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Research Article

Neuroprotective Effect of Monodora myristica on the Lead-induced Injury of the Cerebral Cortex of Male Wistar Rats

Orish CN[1]*, Valentine Chidozie Amasiatu[2] and Chidi U Betrand[3]

[1]Department of Anatomy, Faculty of Basic Medical Science, University of Port-Harcourt, Rivers State, Nigeria

[2]Department of Anatomy, Gregory University Uturu, Uturu, Nigeria

[3]Department of Computer Science, Federal University of Technology - Owerri, Nigeria

***Corresponding Author:** Orish CN, Department of Anatomy, Faculty of Basic Medical Science, University of Port-Harcourt, Rivers State, Nigeria.

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Abstract

Background: Lead (Pb) is one of the most common environmental toxicants, exposure to which can cause significant neurotoxicity and an associated decline in brain function. Plant-derived products with antioxidants activity are useful in reducing lead-induced neurotoxicity. This study investigated neuroprotective and antioxidant properties of the aqueous *Monodora myristica* against lead-induced neurotoxicity in male Wistar rats.

Methods: Six groups of six weight-matched animals each, were used for the study. Group 1 (normal control) was treated with deionized water and group 2 (toxic control) with lead acetate at the doses of 50 mg/kg b.wt, whereas group 3 received *M. myristica* 40 mg/kg only, groups 4,5, and 6 were simultaneously treated with lead (50 mg/kg b.wt), and 40 mg/kg, 80 mg/kg, and 160 mg/kg of *M. myristica* respectively. The treatment was administered orally for 42 days. Their biochemical and histopathological investigations were carried out following animal sacrifice at the end of the study period. The neuroprotective effect of *M. myristica* was assessed by measuring redox status (malondialdehyde), enzymatic antioxidant activities (Superoxide dismutase, Catalase, Glutathione peroxidase and glutathione reductase) and histopathology of the cerebral cortex.

Results: The increase in the malondialdehyde, the decrease in the activity of antioxidant enzymes (Catalase, Glutathione peroxidase and reductase), and the altered histology of the brain induced neurotoxicity by lead acetate were mitigated in the brain of rats treated with *M. myristica*.

Conclusion: Aqueous *M. myristica* has a neuroprotective role against lead-induced neurotoxicity probably mediated through its antioxidant properties.

Keywords: Neurotoxicity; *Monodora myristica*; Antioxidant; Lead

Introduction

Lead (Pb) is ubiquitous in the environment because of its natural occurrence and extensive industrial use. Diverse anthropogenic activities impact the body's burden of lead [1]. Pb has a wide-ranging negative effect on many organs and induces many biochemical, physiological, and behavioural alterations [2]. The brain is particularly susceptible to the deleterious effects of Pb. Oxidative stress has been recognized to be a major indirect mechanism of Pb neurotoxicity [2]. The induction of oxidative stress is characterized by increased levels of reactive oxygen

species (ROS) such as superoxide (O^{2-}) and hydroxyl (•OH) radicals, hydrogen peroxide (H_2O_2), and lipid peroxide [2]. Medicinal plants are important sources of drugs [3]. Modern pharmaceutical industry is founded on compounds identified in medicinal plants [3]. Various crude extracts and drugs of plant origin have shown promise in the treatment of a range of neurological diseases, and many studies have demonstrated how the antioxidant properties of natural products help to mitigate the toxicity of lead acetate. *Monodora myristica* also called calabash, Jamaica or African nutmeg is a tropical plant that belongs to the Annonaceae family. This less-studied the and the greatly under-exploited plant is extensively distributed in Africa, Asia, Australia as well as Central and South America [4]. It is used to manage so many ailments like arthritis, and stomach aches. Previous studies have reported the antioxidant properties of *M. myristica* seeds [5,6].

To date, however, there remains a paucity of data on the neuroprotective effects of *M. myristica* on Pb-induced neurotoxicity. The present study has evaluated the ameliorative effects of *M. myristica*, on Pb-induced neurotoxicity in rat models.

Materials and Methods

Sample identification

Dried fruit *Monodora myristica* was bought from the local market in Port Harcourt, Rivers State, Nigeria. It was identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy, University of Port Harcourt, Rivers State Nigeria.

