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Exploitation of aqueous *Rosmarinus officinalis* extract as a protective agent in the hippocampus of lead acetate-exposed rats

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ABSTRACT

Background: Hippocampal dysfunction can result from defects, degeneration, or toxins like lead (Pb), which crosses the blood-brain barrier, disrupts calcium signaling, and causes oxidative stress, leading to cognitive decline. Dietary antioxidants may help counteract this oxidative injury and protect hippocampal function. **Objective:** This study investigated the protective activity of aqueous *Rosmarinus officinalis* leaf extract on lead acetate induced hippocampal toxicity. **Materials and Methods:** Forty-eight adult Wistar rats were divided into six groups: A (control), B (100 mg/kg Pb only), C (100 mg/kg Pb + 100 mg/kg *R. officinalis*), D (100 mg/kg Pb + 200 mg/kg *R. officinalis*), E (100 mg/kg *R. officinalis* only), and F (200 mg/kg *R. officinalis* only). All administrations, via an orogastric tube, lasted for twenty-eight (28) days. Neurobehavioral tests (Novel Object Recognition, Y-maze, Elevated Plus Maze) were evaluated, after which hippocampi were collected to assess lead levels, antioxidant activity, lipid peroxidation, acetylcholinesterase (AChE), nitric oxide (NO), histology, and apoptosis. **Results:** Pb-exposed rats showed significant (p<0.05) weight loss, cognitive and memory deficits, disrupted antioxidant and AChE activity, increased lipid peroxidation, elevated hippocampal NO and lead levels, along with neuronal atrophy and caspase-3 upregulation, indicating apoptosis. Pretreatment with *R. officinalis* significantly (p<0.05) mitigated weight loss, oxidative stress, lipid peroxidation, neuronal damage, and levels of hippocampal lead, NO, AChE, and caspase-3, highlighting its antioxidant, metal-chelating, NO-scavenging, anti-cholinesterase, and anti-apoptotic effects against Pb-induced neurotoxicity. **Conclusion:** Consequently, this study provides novel evidence supporting *R. officinalis* as a promising neuroprotective agent with potential for drug development against hippocampal dysfunction.

Keywords: *Rosmarinus officinalis*, Lead Acetate, Hippocampus, Neuroprotection, Oxidative Stress, Neurotoxicity.

INTRODUCTION

Lead (Pb) exposure continues to pose a major global public health challenge owing to its environmental persistence, bioaccumulative nature, and multisystem toxicity [1]. As a non-biodegradable heavy metal, Pb persists in soil, water, and air, primarily originating from mining, industrial emissions, and fossil fuel combustion [2,3]. Although regulatory interventions have curtailed Pb usage in several developed regions, exposure levels remain alarmingly high in many low- and middle-income countries where enforcement mechanisms are inadequate. In 2021, Pb exposure was estimated to account for approximately 1.5 million deaths worldwide, with the greatest burden observed among socioeconomically disadvantaged populations [4,5]. Following absorption, Pb is distributed to soft tissues and subsequently sequestered in bone, serving as a long-term reservoir that can gradually release the metal into circulation, thereby sustaining chronic toxicity [6].

The brain, particularly the hippocampus, is exceptionally susceptible to toxic insults owing to its critical role in cognition, emotional regulation, and synaptic plasticity, as well as its high metabolic demand [7,8]. Lead (Pb) exerts neurotoxicity primarily by mimicking calcium ions, thereby disrupting neuronal signalling, synaptic transmission, and neurotransmitter release through interference with calcium-dependent processes [9,10].

In addition, Pb exposure precipitates oxidative stress, characterized by excessive generation of reactive oxygen species (ROS) and depletion of endogenous antioxidant defenses [11]. This oxidative imbalance damages cellular macromolecules, promotes neuroinflammation, and induces mitochondrial dysfunction and neuronal apoptosis [10]. Collectively, these pathological events contribute to the onset and progression of neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, in which hippocampal degeneration is a defining neuropathological feature [11-13].

Given the irreversible nature of many Pb-induced neurotoxic effects, there is an urgent need to identify sustainable and cost-effective interventions to mitigate its impact on the brain, particularly in vulnerable populations. In this context, plant-based antioxidants have emerged as promising therapeutic agents due to their capacity to scavenge ROS, upregulate endogenous antioxidant enzymes, and modulate inflammatory responses [14-16]. Among these, *R. officinalis* L. (commonly known as rosemary) has garnered significant scientific interest. Belonging to the Lamiaceae family, *R. officinalis* is a hardy, aromatic shrub native to the Mediterranean region and traditionally used in culinary and folk medicine [17,18].

Its bioactive profile is characterized by a high concentration of phenolic compounds such as rosmarinic acid, carnosic acid, and carnosol, which are recognized for their strong antioxidant properties [18,19]. These compounds exert their protective actions by directly neutralizing free radicals, inhibiting lipid peroxidation, and enhancing the activity of endogenous antioxidant enzymes. Beyond its antioxidant properties, *R. officinalis* has demonstrated a broad spectrum of pharmacological activities including anti-inflammatory, antinociceptive, antiulcerogenic, antidepressant, anxiolytic, and hepatoprotective effects [20-22]. Despite its promise, the effects of *R. officinalis* specifically against Pb-induced hippocampal damage remain underexplored. Accordingly, this study aimed to investigate the activity of aqueous *R. officinalis* leaf extract on Pb-induced hippocampal toxicity in adult Wistar rats.

MATERIAL AND METHODS

Chemicals and Reagents

Normal saline was manufactured by Unique Pharmaceuticals, Sango-Otta, Nigeria, and lead acetate (99% purity) by Loba Chemie Pvt. Ltd, Mumbai, India. Other reagents were all of the analytical grades.

Extraction of the Aqueous Extract of *Rosmarinus officinalis*

Packets of dried *R. officinalis* leaves were bought at a local market in Benin City, Nigeria, with NAFDAC Number A1-9793 and batch number 206211. Plant extraction was performed as previously described by Enogieru and Iyoha [23], with minor modifications. A total of 5 kg of dried leaf material was pulverized into a fine powder using a mechanical blender. Aliquots of 10 g of the powdered sample were extracted with distilled water using either three successive macerations (3 × 50 mL, 24 hours each at room temperature) or a single decoction step (1 × 50 mL, 10 minutes at 95 °C). Following extraction, the mixtures were filtered using Whatman No. 1 filter paper, and the resulting aqueous extracts were freeze dried. The dried extracts were stored in the dark at 4 °C until further use. Prior to experimentation, the freeze-dried extract was reconstituted in distilled water to the desired working concentration.

