ORIGINAL ARTICLE

# *Mucuna puriens* root extract: A novel therapeutic agent for lead induced polyphagia and hippocampal neurotoxicity in adult Wistar rats

*Vivian Onyinye Atuadu[1], John Chukwuma Oyem[2*], Elizabeth Finbarrs-Bello[1], Cynthia Ezeah[3]*

*[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria, [2]Department of Anatomy, College of Health Sciences, Novena University Ogume, Delta State, Nigeria, [3]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Nsukka, Enugu Campus (UNEC), Enugu State, Nigeria*

**Abstract**

*Background:* Environmental lead exposure is a public health challenge in Nigeria, and chronic lead exposure results in neurological conditions such as headaches, depression, short and long-term memory impairment, loss of motor coordination and sleep disturbances. *Aim and Objective:* This study evaluated the roles of *Mucuna puriens* (MP) root extract on Lead Acetate (LA)-induced neurotoxicity in adult Wistar rats. *Material and Methods:* It employed an *in vivo* model of LA-induced neurotoxicity and administered 100, 150, and 200 mg/kg/bwt of MP to the pretreated groups (C-E). Group A served as the control group and received 1 mg/kg/bwt of normal saline. In contrast, Group F, the reference group, was administered 120 mg/kg/bwt of LA and 100 mg/kg/bwt of Vitamin E. Appetites of animals were assessed by measuring changes in body weight and quantity of left-over feeds at the end of the administration. Furthermore, the activities of Malondialdehyde (MDA) and Glutathione (GSH) concentrations and histopathological investigations were measured. *Results:* Our result revealed neuronal loss and degeneration, characterized by edema, pyknosis, and chromatolysis on the hippocampal cells of experimental rats administered LA. However, following a co-administration with MP, there was a dose-dependent preservation of hippocampal architecture from neuronal degeneration. The beneficial effect was also confirmed by a reduction in MDA activities and an elevation in GSH concentrations. It showed that MP regulates polyphagia by causing a significant decrease in appetite with increased body weights. *Conclusion:* The current study indicates that LA exposure induces neuronal toxicity, oxidative stress, and alters appetite in Wistar rats, which is reversed by daily MP administration. This finding is probably due to the antioxidant activities of MP.

**Keywords:** Lead; Neurotoxicity, Hippocampus, Polyphagia, Oxidative stress, Mucuna puriens

## Introduction

Over the years, environmental exposures to heavy metals have been linked as a significant contributor to the pathogenesis of neurological diseases [1]. Lead is a heavy metal that accumulates in vital organs, with a particular predilection to the kidneys and brain [2]. Occupational and environmental lead exposure is now a public concern due to its ubiquity, long-lasting deleterious effects, and irreversible nature [3].

An annual report on the levels of lead exposure revealed that Africans and Asians have the highest levels of lead exposure that is probably derived from anthropogenic sources, such as the burning of lead gasoline and unregulated lead industrial

emission [4-5]. High lead proportions have been detected in contaminated foods, drinks, and equipment made of lead; water bodies used to supply the public with drinking water, and in equipment made of lead in the Northern part of Bangladesh [4]. Although public water systems have adopted water treatments and purifiers to reduce lead contamination, the concentrations of lead in 3159 samples collected in Ontario were reported to range from < 0.02 to 1320 μg/L [6].

The Central Nervous System (CNS) is the most susceptible to the deleterious effect of lead due to oxidative damage [7]. This may be due to the brain's high oxygen consumption levels and richness in iron and polyunsaturated fatty acids [7-8]. Xue *et al.* (2008) identified lead as a contributing agent in the pathogenesis of lead poisoning [9]. In a study by Halliwell in 2006, lead poisoning induced lipid peroxidation, DNA damage, and signal transduction regulation [9]. Other fundamental mechanisms of lead-induced toxicity include neurotransmission impairment and deregulation of cell signaling by inhibiting protein kinase C [3].

Lead exposure in utero has detrimental effects on the developing brain of infants and children, causing learning impairment and cognitive and neurobehavioral deficits [10]. The World Health Organization (WHO) in 2013 estimated that 600,000 children exposed to lead have intellectual disabilities; 99% of them reside in developing countries [11]. Lead exposure in infancy impedes the child's mental development and causes a low intelligent quotient that may persist to adulthood.

Several studies posited oxidative stress as one of the mechanisms of lead-induced neurotoxicity [10, 12-13]. According to Ghandhi and Abramov (2012), oxidative stress is a significant factor in the pathogenesis of neurodegenerative diseases [13]. Lead reduces tissue Glutathione (GSH) concentrations. It thus impairs the endogenous antioxidant system [10], which may result in brain dysfunctions that may lead to poor verbal skills, intelligence quotient, performance intelligent quotient, academic skills such as mathematics and reading, visual/spatial skills, problem-solving skills, executive functions, motor skills, and memory and language skills [14].

