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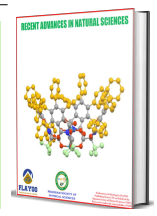

The effects of methanolic extract of *Syzygium guineense* (Wild.) D.C. on lead-induced neurotoxicity on the prefrontal cortex of Wistar rats

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ABSTRACT

Antimicrobial and wound-healing activities of *Syzygium guineense* are well documented, but its use in lead-induced neurotoxicity remains less explored. This study examined the effects of *S. guineense* leaf (SGL) extract on lead-induced prefrontal cortex (PFC) damage in Wistar rats. Twenty-five rats (60–80 g) were assigned to five groups ($n = 5$): control; lead (5 mg/kg); and lead + SGL extract at 100, 250, or 400 mg/kg. Behavior was assessed, and the PFC was excised for hematoxylin and eosin (H&E) and Cresyl Fast Violet (CFV) staining and for measurement of malondialdehyde (MDA), glucose-6-phosphate dehydrogenase (G6PDH), and lactate dehydrogenase (LDH) after two weeks of treatment. PFC MDA was significantly higher ($P < 0.05$) in the lead-only group than in the SGL-treated groups and control, and G6PDH differed significantly ($P < 0.05$) between the lead-only and SGL-treated groups. The lead-only and 100 mg/kg SGL groups showed vacuolation and neuronal cells. Cognitive function, PFC histoarchitecture, and Nissl staining improved in the 250 and 400 mg/kg SGL groups. These findings suggest that higher doses of SGL extract can potentially ameliorate lead-induced PFC damage.

1. INTRODUCTION

Lead (Pb) is a ubiquitous, soft, grey-blue, highly toxic heavy metal that causes poisoning in humans and domestic animals, with deleterious effects on various organs of the body [1]. Exposure to Pb through the gastrointestinal, integumentary, and respiratory systems generates free-radical-induced oxidative stress, which, in turn, elicits inflammatory responses [1]. These re-

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sponses lead to derangement of the antioxidant defense system and alterations in body functions [2]. Several animal and human studies have reported hematological [3] and neurological adverse effects [4], as well as alterations in the antioxidant defense system [5].

Lead induces neurological effects via apoptosis, excitotoxicity, and oxidative stress, which damage cellular components of the nervous system, including neurotransmitter storage and release processes [6]. However, Pb-induced neurological effects such as cognitive impairment, behavioral disorders, learning defects, and decreased intelligence quotient (IQ) are more common in children than in adults [7]. This has been attributed to higher absorption and ingestion rates in children than in adults and may help explain the susceptibility of developing brains to toxicants [7, 8].

Within the brain, Pb-induced neurotoxicity is common in areas involved in higher functions such as reasoning, cognitive abilities, behavior, and decision-making, activities largely associated with the prefrontal cortex [9]. The prefrontal cortex (PFC) is the anterior part of the frontal lobe of the brain [10] and has been implicated in planning complex and higher-order cognitive behavior, personality expression, decision-making, and moderation of social behavior [11].

Complementary and alternative medicines involving herbs and medicinal plants are receiving considerable attention for the management of various ailments [12]. The efficacy of many medicinal plants used in such therapies has been established, and almost 80% of the global population depends on alternative therapy [13]. Among medicinal plants employed in alternative medicine is *Syzygium guineense* (Myrtaceae), which has been widely used to treat infections, diabetes, hypertension, amenorrhoea, diarrhea, rheumatism, and mental disorders [14].

Syzygium guineense (Myrtaceae) is a flowering tree native to sub-Saharan and Southern Africa [15]. In Mali, Senegal, and Sierra Leone, a decoction of its leaves has been used in traditional medicine to treat wounds, ulcers, diarrhea, rheumatism, and infections [15]. It also has reported antivenom, antihypertensive, and antihyperglycaemic effects [16]. In the Benin Republic, *S. guineense* is used for the treatment of mental disorders and amenorrhoea [17]. These diverse therapeutic uses and effects have been attributed to documented flavonoids with antioxidant, immunomodulatory, anti-inflammatory, antibacterial, and antihypertensive activities [14]. Although the antibacterial, antidiabetic, and wound-healing activities of this medicinal plant have been extensively documented, there is a paucity of research on its use in managing lead-induced neurotoxicity, and its presumed neuroprotective effects remain unexplored. Hence, this study investigated the antioxidant and neuroprotective effects of *S. guineense* in the PFC of Wistar rats exposed to lead.

2. MATERIALS AND METHODS

2.1. PLANT SOURCE

Fresh *Syzygium guineense* leaves were collected from Chaza, Niger State, Nigeria, and authenticated at the Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), with voucher number NIPRD/H/6585.

Table 1. Phytochemical analysis of the powdered sample and methanolic extract of *Syzygium guineense* leaf.

Compound	Powdered sample	Plant extract
Tannins	+	+
Glycosides	–	–
Saponins	+	+
Terpenes	–	–
Sterols	–	–
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Resins	+	+
Carbohydrates	+	+

Note: +, present; –, absent.