Procurement and Care of Animals

Wistar rats used for this study were procured and bred in the Animal House, Department of Anatomy, University of Benin. The rats were fed with standard rat chow (Chikun Feed Grower Mash, Olam Agri Holdings Pte Ltd., Lagos State, Nigeria) and water throughout the entire study period. They were weighed weekly before commencement and throughout the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. The animals received humane care following the principle of humane care and the use of laboratory animals. Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Benin City, Nigeria (Approval No. CMS/REC/2021/172).

Research Design

Following acclimatization, forty-eight (48) adult Wistar rats weighing between 140 g to 160 g were divided into six (6) groups of eight (8) animals each. Group A served as Control; Group B was given 100 mg/kg body weight of lead acetate only; Group C was given 100 mg/kg body weight of *R. officinalis* extract + 100 mg/kg body weight of lead acetate; Group D was given 200 mg/kg body weight of *R. officinalis* extract + 100 mg/kg body weight of lead acetate; Group E was given 100 mg/kg body weight of *R. officinalis* extract only; Group F was given 200 mg/kg body weight of *R. officinalis* extract only.

Administration lasted for twenty-eight (28) days. All administrations were carried out via oral route using an orogastric tube. Treatment groups were pre-treated with *R. officinalis* extract, 1 h before the administration of lead acetate.

Neurobehavioral Assessments

Novel Object Recognition Test

This test was carried out in a wooden open box device (80 × 60 × 40 cm), as previously described [24]. Here, rats explored the device for a 2-minute session of familiarization on the 27th day of the experiment. On the 28th day, a first 5 min sample trial test (T1) was carried out, with two similar objects (named familiar objects FO1 and FO2) placed at the corners of the box. In the second 5-minute test (T2), FO2 presented in T1 was substituted with a novel object (NO), and the exploration times for FO1 and NO were recorded. The discrimination between FO1 and NO during T2 was determined by equating the time spent exploring FO1 and NO [18,19]. For quality control, a discrimination index (DI) was calculated as follows: DI = NO−FO1/NO + FO1.

Y-Maze Test

This test was carried out in a wooden apparatus consisting of three identical arms (33×11×12cm each) which are symmetrically separated at 120° with an equilateral triangular central area, as previously described [25]. The rats were placed at the end of one arm and allowed to move freely through the maze for 5 min after which every session was stopped. An arm entry was recorded when the hind paws of the rat were completely within the arm and spontaneous alternation behaviour was defined as three consecutive entries in three different arms. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as the:

Total of alternations / (total arm entries –2×100).

Elevated Plus Maze Test

According to Carobrez and Bertoglio [26], the Elevated Plus Maze (EPM) is commonly employed to assess anxiety-like behavior in laboratory rodents, though it can also be utilized to evaluate cognitive function. The apparatus consists of two open arms (50 × 10 cm) and two closed arms of the same dimensions, with the closed arms enclosed by 30 cm high walls. These arms are arranged in a plus-shaped configuration and connected by a central platform (10 × 10 cm), with the entire maze elevated 60 cm above the ground. Each rat was placed at the center of the maze facing an open arm and allowed to explore freely for 5 minutes. During this period, transfer latency parameters was recorded. Transfer latency is defined as the time taken for the animal to move from the open arm into a closed arm upon initial placement in the maze, often interpreted as an index of

cognitive function or exploratory motivation. An arm entry was recorded when all four paws of the animal (or at minimum, both hind paws) were fully within the arm. To prevent olfactory cues from affecting behavior, the maze was cleaned with 70% ethanol between trials [27,28].

Antioxidant Enzymes and Lipid Peroxidation Assessment

Following the sacrifice of rats under low-level chloroform anaesthesia before cervical dislocation, the brains of experimental rats were removed and the hippocampus was dissected for biochemical and histological investigations. The harvested hippocampus was washed twice in cold phosphate-buffered saline (PBS), homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and centrifuged at 10,000 g for 10 minutes at 4 °C [16,23]. The supernatant was collected and assessed for Malondialdehyde (MDA) [29], Catalase (CAT) [30], Superoxide Dismutase (SOD) [31], Glutathione peroxidase (GPx) [32] and Glutathione (GSH) [33].

Histological Assessment

The hippocampi were fixed in 10% buffered formal saline for 72 h and processed through the Hematoxylin and Eosin staining methods as previously described [34].

Statistical Analysis

The obtained data were analyzed, and graph production was carried out, using GraphPad Prism software, version 9.0 (GraphPad Software, San Diego, CA, USA). All values were analyzed for normality of data using the Shapiro-Wilk test and homogeneity of variance with the Levene test, as previously reported [35]. The data demonstrated normal distribution and homogeneity of variance. Statistical significance was then determined by one-way analysis of variance (ANOVA) for all parameters. Tukey's posthoc test was performed to determine groups involved in statistical differences and results were expressed as mean ± S.E.M. A *p*-value of < 0.05 was defined as statistically significant.

RESULTS

Effect of Treatment on Neurobehavioral Activity

Figure 1-3 shows the findings on cognition and memory from the NOR, Y-Maze and EPM tests respectively. In Pb-exposed rats, a significant decrease ($p<0.05$) was observed in the total exploration time and discrimination index when compared to control. However, a significant increase ($p<0.05$) was observed in pre-treated rats (RO1 + Pb and RO2 + Pb) when compared to Pb-only. Similarly, there was a significant decrease ($p<0.05$) in total arm entries and spontaneous alternation in Pb-exposed rats compared to control. However, a significant increase ($p<0.05$) in discrimination index was observed in pre-treated rats (RO1 + Pb and RO2 + Pb) when compared to Pb-only.

Effect of Treatment on Oxidative Stress

Figure 4 shows the effect of treatment on oxidative parameters. There was significant decrease ($p<0.05$) in hippocampal SOD, CAT, GPx, and GSH and a corresponding increase ($p<0.05$) in MDA, in Pb-exposed rats when compared to control. However, there was a significant decrease ($p<0.05$) in SOD, CAT, GPx, and GSH and a corresponding decrease ($p<0.05$) in MDA, in Pb-exposed rats pre-treated with *R. officinalis* when compared to Pb-only group.

Effect of treatment on AChE, Pb accumulation, NO and Caspase-3

There was a significant increase ($p<0.05$) in AChE, Pb concentration, NO in Pb-exposed rats when compared to control. There was also an upregulation of Caspase-3 activity in Pb-exposed rats when compared to control. However, there was a significant decrease ($p<0.05$) in AChE, Pb accumulation, NO and down-regulation of Caspase-3 in Pb-exposed rats pretreated with *R. officinalis* when compared to Pb-only group.