Sample processing and extraction

The dried *Monodora myristica* was milled into fine powder. After weighing the powder, the extract was prepared using a cold maceration method. One gram of the powder was dissolved in 100 ml of deionized water and left covered for 24 hours. After 24h, the mixture was strained, the marc was pressed, and the extract was filtered and stored in the refrigerator at 4°C. Fresh extracts were prepared every three days.

Experimental design

Six groups of five weight-matched animals each were used for the study. Group 1 was treated with deionized water and Group 2 animals received only 50 mg/kg b.wt. lead acetate, group 3 received 40 mg of *M. myristica* only Group 4-6 received 40, 80 and 160 mg/kg of *M. myristica* p.o. and 50 mg/kg b.wt of lead acetate orally respectively for 42 days [7]. Animals were sacrificed for 24 h under ethyl ether anaesthesia after the last treatment. The brain was excised, and preserved in 10% formalin for histopathological study. All the experimental procedures were performed according to the committee for control and supervision of experiments on animal, norms and approved by the Institutional Animal Ethical Committee University of PortHarcourt Nigeria.

Antioxidant assay

The cerebrum was dissected minced into small pieces and homogenized with ice-cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 rpm for 15 mins at 4°C. The supernatant was collected for the estimation of catalase (CAT) activity using hydrogen peroxide as substrate according to the method of Clairborne (Clairborne 1995) [8]. Superoxide dismutase (SOD) was assayed by the method described by (Misra and Fridovich 1972) [9]. Glutathione-S-transferase (GST) was assayed by the method of Habig., *et al*. 1974[10]. Reduced glutathione (GSH) was determined at 412 nm using the method described by Sedlak and Lindsay 1996 [11]. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa., *et al*. 1978 [12] and the MDA level was calculated according to the method of Todorova., *et al*. (2005) [13]and expressed as µmol MDA/mg protein.

Histopathological studies

Brain from all the experimental groups was fixed in 10% formaldehyde, dehydrated in graded alcohol, cleared in xylene and then embedded in paraffin. Microtome sections (5 µm thick) were prepared from each brain sample and stained with toluidine blue dye according to the method by Orish., *et al.* 2021 [14]. The sections were examined for the pathological findings.

Statistical analysis

The data were expressed as Mean ± Standard deviation (SD) and analyzed using SPSS (Statistical Package for Social Sciences) and one-way analysis of variance (ANOVA) followed by pa post-hoc Turkey test. Values of *$p < 0.05$ were considered statistically significant.

Results

Figure 1 shows the effect of *M. myristica* on the feed intake of lead treated Wistar albino rats. Lead treatment (Group 2) caused a

significant decrease in feed intake when compared with the control group, whereas Gps 4-6 that received both lead and *M. myristica* had a significant decrease in feed intake when compared with control.