2.1.1. Preparation of plant extract

Dried leaves of *S. guineense* were pulverized into a fine powder using a grinding machine. A 400-g portion of the powdered sample was used for extraction by maceration. The sample was soaked in 2.4 L of methanol for 48 h with constant shaking using an orbital shaker. The mixture was then filtered through Whatman filter paper.

The solvent from the total extract was distilled off, and the filtrate was evaporated and concentrated to a paste using a rotary evaporator. The percentage yield of the extract was 27.5%, and the weight yield was 110 g. A 5-g portion of the methanolic plant extract was dissolved in 125 mL of water, yielding a concentration of 40 mg/mL.

2.2. ANIMALS

Twenty-five male Wistar rats weighing 60–80 g were obtained from a Veterinary Institute in Plateau State, Nigeria. The use of these rats for research was approved by the Bingham University Animal House in the Department of Anatomy in accordance with institutional ethics approval (AREC/UB/038/23). The rats were acclimatized for 2 weeks and had access to animal feed and clean water supplied by the University Animal House. They were maintained under standard conditions at room temperature (25–27 °C) and a 12-h light–dark cycle.

2.3. EXPERIMENTAL DESIGN: GROUPING AND DOSING

Twenty-five Wistar rats weighing 60–80 g were randomly assigned to five groups, with five rats per group:

- Group 1 (control): 0.5 mL of distilled water daily.
- Group 2: 5 mg/kg body weight of lead acetate only.
- Group 3: 5 mg/kg body weight of lead acetate and 100 mg/kg body weight of extract (low dose, LD).
- Group 4: 5 mg/kg body weight of lead acetate and 250 mg/kg body weight of extract (middle dose, MD).
- Group 5: 5 mg/kg body weight of lead acetate and 400 mg/kg body weight of extract (high dose, HD).

Table 2. Body weight of Wistar rats before and after the experiment.

Group	Initial mean weight (g), mean ± SEM	Final mean weight (g), mean ± SEM
1 (Control)	70 ± 5.41	106 ± 6.42
2 (Lead only)	74 ± 2.48	$90 \pm 4.07^{*}$
3 (Lead + LD)	74 ± 4.94	$98 \pm 6.04^{*}$
4 (Lead + MD)	68 ± 5.95	$86 \pm 6.42^{*}$
5 (Lead + HD)	74 ± 2.48	$90 \pm 4.35^{*}$

SEM, standard error of the mean; LD, low dose of extract (100 mg/kg body weight); MD, middle dose of extract (250 mg/kg body weight); HD, high dose of extract (400 mg/kg body weight). *Significantly different from the control group at $P < 0.05$.

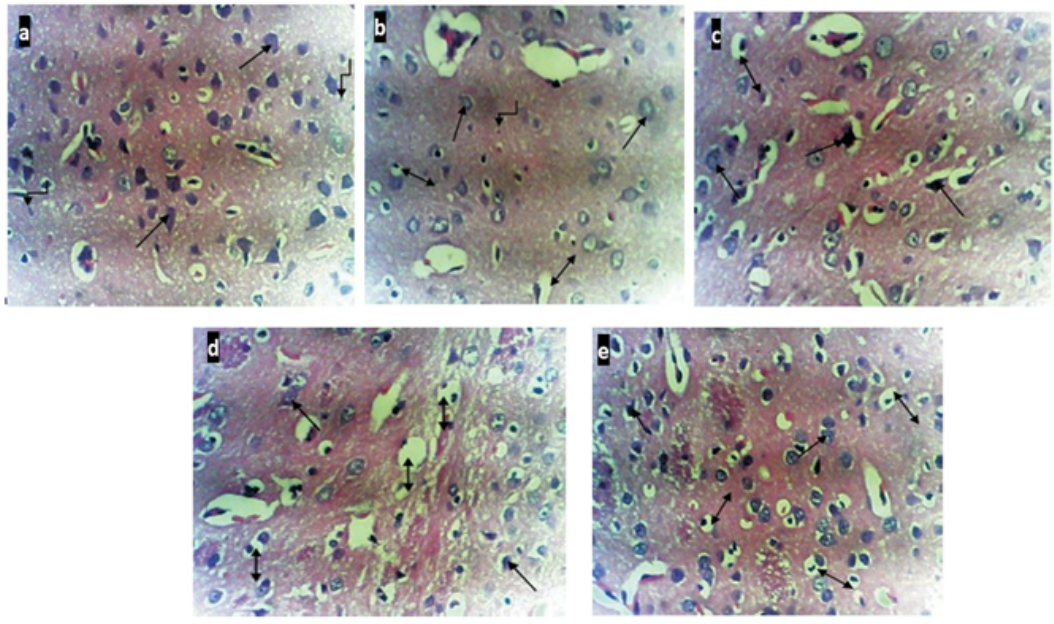

Figure 1. Photomicrographs of prefrontal cortex sections stained with hematoxylin and eosin (H&E): (a) control group; (b) lead-only group; (c) lead + 100 mg/kg extract group; (d) lead + 250 mg/kg extract group; and (e) lead + 400 mg/kg extract group. Single-ended arrows indicate pyramidal neurons; double-ended arrows indicate vacuolations; zig-zag arrows indicate glial cells. Magnification: $\times 100$**.**

Table 3. Effects of leaf extract on spontaneous alternation performance.