Effect of Treatment on Histology of the Hippocampus

Plate 1 (A-F) shows the histology of the hippocampus (CA1) of rats across experimental groups. Pb- exposed rats showed significant alterations exhibited as atrophy and vacuolated pyramidal cells and astrocytes. However, Pb-exposed rats pretreated with *R. officinalis* showed relatively normal histological structures.

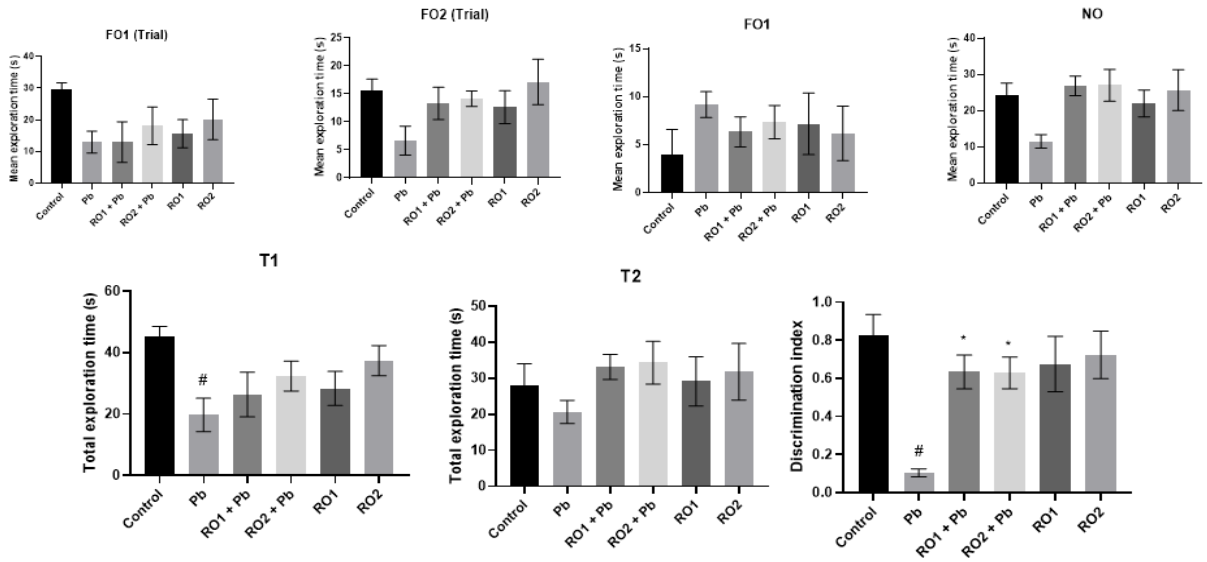

Figure 1: NOR Parameters of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # p<0.05 compared with the control group; * p<0.05 compared with Pb group

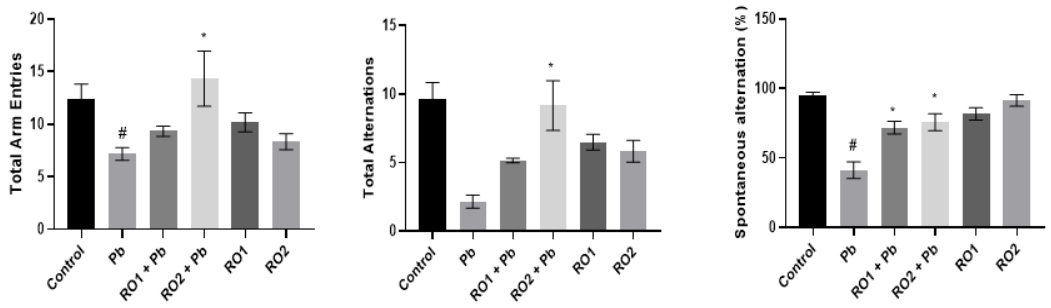

Figure 2: Y-maze Parameters of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # p<0.05 compared with the control group; * p<0.05 compared with Pb group

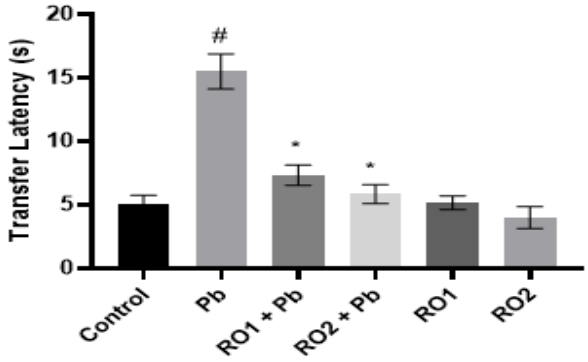

Figure 3: Transfer latency across experimental groups. Values are given as mean ± SEM of each group. # p<0.05 compared with control group; *p<0.05 compared with Pb only group.

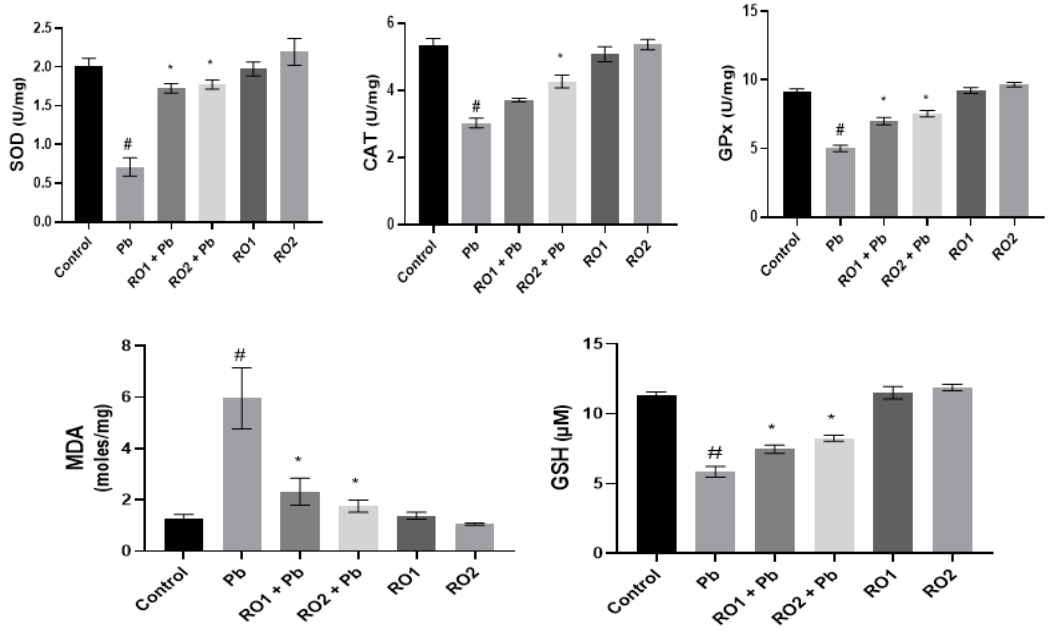

Figure 4: Oxidative Stress Parameters in the hippocampus of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # p<0.05 compared with the control group; * p<0.05 compared with Pb group.

