 2, Lead exposure caused a marked decrease ($p < 0.05$) in percentage DNA fragmentation in the striatum. Administration of *D. mesipiliformis* at 200 mg/kg b.wt however elevated fragmented DNA level.as compared with the control mice. The concentration of nitric oxide was comparable ($p > 0.05$) and caspase – 3 significantly reduced ($p < 0.05$) in lead exposed mice as compared with the control (group 1) mice. Treatment with plant extract and vitamin E increased ($p < 0.05$) striatum caspase – 3 level but did not affect ($p > 0.05$) nitric oxide concentration.

Table 3: Concentrations of Total Protein, Reduced Glutathione and Protein Thiol in Brain Striatum of Lead – induced Mice Treated with Aqueous Extract of *Diospyros mespiliformis*

Groups	Total protein (mg/dl)	Reduced glutathione (Unit/mg Protein)	Protein thiol (mg/g wet tissue)
Group 1	8.18±1.01^{c}	44.82±2.55^{b}	25.03±4.04^{c}
Group 2	12.28±1.63^{a}	53.63±2.83^{a}	32.18±3.71^{b}
Group 3	10.95±1.14^{ab}	28.85±2.94^{c}	33.14±3.36^{b}
Group 4	10.48±0.94^{b}	46.83±2.35^{b}	36.04±3.24^{a}
Group 5	11.16±1.21^{ab}	45.24±1.94^{b}	27.67±0.93^{c}

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 1 = Control; Group 2 = Pb (50 mg/kg b.wt.); Group 3 = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group 4 = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group 5 = (Pb + 100 mg/kg b. wt of vitamin E).

Results in table 3 indicated that induction of lead (Pb) caused an increased ($p < 0.05$) in the concentrations of total protein, reduced glutathione and protein thiol in brain striatum of mice as compared with the control mice (group 1). Administration of aqueous extract of *D. mesipiliformis* according to results in table 3, showed that level of protein thiol markedly increased ($p < 0.05$) in animals receiving 400 mg/kg b.wt (group 4) as compared to control mice. However, reduced glutathione and total protein were decreased. Mice receiving vitamin E (group 5) had comparable ($p > 0.05$) reduced glutathione and protein thiol levels with the control animals (group 1).

Table 4: Activity of Myeloperoxidase and Tumor Necrosis Factor – α in the Brain Striatum of Lead – induced Mice Treated with Aqueous Extract of *D. mesipiliformis*

Groups	Myeloperoxidase (unit/mg protein)	Tumor Necrosis factor-α (pg/ml)
Group 1	4.23 ± 0.34^{a}	13.28 ± 0.93^{a}
Group 2	4.90 ± 0.53^{b}	28.57 ± 0.99^{b}
Group 3	3.74 ± 0.43^{c}	23.60 ± 0.73^{c}
Group 4	3.60 ± 0.37^{c}	30.57 ± 0.56^{d}
Group 5	3.87 ± 0.49^{c}	19.16 ± 1.15^{c}

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 1 = Control; Group 2 = Pb (50 mg/kg b.wt.); Group 3 = (Pb + 200 mg/kg b.wt of D. mesipiliformis extract); Group 4 = (Pb + 400 mg/kg b. wt of D. mesipiliformis extract); Group 5 = (Pb + 100 mg/kg b. wt of vitamin E).

Table 4 shows that Pb exposure significantly elevated myeloperoxidase activity and levels of Tumor necrosis factor - α compared to control. Treatment with *D. mespiliformis* and vitamin E significantly ($p < 0.0$) reduces TNF – α in mice administered with the 200 mg/kg b.wt. All three treated groups showed lower ($p < 0.05$) activity of myeloperoxidase.

DISCUSSION

This study shows that *Diospyros mespiliformis* extract protects the brain striatum from lead (Pb)-induced neurotoxicity by restoring antioxidant balance, maintaining mitochondrial function, and reducing inflammation. Pb exposure elevated reduced glutathione (GSH), total protein, and protein thiols, indicating oxidative stress and a compensatory defense response. Pb generates reactive oxygen species (ROS) by impairing mitochondrial enzymes and depleting endogenous antioxidants, leading to lipid and protein oxidation (Flora *et al*., 2012; Valko *et al*., 2007).

Treatment with *D. mespiliformis* normalized GSH and protein thiols, showing strong antioxidant effects likely due to its flavonoids and phenolic compounds, which scavenge ROS and may chelate Pb ions (Akinmoladun et al., 2010). Pb also suppressed caspase-3 activity, suggesting impaired apoptosis and a shift toward necrosis, which promotes uncontrolled neuronal damage and inflammation (Garza *et al*., 2006). The extract restored caspase-3 activity, indicating preservation of mitochondrial integrity and controlled cell death. Additionally, Pb-induced increases in TNF-α and MPO confirmed a strong inflammatory response. *D. mespiliformis* significantly reduced these markers, demonstrating anti-inflammatory properties comparable to vitamin E, but with broader effects due to its multiple bioactive compounds (Adebayo-Tayo *et al*., 2017).

CONCLUSION

Diospyros mespiliformis extract significantly protects the brain striatum against Pb-induced neurotoxicity by reducing oxidative stress, restoring apoptosis, and suppressing inflammation. Its effects were comparable to or greater than vitamin E, highlighting its therapeutic potential as a natural neuroprotective agent. These findings support further research into its active compounds and clinical applications for heavy metal-induced neurodegenerative conditions.

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