Group	Spontaneous alternation performance, mean ± SEM
1 (Control)	80 ± 1.30
2 (Lead only)	$56 \pm 2.43^{*}$
3 (Lead + LD)	59 ± 2.14
4 (Lead + MD)	$68 \pm 2.26^{**}$
5 (Lead + HD)	$75 \pm 1.60^{**}$

SEM, standard error of the mean; LD, low dose of extract (100 mg/kg body weight); MD, middle dose of extract (250 mg/kg body weight); HD, high dose of extract (400 mg/kg body weight). *Significantly different from the control group at $P < 0.05$. **Significantly different from the lead-only group at $P < 0.05$.

2.3.1. Route of administration, schedule, and volume

Neurotoxicity was induced by oral administration of lead acetate for 28 days, following the methodology described in Ref. [18]. Subsequently, the extract was administered orally once daily for two weeks, with the dose volume adjusted according to the body weight of each animal.

2.4. BEHAVIORAL STUDIES FOR COGNITIVE FUNCTIONS

Working memory and exploratory behavior were assessed using the Y-maze test, which measured spontaneous alternation performance. The Y-maze apparatus consisted of three arms labeled A, B, and C, each measuring 35 cm in length, 15 cm in height, and 10 cm in width and interconnected at 120-degree angles. Each rat was placed in the center of the apparatus and allowed to move freely for 5 min, during which the number of entries into each arm was recorded using a video camera. Independent observers documented the data from the computer system, counting one entry each time the entire body of the rat entered a specific arm. Spontaneous alternation performance (SAP) was analyzed according to the method described by Typlt *et al.* [19]. A successful alternation occurred when an animal visited three different arms (A, B, and C) in succession.

$$\text{SAP}\,(\%) = \frac{\text{Number of alternations} \times 100}{\text{Total arm entries} - 2}. \quad (1)$$

The number of alternations was the number of sequences in which the animal entered all three arms (A, B, and C) in succes-

Table 4. LDH, G6PDH, and MDA levels in the experimental groups (mean ± SEM).

Group	LDH (IU/L)	G6PDH (IU/L)	MDA (μmol/L)
1 (Control)	1326.0 ± 35.13	1092.0 ± 77.22	30.5 ± 0.31
2 (Lead only)	1321.0 ± 25.61	$1013.5 \pm 25.62^{*}$	$39.0 \pm 2.24^{*}$
3 (Lead + LD)	1314.5 ± 3.78	$1177.0 \pm 1.83^{**}$	$30.5 \pm 1.12^{**}$
4 (Lead + MD)	1323.5 ± 4.09	$1124.0 \pm 24.50^{**}$	33.5 ± 0.31
5 (Lead + HD)	1320.5 ± 25.21	$1186.0 \pm 16.94^{**}$	$28.5 \pm 2.35^{**}$

SEM, standard error of the mean; LDH, lactate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; MDA, malondialdehyde; LD, low dose of extract (100 mg/kg body weight); MD, middle dose of extract (250 mg/kg body weight); HD, high dose of extract (400 mg/kg body weight). *Significantly different from the control group at $P < 0.05$. **Significantly different from the lead-only group at $P < 0.05$.