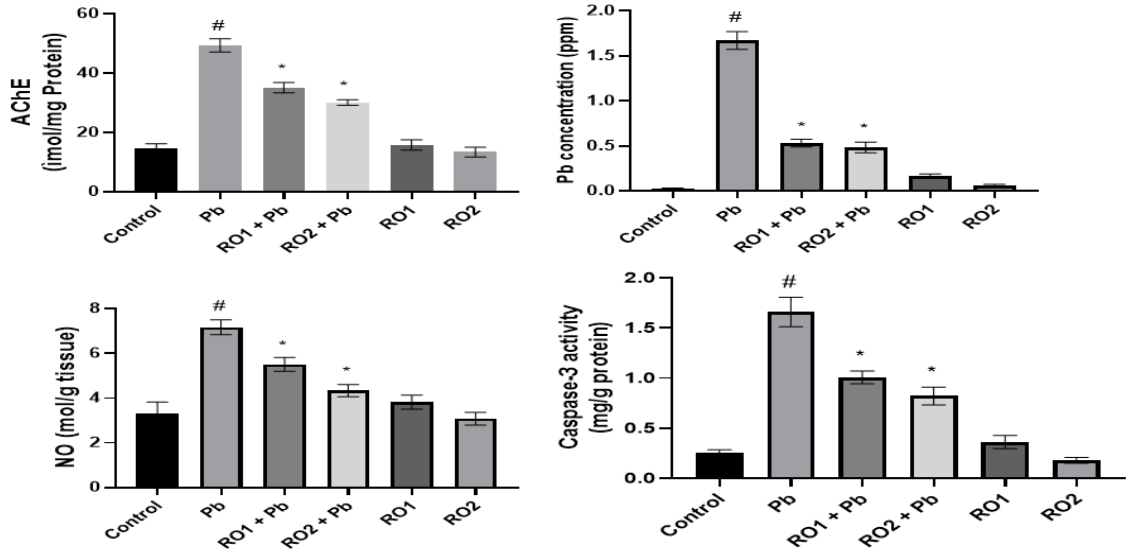

Figure 5: AChE, Pb accumulation, NO and Caspase-3 assessments in the hippocampus of control and treatment groups after 28 days. Values are given as mean ± SEM of each group. # $p<0.05$ compared with the control group; * $p<0.05$ compared with Pb group.

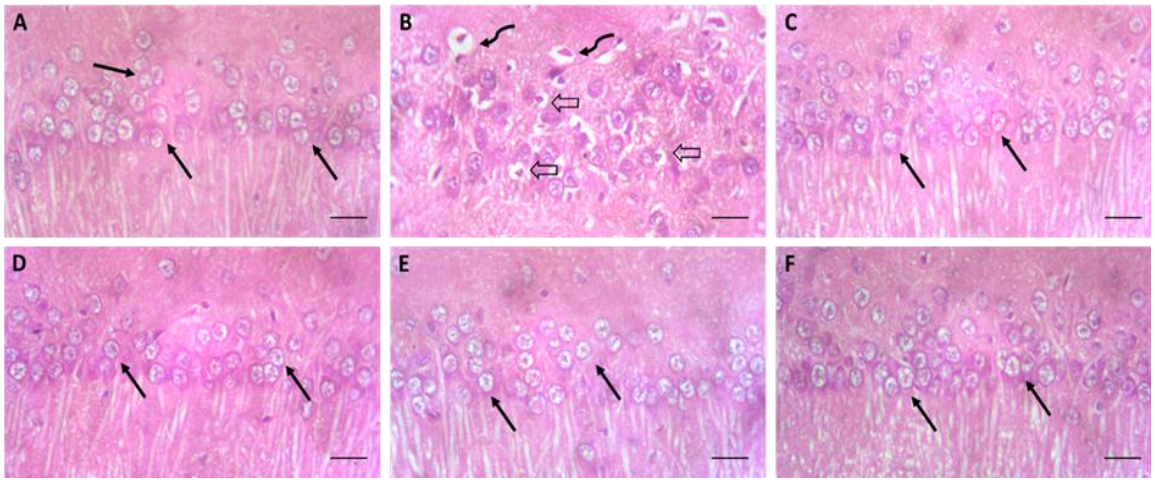

Plate 1: Representative histology of the hippocampus CA1 in control and treatment rats. (A) Control group revealing normal structure of pyramidal cells (arrows). (B) Pb-treated rats showing atrophy and vacuolated pyramidal cells (transparent double arrows) and astrocytes (curved arrows). (C-F) Relatively normal histological structure of the hippocampus observed. (HandE; Scale bar: 25µm)

DISCUSSION

This study investigated the potential neuroprotective effect of *R. officinalis* against Pb-induced hippocampal toxicity in Wistar rats. The hippocampus plays a central role in memory formation, spatial learning, and emotional regulation, making it highly susceptible to neurotoxic damage [36]. Understanding its vulnerability to lead exposure is essential for developing strategies to preserve cognitive function and prevent metal-induced neurodegeneration.

Behavioral tests are key for detecting neurotoxic effects, providing early insights into CNS dysfunction [37,38]. Pb, a known neurotoxin, impairs cognition, mood, and motor skills [10]. The Novel Object Recognition (NOR) test, reliant on hippocampal and prefrontal cortex function, evaluates recognition memory [39,40]. Pb exposure reduced exploration time and the discrimination index (DI), indicating memory and attention deficits [41-43]. *R. officinalis* pretreatment significantly improved DI, suggesting protection against Pb-induced memory loss. The Y-maze assesses hippocampal-dependent spatial memory. Pb-only rats showed reduced total arm entries and spontaneous alternation, indicating impaired working memory and exploration [44-46]. *R. officinalis* restored these behaviors, enhancing cognitive performance. The Elevated Plus Maze (EPM) test, measuring transfer latency, reflects learning and anxiety levels [47,48]. Pb increased transfer latency, consistent with hippocampal dysfunction, anxiety, and synaptic impairment [44,49,50]. *R. officinalis* pretreatment significantly reduced latency, indicating cognitive improvement and anxiety reduction. Overall, Pb disrupts hippocampal-dependent behaviors, impairing recognition and spatial memory as well as stress response. *R. officinalis* effectively mitigated these effects, demonstrating antioxidant and neuroprotective properties, and highlighting its therapeutic potential against Pb-induced neurotoxicity.