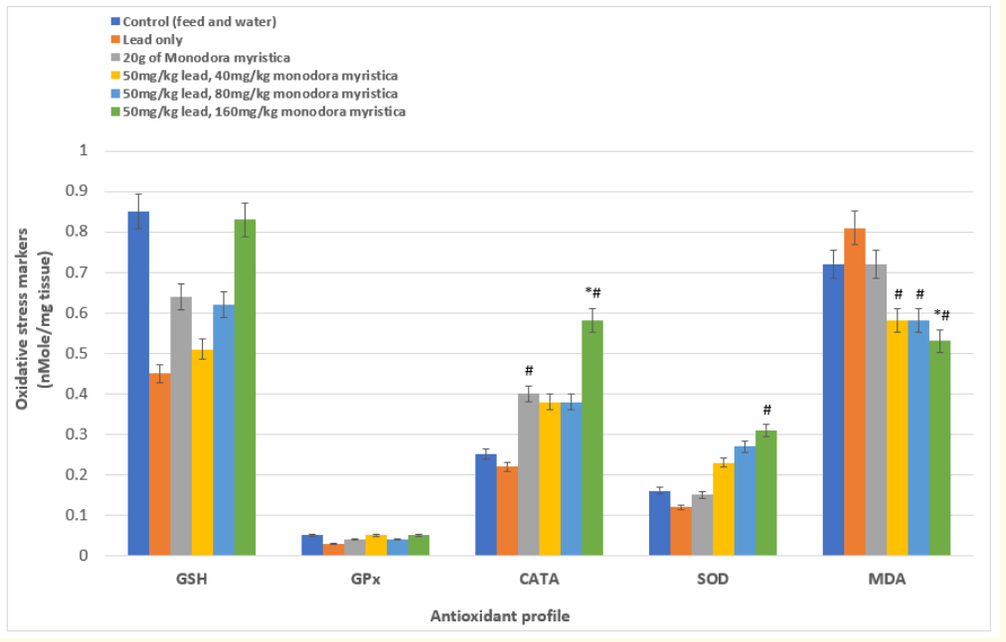

Figure 1: Bar chart showing the effect of *Monodora myristica* on the oxidative stress markers. Each value represents mean±SD, n = 3, CATA = Catalase, GSH = Reduced Glutathione, SOD = Superoxidase dismutase, GPX = Glutathione Peroxidase, MDA = Malondialdehyde. Each value represents mean±SD, Values marked with asterisk (*) differ significantly from control value (*p < 0.05) while those marked with (#) differ significantly from lead only group (#p < 0.05).

Figure 2 shows the effect of *M. myristica* on the fluid intake of lead treated Wistar albino rats. Lead treatment (Group 2) caused a significant decrease in fluid intake when compared with the control group, whereas Gps 4-6 that received both lead and *M. myristica* had a significant increase in fluid intake when compared with control.

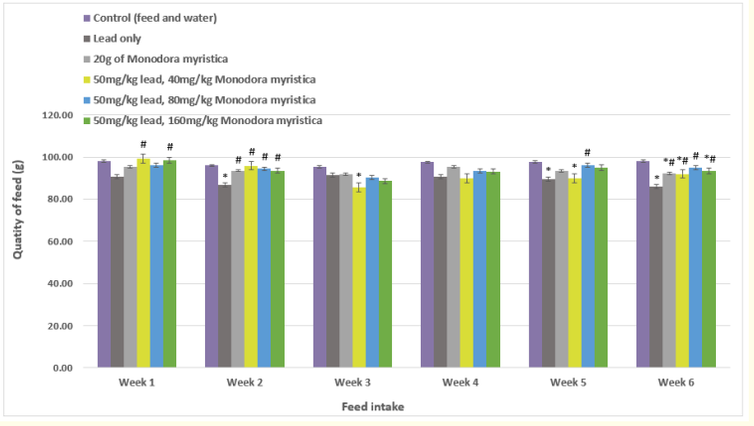

Figure 2: Bar chart showing the effect of *Monodora myristica* on the feed intake of albino Wistar rats.

Each value represents mean±SD, Values marked with an asterisk (*) differ significantly from the control value (*p < 0.05) while those marked with (#) differ significantly from the lead only group (#p < 0.05).

The effect of *M. myristica* on the oxidative stress markers of lead treated Wistar rats is shown in figure 4. Lead treatment (Group 2 caused a decrease in antioxidant enzymes namely Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Glutathione-S-transferase (GST) when compared with the normal control group, whereas Gps 4-6 that received both lead and *M. myristica* had significant increased antioxidant enzymes namely Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Glutathione-S-transferase (GST) when compared with toxic control (lead acetate only). Lead treatment caused an increase in malondialdehyde (MDA) when compared with control, whereas Gps 4-6 that received both lead and *Monodora myristica* had a significant decrease in malondialdehyde (MDA) when compared with control

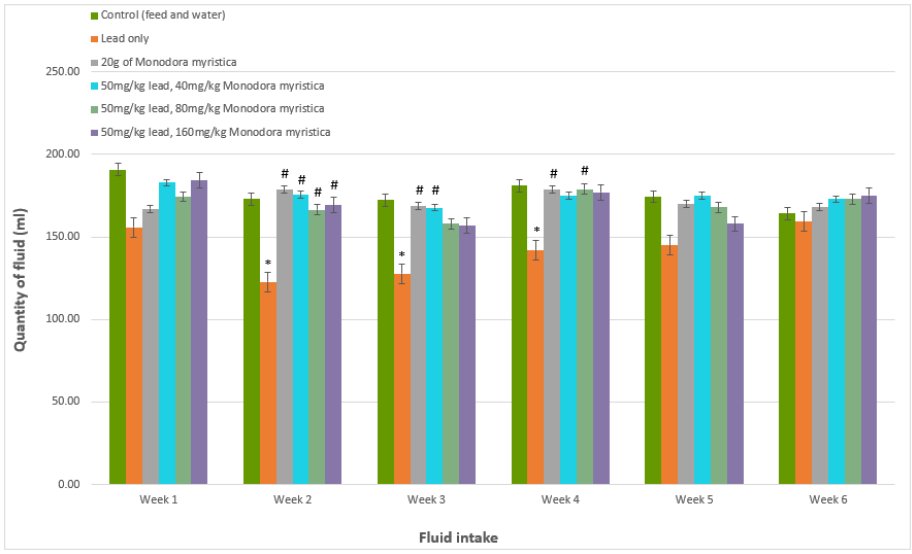

Figure 3: Bar chart showing the effect of *Monodora myristica* on the fluid intake of albino Wistar rat.