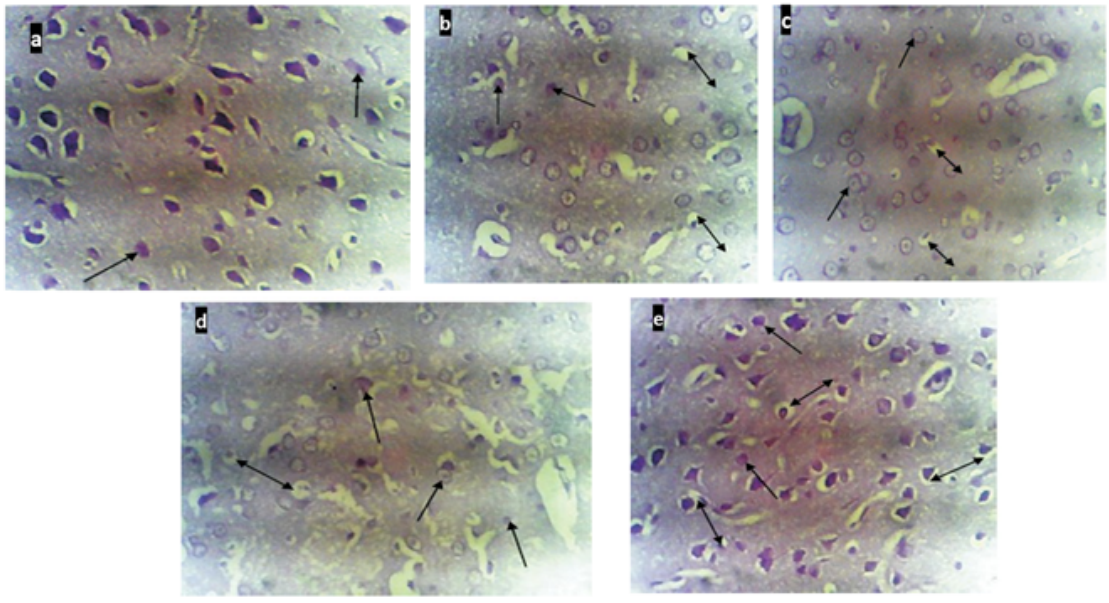

Figure 2. Photomicrographs of prefrontal cortex sections stained with Nissl stain: (a) control group; (b) lead-only group; (c) lead + 100 mg/kg extract group; (d) lead + 250 mg/kg extract group; and (e) lead + 400 mg/kg extract group. Single-ended arrows indicate pyramidal neurons; double-ended arrows indicate vacuolations. Magnification: $\times 100$**.**

sion. Total arm entries was the absolute number of entries into any arm regardless of sequence. Subtracting two accounts for the fact that the first two arm entries of a session cannot form a valid three-arm sequence.

2.5. EUTHANASIA PROCEDURE AND BRAIN HISTOLOGICAL STUDY

Twenty-four hours after the last administration, the experimental animals were euthanized using isoflurane in a closed container. The animals were observed until breathing ceased and were humanely euthanized according to UB-AREC-approved guidelines. Tissue samples were collected for biochemical analyses.

Histological analysis of the PFC was performed using H&E and Cresyl Fast Violet (CFV; Nissl) stains. Sections of the PFC were obtained for histological assessment following lead exposure and treatment with the plant extract. Brain tissues were processed according to standard light-microscopy protocols. Serial 3-μm sections were cut using a rotary microtome (Leica 1900) and stained with H&E and CFV. The slides were mounted in DPX. Photomicrographs were taken at $\times 100$ magnification.

2.6. TISSUE PROCESSING FOR BIOCHEMICAL STUDIES

Sections of the PFC were homogenized with 5% sucrose solution using a tissue homogenizer. The tissue homogenates were assayed as described below.

2.6.1. Malondialdehyde

Malondialdehyde (MDA) levels in tissues were measured according to the protocol outlined by Stocks and Dormandy [20].

2.6.2. Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase (G6PDH) activity in the homogenate was measured using the method of Adem and Ciftci [21].

2.6.3. Lactate dehydrogenase

Lactate dehydrogenase (LDH) activity in the homogenate was measured according to the method of Shahriari *et al.* [22].

2.7. PHYTOCHEMICAL ANALYSIS

2.7.1. Tests for tannins, alkaloids, flavonoids, saponins, glycosides, and carbohydrates

Tests for these phytochemical constituents were carried out using methods described by Balamurugan *et al.* [23].

2.7.2. Test for phenols

The test for phenols was carried out according to the method of Yadav and Agarwala [24].

2.7.3. Tests for terpenes and sterols

The tests for terpenes and sterols were carried out using methods described by Balamurugan *et al.* [23].

2.7.4. Test for resins

The test for resins was carried out using the method described by Santhi and Sengottuvel [25].

2.8. STATISTICAL ANALYSIS

Data were statistically evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test in SPSS version 17.0 (SPSS Inc., Chicago, USA), and are expressed as mean ± standard error of the mean (SEM). A value of $P < 0.05$ was considered statistically significant. Pairwise comparisons comprised group 1 vs group 2; group 2 vs groups 3, 4, and 5; group 3 vs groups 4 and 5; and group 4 vs group 5.

3. RESULTS

3.1. PHYTOCHEMICAL ANALYSIS

Table 1 presents the phytochemical analysis of the powdered sample and methanolic extract of *S. guineense* leaf. The results show the active components detected in the powdered sample and plant extract. No difference was observed in the detected components before and after methanol extraction, indicating that the extraction solvent did not alter the qualitative phytochemical profile assessed in this study.

3.2. EFFECTS OF LEAD AND *SYZYGIUM GUINEENSE* LEAF EXTRACT ON BODY WEIGHT

There was no statistically significant difference in body weight between the SGL-treated groups and the lead-only group. However, all treated groups showed a significant reduction in final body weight compared with the control group (Table 2).

3.3. EFFECTS OF LEAD AND *SYZYGIUM GUINEENSE* LEAF EXTRACT ON SPONTANEOUS ALTERNATION PERFORMANCE

Table 3 shows a significant decrease in spontaneous alternation performance in group 2 (lead only) compared with the control group (group 1). Notably, spontaneous alternation performance was significantly higher in groups 4 (lead + MD) and 5 (lead + HD) than in group 2 (lead only).