Oxidative stress is a primary mechanism by which heavy metals, including Pb, induce neurotoxicity [13]. Pb enhances reactive oxygen species (ROS) production, disrupting the balance between oxidative agents and antioxidant defenses [51,52]. Excess ROS interact destructively with lipids, proteins, and DNA due to their unpaired electrons [53]. The brain, with its high oxygen consumption and lipid content, is particularly susceptible to oxidative injury [54]. Pb crosses the blood–brain barrier, impairing enzyme systems crucial for brain function [55]. Antioxidants—particularly superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)—are the body's main defense against ROS [56,57]. SOD converts superoxide radicals into hydrogen peroxide [58], which CAT then breaks down into water and oxygen, preserving SOD function and preventing tissue damage [59,60]. GSH directly neutralizes free radicals, while malondialdehyde (MDA), a

lipid peroxidation by-product, serves as a biomarker for oxidative stress [61]. This study observed decreased SOD, CAT, and GPx activity, reduced GSH levels, and increased MDA concentrations in Pb-exposed rats, consistent with previous reports linking Pb neurotoxicity to oxidative stress [13,62,63]. However, pretreatment with *R. officinalis* significantly restored antioxidant enzyme activity, increased GSH, and reduced MDA levels, demonstrating its strong antioxidant properties and potential in mitigating Pb-induced oxidative damage.

Acetylcholinesterase (AChE) terminates synaptic transmission by hydrolyzing acetylcholine, a process essential for cholinergic signaling, especially in the hippocampus [64,65]. Pb exposure increases hippocampal AChE activity, accelerating acetylcholine breakdown, reducing cholinergic signaling, and impairing synaptic plasticity and cognition [66]. This study found elevated AChE activity in the hippocampus of Pb-exposed rats, indicating cholinergic disruption and neuronal stress. Pre-treatment with *R. officinalis* significantly reduced AChE activity, suggesting its neuroprotective role in restoring cholinergic balance. Pb also accumulates in the hippocampus, causing neurochemical changes and structural damage linked to learning deficits [13]. Chelators, which bind metals for excretion, are explored to counter Pb neurotoxicity [67-69]. This study showed *R. officinalis* significantly lowered hippocampal Pb levels, likely due to its flavonoids and polyphenols forming stable Pb complexes, limiting its absorption and brain deposition [70,71]. Nitric oxide (NO), synthesized mainly by neuronal nitric oxide synthase (nNOS), has dual roles in neuroprotection and neurotoxicity [72,73]. Excess NO forms reactive species, contributing to oxidative stress and neuroinflammation via inducible NOS (iNOS) in glial and immune cells [74-77]. This study observed increased NO levels in the hippocampus of Pb-exposed rats, which were significantly reduced by *R. officinalis*, indicating its potential to scavenge NO and mitigate inflammation.

Apoptosis is a controlled form of cell death crucial for development, immune regulation, and tissue homeostasis [78,79]. In neurodegenerative diseases like Alzheimer's, Parkinson's, and ALS, apoptosis contributes to progressive neuronal loss [80,81]. Key mechanisms include caspase activation, protein misfolding, and calcium imbalance [82,83]. Caspase-3, a central executioner of apoptosis, is activated via extrinsic (death receptor-mediated) or intrinsic (mitochondrial) pathways, both converging on substrate cleavage and cell death [84-87]. Caspase-3 expression is elevated in the hippocampus of Alzheimer's patients, linking apoptosis to neurodegeneration [88,89]. This study similarly found increased hippocampal caspase-3 levels in Pb-exposed rats, consistent with Pb-induced apoptotic damage [62,90-92]. However, *R. officinalis* pre-treatment significantly reduced caspase-3 expression, indicating its potential anti-apoptotic and neuroprotective effects [93].

The hippocampus is particularly vulnerable to Pb toxicity due to its complex neural circuits and sensitivity to cellular damage [10,94]. Pyramidal neurons, essential for learning and memory, are especially susceptible, with Pb exposure impairing synaptic transmission and cognitive function [23,95]. Results from this study showed significant histological damage in the hippocampus of Pb-exposed rats, including atrophy, vacuolated pyramidal cells, and reactive astrocytes—indicative of disrupted neuronal integrity and impaired connectivity [96,97]. In contrast, rats pre-treated with *R. officinalis* showed preserved hippocampal structure with minimal damage. This aligns with prior research on the neuroprotective effects of plant-derived antioxidants [16,98]. The protective effects of *R. officinalis* may stem from its phenolic and flavonoid content, which help reduce oxidative stress and inflammation, preserving neuronal architecture [74,99,100].

CONCLUSION

Findings from this study indicate that *R. officinalis* attenuates Pb-induced hippocampal toxicity primarily through its antioxidant, metal-chelating, anti-cholinesterase, and anti-apoptotic effects. Therefore, *R. officinalis* may be a promising candidate for developing novel therapeutic agents to manage Pb neurotoxicity and its associated cognitive impairments.

Author Contributions

CCO carried out the animal experiments, plant extraction, and data collection. JEA prepared the first draft of the manuscript and participated in manuscript editing. FOT conducted the behavioural assessments and biochemical assays. ARO and OUI contributed to data collection and laboratory assistance. ABE conceived the study, designed the methodology, performed the statistical analysis, interpreted the data, and supervised the research. All authors approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available and will be made available upon request.

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declared no conflict of interest.

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REFERENCES

1. Fatima G, Raza AM, Dhole P. Heavy metal exposure and its health implications: a comprehensive review. *Indian J Clin Biochem*. 2025;1-29.
2. Niede R, Benbi DK. Integrated review of the nexus between toxic elements in the environment and human health. *AIMS Public Health*. 2022;9(4):758-89.
3. Howard JA, David L, Lux F, Tillement O. Low-level, chronic ingestion of lead and cadmium: the unspoken danger for at-risk populations. *J Hazard Mater*. 2024;135361.
4. World Health Organization. Exposure to lead: a major public health concern. Geneva: World Health Organization; 2023.
5. Institute for Health Metrics and Evaluation. GBD 2021 lead exposure estimates 1990-2021. *Global Health Data Exchange*; 2024.
6. Srivastava H, Saini P, Singh A, Yadav S. Heavy metal pollution and biosorption. In: *Biosorption processes for heavy metal removal*. 2024. p. 1-38.
7. Song J. Amygdala activity and amygdala-hippocampus connectivity: metabolic diseases, dementia, and neuropsychiatric issues. *Biomed Pharmacother*. 2023;162:114647.
8. Popa LL, Chira D, Asaftei A, Strilciuc S, Mureșanu D. The modulation of hippocampus plasticity. In: Hippocampus: More Than Just Memory. 2023. p. 107.
9. Penticoff HB, Fortin JS. Toxic/metabolic diseases of the nervous system. In: *Neurobiology of Brain Disorders*. 2023. p. 379-401.
10. Gudadhe S, Singh SK, Ahsan J. Cellular and neurological effects of lead (Pb) toxicity. In: *Lead Toxicity Mitigation: Sustainable Nexus Approaches*. 2024. p. 125-45.