Each value represents mean±SD, Values marked with an asterisk (*) differ significantly from the control value (*p < 0.05) while those marked with (#) differ significantly from the lead only group (#p < 0.05).

Figure 4

The neuroprotective effect of *M. myristica* on the lead-induced injury on the cerebrum in male Wistar rats is shown in figure 1(a-d).

The control group (Figure 1a) showed intact neuronal cells with no degeneration whereas in the Pb only treated group, there was neuronal degeneration evidenced by cytoplasmic vacuolation (Figure 1b). Group treated with *M. myristica* only, the neuronal cells in various layers appear intact with increased regeneration (Figure 1c). The concurrent treatment with *M. myristica* showed neuronal regeneration in cerebral histology (Figure d,) evidenced by reduced cell vacuolation.

Discussion

Lead exerts some of its neurotoxic effects by promoting oxidative damage and peroxidation of the lipids in the cell membranes, thus compromising cellular functions by impairing the physiological functions, fluidity, and integrity of cell membranes, thereby increasing the cell vulnerability to lipid peroxidation and cell death [1]. Both animal and human studies have suggested that exposure to Pb is associated with increased oxidative stress and a heightened incidence of neurotoxicity. Numerous natural products are effective antioxidants and many medicinal plants with a long history of use in folk medicine in different countries against a variety of diseases have turned out to be rich sources of antioxidants [13,14]. Recently, great interest has been given to naturally occurring antioxidants which played prominent roles in inhibiting both free radicals generation and oxidative chain reactions within tissues and membranes [15].

Antioxidants exert their effects via several basic mechanisms, which include scavenging the species that initiate peroxidation, quenching singlet oxygen, chelating metals, breaking free radical chain reactions, and reducing the concentration of O_2. As powerful free radical scavengers, they can act as very effective neuroprotective agents against lead-induced oxidative stress. The presence of alkaloids, saponin, tannins, flavonoids, cardiac glycosides and phenols in varying quantities in the aqueous extract of *M. myristica* seeds has accounted for its antioxidant property [16] yet there is paucity of information as regards *M. myristica*.

Exposure to lead has been reported to cause oxidative stress and cause neurotoxicity. An increase in MDA with a concomitant decrease in the activities of SOD and CAT and decreased GSH levels was observed in the study of the protective effect of curcumin against lead neurotoxicity [17]. Our observation of increased malondialdehyde (MDA) by lead treatment in rat cerebral cortex and a significant decrease in total antioxidants with lead confirms the role of oxidative stress in lead-induced neurotoxicity This is consistent with earlier studies [2]. Lead treatment of the rats increased cerebral tissue (L-MDA) levels as compared with the *Monodora myristica* extract treated groups ($p < 0.05$) and a decrease in antioxidant glutathione, glutathione peroxidase, catalase and superoxide dismutase activity which had been reported by others [18,19]. This is similar to the present study.

M. myristica seed is a rich source of flavonoids which have been shown to exhibit both antioxidant and anti-inflammatory properties [9]. *M. myristica* has strong antioxidants which ameliorated hepatocellular damage caused by cadmium intoxication in experimental rats in a dose-dependent manner by improving the antioxidant defence systems as well as mitigating lipid peroxidation associated with cadmium toxicity [16]. Lead was administrated orally in the doses of 50 mg/kg for a period of 8 weeks and study was performed at the end of exposure. The present study also corresponds to research work on title Lead intoxication: Histological and oxidative damage in rat cerebrum and cerebellum which showed decrease in the concentration of all the antioxidant enzymes after lead treatment, as well as degeneration of neurons [20]. This is consistent with our study. *M. myristica* essential oils were found to reduce stress-related disorders like depression significantly restoring the hippocampal redox balance state and attenuating CUMS-induced oxidative damage which is inconsistent with this present study on restoring oxidative enzymes [21].

Conclusion

M. myristica seeds aqueous extract may have a protective role against Lead-induced neurotoxicity probably mediated through its antioxidant properties.

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