3.4. EFFECTS OF LEAD AND *SYZYGIUM GUINEENSE* LEAF EXTRACT ON BIOMARKERS

Compared with the control, the lead-only group showed a significant increase in MDA and a significant decrease in G6PDH. MDA was significantly lower in the low- and high-dose SGL groups than in the lead-only group, whereas the reduction in the middle-dose group was not statistically significant. G6PDH was significantly higher in all SGL-treated groups than in the lead-only group. There were no significant differences in LDH levels among the groups (Table 4).

3.5. HISTOLOGICAL STUDIES

3.5.1. H&E staining of pyramidal neurons

Figure 1(b) reveals cytoplasmic vacuolations and degenerating pyramidal neurons in the lead-treated group compared with the control group (Figure 1(a)). These findings show that lead acetate caused histomorphological distortions in neuronal cells of the PFC. The distortions were not ameliorated in group 3 treated with 100 mg/kg (low dose) of SGL (Figure 1(c)), which showed some vacuolated pyramidal neuronal cells. However, group 4 (lead + 250 mg/kg extract) and group 5 (lead + 400 mg/kg extract) showed fewer vacuolated pyramidal cells and more normal neuronal cells.

3.5.2. Nissl staining of pyramidal neurons

The Nissl-stained sections of the PFC are shown in Figure 2. The control group stained more densely, revealing the Nissl bodies of pyramidal neurons, than group 2 (lead only), which stained poorly. Pale-staining pyramidal PFC sections were observed in group 3 (lead + 100 mg/kg extract) and group 4 (lead + 250 mg/kg extract). The PFC section in group 5 (lead + 400 mg/kg extract) showed densely stained pyramidal neurons similar to those in the control group.

4. DISCUSSION

Lead is highly toxic and has been documented to disrupt neurological, biochemical, physiological, and cognitive functions [26]. Lipid peroxidation is a well-known mechanism of cellular damage in plants and animals and serves as an indicator of oxidative stress in cells and tissues. Lipid peroxidation of polyunsaturated fatty acids generates unstable products that break down to form a complex array of compounds, including reactive carbonyl compounds, of which malondialdehyde (MDA) is among the most abundant. Consequently, MDA is commonly measured as an indicator of lipid peroxidation [27] and as an index of the degree of oxidative damage to cellular structures [27]. The present study showed that the lead-only group had elevated MDA levels compared with the control group.

An increase in MDA levels indicates ongoing lipid peroxidation resulting from excessive free-radical production. The effect of lead on brain tissue observed in this study is consistent with previous reports [28, 29].

Increased oxidative stress in the PFC has also been associated with cognitive impairment [30]. Cognitive function was assessed using a spontaneous alternation performance test, and poorer performance was observed in the lead-only group than in the treated groups. This finding is consistent with the elevated PFC MDA level and associated cognitive deficits observed in these animals. *Syzygium guineense* reduced the elevated MDA levels at all doses tested, but the reduction relative to the lead-only group was significant only at the low (100 mg/kg) and high (400 mg/kg) doses. At the middle dose, a lower mean MDA value was observed, although it was not significantly different from the lead-only group

or the control group. These findings suggest that the extract alleviated oxidative tissue injury caused by lead in the PFC. The high dose of the extract had a positive effect on oxidative stress, significantly reducing the MDA level in group 5 compared with the lead-only group. Similarly, the group treated with the high dose of the extract showed a significant improvement in cognitive function. This finding is consistent with the study by Feudjio *et al.* [31], which reported an acute median lethal dose (LD_{50}) exceeding 2000 mg/kg body weight for *S. guineense* bark extract and assessed its safety in acute and subchronic toxicity studies in rats.

LDH levels in all groups treated with *S. guineense* were similar to those in the lead-only group, with no significant differences among the groups; however, the mean LDH level was slightly lower in the high-dose SGL group. This indicates that *S. guineense* extract had little or no effect on LDH levels in the PFC under the conditions of this study. The slightly lower LDH level observed in the high-dose group may have been associated with the attenuation of lead-induced PFC tissue damage observed in this study. Studies have reported an association between high LDH levels and tissue or organ damage [32].

A decrease in glucose-6-phosphate dehydrogenase (G6PDH) level was observed in the lead-only group compared with the control group. This decrease could suggest suppression of antioxidant G6PDH activity. Lead has also been shown to suppress blood levels of antioxidant enzymes. G6PDH levels in group 3 (lead + low dose), group 4 (lead + middle dose), and group 5 (lead + high dose) were higher than those in group 2 (lead only). This may indicate that the extract has the potential to elevate G6PDH enzyme activity and, in turn, prevent tissue injury. This finding also suggests that lead suppressed G6PDH levels in the PFC, thereby contributing to the tissue alterations observed in this study. G6PDH is a cytoplasmic enzyme that plays a protective role during oxidative stress and provides coenzymes and substrates to primary antioxidant enzymes [27].