11. Jing H, Zhang Q, Li S, Gao XJ. Pb exposure triggers MAPK-dependent inflammation by activating oxidative stress and miRNA-155 expression in carp head kidney. *Fish Shellfish Immunol*. 2020;106:219-27.
12. Chen Z, Huo X, Chen G, Luo X, Xu X. Lead (Pb) exposure and heart failure risk. *Environ Sci Pollut Res*. 2021;28:28833-47.
13. Pyatha S, Kim H, Lee D, Kim K. Association between heavy metal exposure and Parkinson's disease: a review of the mechanisms related to oxidative stress. *Antioxidants*. 2022;11(12):2467;1-18.
14. Abeyrathne EDNS, Nam K, Huang X, Ahn DU. Plant- and animal-based antioxidants' structure, efficacy, mechanisms, and applications: a review. *Antioxidants*. 2022;11(5):1025.
15. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. *Biofactors*. 2022;48(3):611-33.
16. Enogieru AB, Momodu O. Neuroprotective effects of Zingiber officinale against lead-induced toxicity in Wistar rats. *Nutrire*. 2022;48(1):2.
17. Allegra A, Tonacci A, Pioggia G, Musolino C, Gangemi S. Anticancer activity of *R. officinalis* L.: mechanisms of action and therapeutic potentials. *Nutrients*. 2020;12(6):1739.
18. Adepoju A, Ogunkunle T, Femi-Adepoju A, Ejigboye E. Scientific common names (SCNS) for selected medicinal plants: an improved method of botany: scientific common names for medicinal plants. *Arab J Med Aromat Plants*. 2024;10(1):189-251.
19. Mehta N, Meena PD, Saharan GS. Geographical distribution and host range of Sclerotinia sclerotiorum causing stem rot of crucifers. *Plant Dis Res*. 2023;38(1):11-46.
20. Lawal IO, Rafiu BO, Ale JE, Majebi OE, Aremu AO. Ethnobotanical survey of local flora used for medicinal purposes among indigenous people in five areas in Lagos State, Nigeria. *Plants*. 2022;11(5):633;1-19.
21. Kamli MR, Sharaf AAM, Sabir JS, Rather IA. Phytochemical screening of *R. officinalis* L. as a potential anticholinesterase and antioxidant medicinal plant for cognitive decline disorders. *Plants*. 2022;11(4):514;1-15.
22. Jeevalatha A, Kalaimathi RV, Basha AN, Kandeepan C, Ramya S, Loganathan T, *et al*. Profile of bioactive compounds in Rosmarinus officinalis. *J Drug Deliv Ther*. 2022;12(1):114-22.
23. Enogieru AB, Iyoha EN. Role of nitric oxide, TNF-α and caspase-3 in lead acetate-exposed rats pretreated with aqueous *R. officinalis* leaf extract. *Biol Trace Elem Res*. 2023;1-11.
24. Enogieru AB, Omoruyi SI. Exploration of aqueous Phyllanthus amarus leaf extract as a protective agent in mercury chloride-exposed Wistar rats: a neurobehavioural study. *J Appl Sci Environ Manag*. 2022;26(4):629-37.
25. Prieur EA, Jadavji NM. Assessing spatial working memory using the spontaneous alternation Y-maze test in aged male mice. *Bio Protoc*. 2019;9(3):e3162.
26. Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci Biobehav Rev*. 2005;29(8):1193-205.
27. Casteller G, Fraile M, Laconi M, Landa AI, Cabrera R, Gargiulo PA. Desinhibitory effect of allopregnanolone within the medial prefrontal cortex of male rats on the plus maze test. *Int J Neurodegener Disord*. 2006;2:120-26.
28. Naqvi F, Haider S, Batool Z, Perveen T, Haleem D. Sub-chronic exposure to noise affects locomotor activity and produces anxiogenic and depressive-like behavior in rats. *Pharmacol Rep*. 2012;64(1):64-69.
29. Fauziah PN, Maskoen AM, Yuliati T, Widiarsih E. Optimized steps in determination of malondialdehyde (MDA) standards on diagnostic of lipid peroxidation. *Padjadjaran J Dent*. 2018;30(2):136-39.
30. Hadwan MH, Hussein MJ, Mohammed RM, Hadwan AM, Saad Al-Kawaz H, Al-Obaidy SS, *et al*. An improved method for measuring catalase activity in biological samples. *Biol Methods Protoc*. 2024;9(1):15;1-12.
31. Zhao H, Zhang R, Yan X, Fan K. Superoxide dismutase nanozymes: an emerging star for anti-oxidation. *J Mater Chem B*. 2021;9(35):6939-57.
32. Ahmed AY, Aowda SA, Hadwan MH. A validated method to assess glutathione peroxidase enzyme activity. *Chem Pap*. 2021;75:6625-37.
33. Nuhu F, Gordon A, Sturmey R, Seymour AM, Bhandari S. Measurement of glutathione as a tool for oxidative stress studies by high performance liquid chromatography. *Molecules*. 2020;25(18):4196.
34. Drury RAB, Wallington EA. General staining procedures. In: Carleton's histological techniques. Oxford: *Oxford University Press*; 1980. p. 125-50.
35. Enogieru AB, Idemudia OU. Comparative protective activity of aqueous Zingiber officinale root and Theobroma cacao seed extracts on lead acetate-induced cerebellar toxicity in rats. *J Trace Elem Miner*. 2024;10:100190.
36. Albadawi EA. Structural and functional changes in the hippocampus induced by environmental exposures. *Neurosciences (Riyadh)*. 2025;30(1):5-19.
37. Roberts GK, Stout MD, editors. Specifications for the conduct of toxicity studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences. Research Triangle Park (NC): National Institute of Environmental Health Sciences; 2023.
38. Fitzgerald JA, Könemann S, Krümpelmann L, Županič A, Vom Berg C. Approaches to test the neurotoxicity of environmental contaminants in the zebrafish model: from behavior to molecular mechanisms. *Environ Toxicol Chem*. 2021;40(4):989-1006.
39. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp*. 2017;(126):e55718;1-9.
40. Chao OY, Nikolaus S, Yang YM, Huston JP. Neuronal circuitry for recognition memory of object and place in rodent models. *Neurosci Biobehav Rev*. 2022;141:104855.
41. Dumková J, Smutná T, Vrlíková L, Le Coustumer P, Večeřa Z, Dočekal B, *et al*. Sub-chronic inhalation of lead oxide nanoparticles revealed their broad distribution and tissue-specific subcellular localization in target organs. *Part Fibre Toxicol*. 2017;14(1):1-19.
42. Alves Oliveira AC, Dionizio A, Teixeira FB, Bittencourt LO, Nonato Miranda GH, Oliveira Lopes G, *et al*. Hippocampal impairment triggered by long-term lead exposure from adolescence to adulthood in rats: insights from molecular to functional levels. *Int J Mol Sci*. 2020;21(18):6937.
43. Shvachiy L, Amaro-Leal Â, Outeiro TF, Rocha I, Geraldes V. From molecular to functional effects of different environmental lead exposure paradigms. *Biology (Basel).* 2022;11(8):1164;1-18.
44. Enogieru AB, Williams BT. Cognitive- and memory-enhancing activity of Cinnamomum zeylanicum aqueous extract in lead acetate-exposed rats. *J Trace Elem Miner*. 2024;9:100189.
45. Wang M, Xia Y, Ai S, Gu X, Wang HL. Kaempferol improves Pb-induced cognitive impairments via inhibiting autophagy. *J Nutr Biochem*. 2024;125:109556.
46. Zygan-Filipiak K, Adamczuk P, Łukawski K. The effects of coexposure to caffeine and heavy metals on learning and oxidative stress in mice. *Acta Neurobiol Exp (Wars).* 2025;85(1):49-57.
47. Mantanona CP, Alsiö J, Elson JL, Fisher BM, Dalley JW, Bussey T, *et al*. Altered motor, anxiety-related and attentional task performance at baseline associate with

multiple gene copies of the vesicular acetylcholine transporter and related protein overexpression in ChAT::Cre+ rats. *Brain Struct Funct*. 2019;224:3095-116.
48. d'Isa R, Comi G, Leocani L. Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze. *Sci Rep*. 2021;11(1):21177.
49. Larosa A, Wong TP. The hippocampus in stress susceptibility and resilience: reviewing molecular and functional markers. *Prog Neuropsychopharmacol Biol Psychiatry*. 2022;119:110601.
50. Dues DJ, Nguyen APT, Becker K, Ma J, Moore DJ. Hippocampal subfield vulnerability to α-synuclein pathology precedes neurodegeneration and cognitive dysfunction. *npj Parkinsons Dis*. 2023;9(1):125;1-11.
51. Nowicka B. Heavy metal-induced stress in eukaryotic algae—mechanisms of heavy metal toxicity and tolerance with particular emphasis on oxidative stress in exposed cells and the role of antioxidant response. *Environ Sci Pollut Res*. 2022;29(12):16860-911.
52. Liu T, Sun L, Zhang Y, Wang Y, Zheng J. Imbalanced GSH/ROS and sequential cell death. *J Biochem Mol Toxicol*. 2022;36(1):e22942.
53. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int J Mol Sci*. 2021;22(9):4642.
54. Collin MS, Venkatraman SK, Vijayakumar N, Kanimozhi V, Arbaaz SM, Stacey RS, *et al*. Bioaccumulation of lead (Pb) and its effects on human: a review. *J Hazard Mater Adv*. 2022;7:100094.
55. Li Z, Wang Z, Xue K, Wang Z, Guo C, Qian Y, *et al*. High concentration of blood cobalt is associated with the impairment of blood-brain barrier permeability. *Chemosphere*. 2021;273:129579.
56. Shaw P, Kumar N, Sahun M, Smits E, Bogaerts A, Privat-Maldonado A. Modulating the antioxidant response for better oxidative stress-inducing therapies: how to take advantage of two sides of the same medal? *Biomedicines*. 2022;10(4):823;1-27.
57. Rao MJ, Duan M, Zhou C, Jiao J, Cheng P, Yang L, *et al.* Antioxidant defense system in plants: reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. *Horticulturae*. 2025;11(5):477;1-33.
58. Saxena P, Selvaraj K, Khare SK, Chaudhary N. Superoxide dismutase as multipotent therapeutic antioxidant enzyme: role in human diseases. *Biotechnol Lett*. 2022;1-22.
59. Anwar S, Alrumaihi F, Sarwar T, Babiker AY, Khan AA, Prabhu SV, *et al*. Exploring therapeutic potential of catalase: strategies in disease prevention and management. *Biomolecules*. 2024;14(6):697;1-31.
60. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol*. 2024;98(5):1323-67.
61. Zhang X, Zhang W, Liu Y, Yang W, Cao J, Guo M, *et al*. Spermidine treatment delays postharvest senescence of prune (Prunus domestica L.) fruit by regulating reactive oxygen species and membrane lipid metabolism. *Postharvest Biol Technol*. 2025;228:113670.
62. Ortega DR, Esquivel DFG, Ayala TB, Pineda B, Manzo SG, Quino JM, *et al*. Cognitive impairment induced by lead exposure during lifespan: mechanisms of lead neurotoxicity. *Toxics*. 2021;9(2):23;1-30.
63. Li Y, Cai W, Ai Z, Xue C, Cao R, Dong N. Protective effects of sinomenine hydrochloride on lead-induced oxidative stress, inflammation, and apoptosis in mouse liver. *Environ Sci Pollut Res*. 2023;30(3):7510-21.
64. Pawar AT, Jogdeo C, Upaganlawar A, Chandrasekar SB, Putta S. Cholinergic neurotransmission. In: Neurochemical Systems and Signaling. 2023. p. 19-31.
65. Liu Y, Yang W, Xue J, Chen J, Liu S, Zhang S, *et al*. Neuroinflammation: the central enabler of postoperative cognitive dysfunction. Biomed Pharmacother. 2023;167:115582.
66. Huang Q, Liao C, Ge F, Ao J, Liu T. Acetylcholine bidirectionally regulates learning and memory. *J Neurorestoratol*. 2022;10(2):100002.
67. Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, *et al*. Essential metals in health and disease. *Chem Biol Interact*. 2022;367:110173.
68. Leal MFC, Catarino RI, Pimenta AM, Souto MRS. Metal chelators as part of a strategy for the treatment of neurodegenerative diseases. *Trace Elem Electrolytes*. 2023;40(3):126.
69. Li Y, Li B, Chen L, Dong J, Xia Z, Tian Y. Chelating decorporation agents for internal contamination by actinides: designs, mechanisms, and advances. *J Inorg Biochem*. 2023;238:112034.
70. Liu Y, Chen Z, Li A, Liu R, Yang H, Xia X. The phytochemical potential for brain disease therapy and the possible nanodelivery solutions for brain access. *Front Oncol*. 2022;12:936054.
71. Mishra DK, Awasthi H, Srivastava D, Fatima Z. Phytochemical: a treatment option for heavy metal induced neurotoxicity. *J Complement Integr Med*. 2022;19(3):513-30.
72. Andrabi SM, Sharma NS, Karan A, Shahriar SS, Cordon B, Ma B, *et al*. Nitric oxide: physiological functions, delivery, and biomedical applications. *Adv Sci*. 2023;10(30):2303259.
73. Yang Y, Ma K, Li S, Xiong T. Multifaceted role of nitric oxide in vascular dementia. *Med Gas Res*. 2025;15(4):496-506.
74. Kölliker-Frers R, Udovin L, Otero-Losada M, Kobiec T, Herrera MI, Palacios J, *et al*. Neuroinflammation: an integrating overview of reactive-neuroimmune cell interactions in health and disease. *Mediators Inflamm*. 2021;2021:9999146.
75. Liy PM, Puzi NNA, Jose S, Vidyadaran S. Nitric oxide modulation in neuroinflammation and the role of mesenchymal stem cells. *Exp Biol Med (Maywood).* 2021;246(22):2399-406.
76. Iova OM, Marin GE, Lazar I, Stanescu I, Dogaru G, Nicula CA, *et al*. Nitric oxide/nitric oxide synthase system in the pathogenesis of neurodegenerative disorders—an overview. *Antioxidants*. 2023;12(3):753;1-23.
77. Kıran TR, Otlu O, Karabulut AB. Oxidative stress and antioxidants in health and disease. *J Lab Med*. 2023;47(1):1-11.
78. Kalkan H. The program cell death (apoptosis) and the therapy of cancer. In: Tutar Y, editor. Regulation and Dysfunction of Apoptosis. London: IntechOpen; 2021, p. 25.
79. Hajibabaie F, Abedpoor N, Mohamadynejad P. Types of cell death from a molecular perspective. *Biology (Basel)*. 2023;12(11):1426;1-29.
80. Pathak N, Vimal SK, Tandon I, Agrawal L, Hongyi C, Bhattacharyya S. Neurodegenerative disorders of Alzheimer, Parkinsonism, amyotrophic lateral sclerosis and multiple sclerosis: an early diagnostic approach for precision treatment. *Metab Brain Dis*. 2021;1-38.
81. Erekat NS. Apoptosis and its therapeutic implications in neurodegenerative diseases. *Clin Anat*. 2022;35(1):65-78.
82. Gupta R, Ambasta RK, Kumar P. Autophagy and apoptosis cascade: which is more prominent in neuronal death? Cell *Mol Life Sci*. 2021;78(24):8001-47.
83. Saramowicz K, Siwecka N, Galita G, Kucharska-Lusina A, Rozpędek-Kamińska W, Majsterek I. Alpha-synuclein