The histological morphology in the H&E-stained section of the group treated only with lead acetate (group 2) showed pyramidal cells with cytoplasmic vacuolations. These distortions in pyramidal cells, together with light staining, suggest cell injury. This result is similar to that reported by Bauchi *et al.* [33]. Whereas Bauchi *et al.* [33] observed several vacuolations and pyknosis in cortical neurons at a dose of 60 mg/kg lead acetate, the present study showed lead-induced toxicity at 5 mg/kg lead acetate.

Wang *et al.* [5] observed by transmission electron microscopy that lead acetate produced prominent tissue damage characterized by nuclear membrane fragmentation, mitochondrial destruction, rough endoplasmic reticulum dilation, and capillary lumen narrowing. The group administered lead + low-dose (100 mg/kg) SGL extract showed more densely stained pyramidal cells and nuclei than the lead-only group, although vacuolations persisted. This suggests a limited effect of the extract on lead-induced damage in the PFC at this dose. Group 4 (lead + middle-dose SGL) showed the densest staining among the treated groups and showed normal cells and pyramidal neurons similar to those in the control group. This suggests that at a dose higher than 100 mg/kg, *S. guineense* may ameliorate the effects of lead-induced toxicity. Group 5 (lead + high dose) showed PFC tissue with fewer vacuolations and more pyramidal neurons.

Similarly, Nissl staining revealed vacuolated and poorly stained Nissl bodies of pyramidal cells in group 2 (lead only). This finding suggests chromatolysis, a process triggered by axotomy, ischemia, cellular toxicity, and cell exhaustion. Chromatolysis is characterized by dissolution of Nissl bodies in the neuronal cell body and an eccentrically displaced nucleus [34]. Chromatolysis in the PFC in this study was associated with induction of reactive oxygen species (ROS), as indicated by a significantly increased MDA level, which was used as an oxidative-stress marker. Group 3 (lead + 100 mg/kg) also showed weak staining with a few cytoplasmic vacuolations, whereas the group administered lead + 250 mg/kg showed improved Nissl-body staining with a few cytoplasmic vacuolations. The group treated with lead + 400 mg/kg extract showed numerous, densely stained pyramidal neurons similar to the control group. The increase in Nissl bodies indicates that pyramidal neurons were preserved at the higher dose of SGL extract. Studies have shown that neuronal recovery through regeneration can occur after chromatolysis [34, 35].

5. CONCLUSION

This study demonstrates that lead acetate has a deleterious effect on the PFC of Wistar rats, generating free radicals and distorting its histomorphology. However, administration of *S. guineense* showed the ability to significantly reduce the generation of these free radicals and showed potential to alleviate the distorted histoarchitecture of the PFC at higher doses.

5.1. LIMITATIONS

Although an extract-only group would provide an ideal positive neuroprotective baseline, strict regulatory caps on animal numbers precluded its inclusion. The current design instead evaluates the extract's efficacy directly against the lead-exposed groups.

ETHICS STATEMENT

The Bingham University Animal Research Committee approved this study (AREC/UB/038/23).

DATA AVAILABILITY

Data are available upon reasonable request.

DECLARATION OF COMPETING INTEREST

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

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References

[1] M. Balali-Mood, K. Naseri, Z. Tahergorabi, M. R. Khazdair & M. Sadeghi, "Toxic mechanisms of five heavy metals: mercury, lead, chromium, cad-

mium, and arsenic'', Frontiers in Pharmacology **12** (2021) 643972. https://doi.org/10.3389/fphar.2021.643972.

[2] S. Kianoush, M. Balali-Mood, S. R. Mousavi, V. Moradi, M. Sadeghi, B. Dadpour, O. Rajabi & M. T. Shakeri, ''Comparison of therapeutic effects of garlic and D-penicillamine in patients with chronic occupational lead poisoning'', Basic & Clinical Pharmacology & Toxicology **110** (2012) 476. https://doi.org/10.1111/j.1742-7843.2011.00841.x.

[3] N. N. Dongre, A. N. Suryakar, A. J. Patil, J. G. Ambekar & D. B. Rathi, ''Biochemical effects of lead exposure on systolic & diastolic blood pressure, heme biosynthesis and hematological parameters in automobile workers of north Karnataka (India)'', Indian Journal of Clinical Biochemistry **26** (2011) 400. https://doi.org/10.1007/s12291-011-0159-6.

[4] K. Shin, G. Lim, Y. S. Hong, S. Kim, S. Hwang, J. Lee, S. Sin, A. Cho, Y. Kim, R. Gautam & J. Jo, ''Exposure to lead on expression levels of brain immunoglobulins, inflammatory cytokines, and brain-derived neurotropic factor in fetal and postnatal mice with autism-like characteristics'', Journal of Toxicology and Environmental Health, Part A **84** (2021) 891. https://doi.org/10.1080/15287394.2021.1945985.