contribution to neuronal and glial damage in Parkinson's disease. *Int J Mol Sci*. 2024;25(1):360.
84. Orning P, Lien E. Multiple roles of caspase-8 in cell death, inflammation, and innate immunity. *J Leukoc Biol*. 2021;109(1):121-41.
85. Eskandari E, Eaves CJ. Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. *J Cell Biol*. 2022;221(6):e202201159.
86. Pang J, Vince JE. The role of caspase-8 in inflammatory signalling and pyroptotic cell death. *Semin Immunol*. 2023;70:101832.
87. Sharma P, Kaushal N, Saleth LR, Ghavami S, Dhingra S, Kaur P. Oxidative stress-induced apoptosis and autophagy: balancing the contrary forces in spermatogenesis. *Biochim Biophys Acta Mol Basis Dis*. 2023;1869(6):166742.
88. Španić E, Langer Horvat L, Ilić K, Hof PR, Šimić G. NLRP1 inflammasome activation in the hippocampal formation in Alzheimer's disease: correlation with neuropathological changes and neuronal loss. *Cells*. 2022;11(14):2223.
89. Wójcik P, Jastrzębski MK, Zięba A, Matosiuk D, Kaczor AA. Caspases in Alzheimer's disease: mechanism of activation, role, and potential treatment. *Mol Neurobiol*. 2024;61(7):4834-53.
90. Jayaraman A, Htike TT, James R, Picon C, Reynolds R. TNF-mediated neuroinflammation is linked to neuronal necroptosis in Alzheimer's disease hippocampus. *Acta Neuropathol Commun*. 2021;9:1-21.
91. Sharma VK, Singh TG, Singh S, Garg N, Dhiman S. Apoptotic pathways and Alzheimer's disease: probing therapeutic potential. *Neurochem Res*. 2021;46(12):3103-22.
92. Balusu S, De Strooper B. The necroptosis cell death pathway drives neurodegeneration in Alzheimer's disease. *Acta Neuropathol*. 2024;147(1):96;p1-17.
93. Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M, *et al*. Caspase-3: structure, function, and biotechnological aspects. *Biotechnol Appl* Biochem. 2022;69(4):1633-45.
94. Abed M. A comprehensive examination of human brain disorders. *J Biomed Sustain Healthc Appl*. 2023;3(2):141-52.
95. Naqi SZ. A comparative study of the histological changes in cerebral cortex, hippocampus, cerebellum, pons and medulla of the albino rat due to lead toxicity. *Int J Anat Res*. 2015;3(2):1173-8.
96. Ramírez Ortega D, González Esquivel DF, Blanco Ayala T, Pineda B, Gómez Manzo S, Quino JM, *et al.* Cognitive impairment induced by lead exposure during lifespan: mechanisms of lead neurotoxicity. Toxics. 2021;9(2):23;1-30.
97. Carmona A, Roudeau S, Ortega R. Molecular mechanisms of environmental metal neurotoxicity: a focus on the interactions of metals with synapse structure and function. *Toxics*. 2021;9(9):198.
98. Enogieru AB, Egbon FO. Neurobehavioural and histological alterations in lead acetate-exposed rats pretreated with aqueous leaf extract of Vernonia amygdalina. *Afr J Biomed Res*. 2022;25(2):205-13.
99. Oyeyemi IT, Akinlabi AA, Adewumi A, Aleshinloye AO, Oyeyemi OT. Vernonia amygdalina: a folkloric herb with anthelminthic properties. *Beni-Suef Univ J Basic Appl Sci*. 2018;7(1):43-9.
100. Bellavite P. Neuroprotective potentials of flavonoids: experimental studies and mechanisms of action. *Antioxidants*. 2023;12(2):280.

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