[5] J. Wang, H. Zhu, Z. Yang & Z. Liu, ''Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats'', Indian Journal of Pharmacology **45** (2013) 395. https://doi.org/10.4103/0253-7613.115015.

[6] J. Wei, K. Du, Q. Cai, L. Ma, Z. Jiao, J. Tan, Z. Xu, J. Li, W. Luo, J. Chen & J. Gao, ''Lead induces COX-2 expression in glial cells in a NFAT-dependent, AP-1/NFκB-independent manner'', Toxicology **325** (2014) 67. https://doi.org/10.1016/j.tox.2014.08.012.

[7] D. Ramírez Ortega, D. F. González Esquivel, T. Blanco Ayala, B. Pineda, S. Gómez Manzo, J. Marcial Quino, P. Carrillo Mora & V. Pérez de la Cruz, ''Cognitive impairment induced by lead exposure during lifespan: mechanisms of lead neurotoxicity'', Toxics **9** (2021) 23. https://doi.org/10.3390/toxics9020023.

[8] Y. Fang, L. Lu, Y. Liang, D. Peng, M. Aschner & Y. Jiang, ''Signal transduction associated with lead-induced neurological disorders: a review'', Food and Chemical Toxicology **150** (2021) 112063. https://doi.org/10.1016/j.fct.2021.112063.

[9] C. J. Brubaker, K. N. Dietrich, B. P. Lanphear & K. M. Cecil, ''The influence of age of lead exposure on adult gray matter volume'', Neurotoxicology **31** (2010) 259. https://doi.org/10.1016/j.neuro.2010.03.004.

[10] B. Baars & N. M. Gage, *Fundamentals of Cognitive Neuroscience: A Beginner's Guide*, Academic Press, 2013. https://doi.org/10.1016/C2011-0-04186-8.

[11] Y. Yang & A. Raine, ''Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: a meta-analysis'', Psychiatry Research: Neuroimaging **174** (2009) 81. https://doi.org/10.1016/j.pscychresns.2009.03.012.

[12] P. Tugume, E. K. Kakudidi, M. Buyinza, J. Namaalwa, M. Kamatenesi, P. Mucunguzi & J. Kalema, ''Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda'', Journal of Ethnobiology and Ethnomedicine **12** (2016) 5. https://doi.org/10.1186/s13002-015-0077-4.

[13] M. Ekor, ''The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety'', Frontiers in Pharmacology **4** (2014) 177. https://doi.org/10.3389/fphar.2013.00177.

[14] I. C. Ezenyi & J. O. Igoli, ''Antidiarrhoeal properties of *Syzygium guineense* leaf extract and identification of chemical constituents in its active column fractions'', Journal of Complementary and Integrative Medicine **16** (2019) 20160074. https://doi.org/10.1515/jcim-2016-0074.

[15] P. Ghildyal, T. E. Grønhaug, A. Rusten, M. Skogsrud, B. Rolstad, D. Diallo, T. E. Michaelsen, M. Inngjerdingen & B. S. Paulsen, ''Chemical composition and immunological activities of polysaccharides isolated from the Malian medicinal plant *Syzygium guineense*'', Journal of Pharmacognosy and Phytotherapy **2** (2010) 76. https://doi.org/10.5897/JPP.9000018.

[16] J. Omale, A. Ogohi & G. Ebiloma, ''Anti-venom studies on *Olax viridis* and *Syzygium guineense* extracts against *Naja katiensis* venom in rats'', Planta Medica **79** (2013) PE23. https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0033-1352042.

[17] J.-P. Noudogbessi, P. Yédomonhan, D. C. Sohounhloué, J.-C. Chalchat & G. Figuérédo, ''Chemical composition of essential oil of *Syzygium guineense* (Willd.) DC. var. *guineense* (Myrtaceae) from Benin'', Records of Natural Products **2** (2008) 33. Available online: https://www.researchgate.net/publication/26529875_Chemical_composition_of_essential_oil_of_Syzygium_guineense_Willd_DC_var_guineense_Myrtaceae_from_Benin.

[18] M. M. Draid, M. O. Bushwereb & S. G. Ramadan, ''Lead acetate toxicity on glucose level and liver enzymes ameliorated by camel's milk in wistar albino rat'', International Journal of Basic & Clinical Pharmacology **5** (2016) 1125. https://doi.org/10.18203/2319-2003.ijbcp20161580.

[19] M. Typlt, M. Mirkowski, E. Azzopardi, L. Ruettiger, P. Ruth & S. Schmid, ''Mice with deficient BK channel function show impaired prepulse inhibition and spatial learning, but normal working and spatial reference memory'', PLoS ONE **8** (2013) e81270. https://doi.org/10.1371/journal.pone.0081270.

[20] J. Stocks & T. Dormandy, ''The autoxidation of human red cell lipids induced by hydrogen peroxide'', British Journal of Haematology **20** (1971) 95. https://doi.org/10.1111/j.1365-2141.1971.tb00790.x.

[21] S. Adem & M. Ciftci, ''Purification of rat kidney glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase enzymes using 2',5'-ADP Sepharose 4B affinity in a single chromatography step'', Protein Expression and Purification **81** (2012) 1. https://doi.org/10.1016/j.pep.2011.08.031.

[22] A. Shahriari, N. J. Dawson, R. A. Bell & K. B. Storey, ''Stable suppression of lactate dehydrogenase activity during anoxia in the foot muscle of *Littorina littorea* and the potential role of acetylation as a novel post-translational regulatory mechanism'', Enzyme Research **2013** (2013) 461374. https://doi.org/10.1155/2013/461374.

[23] V. Balamurugan, S. Fatima & S. Velurajan, ''A guide to phytochemical analysis'', International Journal of Advance Research and Innovative Ideas in Education **5** (2019) 236. Available online: https://ijariie.com/manuscript/9408.

[24] R. N. S. Yadav & M. Agarwala, ''Phytochemical analysis of some medicinal plants'', Journal of Phytology **3** (2011) 10. Available online: https://updatepublishing.com/journal/index.php/jp/article/view/2737.

[25] K. Santhi & R. Sengottuvel, ''Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo'', International Journal of Current Microbiology and Applied Sciences **5** (2016) 633. https://doi.org/10.20546/ijcmas.2016.501.064.

[26] Y. Deng, X. Lin, J. Zhou, M. Li, Z. Fu & D. Song, ''Concurrent serum lead levels and cognitive function in older adults'', Frontiers in Neuroscience **17** (2023) 1180782. https://doi.org/10.3389/fnins.2023.1180782.

[27] R. Agarwal & A. Rami, ''Redistribution of glucose-6-phosphate dehydrogenase in response to cerebral ischemia in rat brain'', Indian Journal of Clinical Biochemistry **18** (2003) 64. https://doi.org/10.1007/BF02867369.

[28] E. M. M. Abd-Ella, S. F. El-Sisy & H. El-Dash, ''Protective effects of alpha lipoic acid (α-LA) against lead neuro-toxicity in albino rats'', International Journal of Pharmaceutical Science Invention **5** (2016) 5. Available online: https://www.ijpsi.org/Papers/Vol5(2)/B050205013.pdf.

[29] O. Owoeye & S. K. Onwuka, ''Lead toxicity: effect of *Launaea taraxacifolia* on the histological and oxidative alterations in rat regio III Cornu ammonis and cerebellum'', Anatomy Journal of Africa **5** (2016) 783. https://www.semanticscholar.org/paper/Lead-Toxicity%3A-Effect-of-Launaea-taraxacifolia-on-Owoeye-Onwuka/90b523d11c896870606017113b24b887e6528e9e

[30] D. Praticò, C. M. Clark, F. Liun, J. Rokach, V. Y.-M. Lee & J. Q. Trojanowski, ''Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease'', Archives of Neurology **59** (2002) 972. https://doi.org/10.1001/archneur.59.6.972.

[31] A. F. Feudjio, R. Koularambaye, F. M. Jenny, A. Choupo, P. A. Z. Emini, D. L. M. Tsague, F. T. Nzufo & C. A. Pieme, ''Safety assessment of the aqueous bark extract of *Syzygium guineense* (Myrtaceae): acute and sub-chronic toxicity studies in rats'', Pharmacological Research–Natural Products **11** (2026) 100606. https://doi.org/10.1016/j.prenap.2026.100606.

[32] A. Ashraf, A. Liaquat, S. Shabbir, S. A. Bokhari, Z. Tariq, Z. Furrukh, A. A. Raja & M. J. Khan, ''High level of lactate dehydrogenase and ischaemia–reperfusion injury regulate the multiple organ dysfunction in patients with COVID-19'', Postgraduate Medical Journal **99** (2023) 576. https://doi.org/10.1136/postgradmedj-2022-141573.

[33] Z. Bauchi, D. Kizito, A. Alhassan, S. Akpulu & J. Timbuak, ''Effect of aqueous seed extract of *Nigella sativa* on lead-induced cerebral cortex toxicity in Long Evans rats'', Bayero Journal of Pure and Applied Sciences **9** (2016) 48. https://doi.org/10.4314/bajopas.v9i1.8.

[34] T. A. Etibor, M. I. Ajibola, M. O. Buhari, A. A. Safiriyu, O. B. Akinola & E. A. Caxton-Martins, ''*Datura metel* administration distorts medial prefrontal cortex histology of Wistar rats'', World Journal of Neuroscience **5** (2015) 282. https://doi.org/10.4236/wjns.2015.54026.

[35] I. Gersh & D. Bodian, ''Some chemical mechanisms in chromatolysis'', Journal of Cellular and Comparative Physiology **21** (1943) 253. https://doi.org/10.1002/jcp.1030210305.