*Mucuna pruriens* (MP), also known as "the cowhage" or "velvet" bean, is an established traditional medicine that is used as an aphrodisiac and in the management of male infertility and nervous disorders [15]. In ancient Indian medicine, MP was used to treat Parkinson's disease, probably due to its high concentration of l-3,4-dihydroxyphenylalanine (L-DOPA); a precursor of neurotransmitter dopamine [16]. It is well known that dopamine is a neurotransmitter; there is a reduction in the dopamine content in the brain when there is a disruption in tyrosine conversion [17]. MP enters the CNS through L-DOPA, a dopamine precursor. It transverses the blood-brain barrier and undergoes conversion to dopamine, thereby restoring neurotransmission [17-18].

It was also used as an anti-tumour agent, an insecticide, and an anti-venom that neutralizes the venom from scorpion stings [19-20]. Several studies revealed the presence of chemical constituents in MP extracts such as L-DOPA, hallucinogenic tryptamines, phenols, and tannins, which exhibited anti-diabetic, anti-inflammatory, neuroprotective and antioxidant properties [15-16, 20, 22]. A study by Gupta *et al*. reported

antiepileptic and anti-neoplastic activities for the methanol extract of MP; another study posited that the methanol extract of MP seeds possessed potent *in vitro* antioxidant activities, which indicates it may be a potential source of natural antioxidants and anti-microbial agents [23].

Phytochemical screening on MP revealed the presence of n-hexadecanoic acid (48.21%), squalene (7.87%), oleic acid (7.62%), ascorbic acid (3.80%), and octadecanoic acid (6.21%) [24]. Sulphur-containing amino acids and other compounds such as polyphenols, trypsin inhibitors, phytate, cyanogenic glycosides, oligosaccharides, saponins, lectins, and alkaloids have been reported [25]. In addition, mucunine, mucunadine, prurienine, prurieninine, glutathione, garlic acid, beta-sitosterol, and serotonin have been reported in extracts of MP [26].

Among its numerous health benefits, several studies have shown a therapeutic benefit for this plant's antioxidant properties in Parkinsonism [27-29]. Kumar and Muthu (2010) showed that ethyl acetate and methanolic extract of MP contain large quantities of phenolic compounds and possess high antioxidant and free radical scavenging activities [30]. This suggests that this plant extract is an essential source of natural antioxidants that can be useful in preventing different oxidative stressors [30]. However, based on the existing literature, it is unknown if the root extract of MP will avert hippocampal damage and polyphagia in lead acetate induced Wistar rat models of neurotoxicity. Therefore, the current study investigated the neuroprotective properties of MP root extract on a lead acetate-induced oxidative stress and polyphagia on the hippocampal brain tissue of adult Wistar rats. This research explores intervention strategies and therapeutic design targets imperative to combat lead-induced neurodegenerative diseases.

## Material and Methods

### Plant collection and authentication

Fresh roots of MP were purchased from farmland in Obinagu, Amechi-Idodo in Nkanu-East LGA of Enugu State, South-Eastern part of Nigeria. It was identified and authenticated in the Department of Agricultural Science, Enugu State University of Science and Technology, Nigeria, with a reference number; University of Nigeria Herbarium (UBN 162).

### Chemical compound collection

Lead Acetate (LA) (catalogue number-316512, purity level-99-103%), produced by Indian platinum limited with a molecular weight of 379.333g/mol and a density of 3.25 g/cm$^3$, was purchased from a registered chemical dealer in Ogbete main market, Enugu, Nigeria. Vitamin E supplements (catalogue-47786, purity level-97.5%, Jiangang Natural Vitamin E soft gels, China) were also purchased from a registered pharmacy at the same location. The manufacturers' details, batch number, and expiry dates of both compounds were considered.

### Ethical approval

Approval for this study was obtained from the Basic Medical Science Ethical Committee (Ref number: ESUCOM/FBMS/ETR/2019/012), Enugu State University of Technology Enugu, Nigeria. Experimental animals were handled and cared for as per the guidelines of Animal Research Ethics [31]. The research procedures complied strictly and conform to the "Guide to the care and use of laboratory animals in research and

teaching" as prescribed in NIH publications volume 25 No. 28 and as recommended by Helsinki declarations.

**Plant extract preparation**

Plant extraction was carried out according to Altemimi *et al*. [32]. The fresh roots of MP were washed under continuous flowing currents of tap water to remove dust and dirt. The roots were cut into small pieces, air-dried for about three weeks in a well-aerated atmosphere, and prevented from sunlight to avoid vital phytoconstituents denaturation. The dry roots were ground into a fine powder using an electronic grinder. Then, 100 g of the fine powder was soaked in 100 ml of absolute ethanol in a well-covered flask and was vigorously shaken every 12 hour to allow adequate extraction for the next 48 hour. The mixture was filtered twice with a Whatmann no 1 filter paper. After that, the decoction was evaporated in a water bath at 50°C and dried into a pulp using a vacuum desiccator. The extract was transferred to an airtight bottle and stored in a refrigerator at 4°C before use.

**Animal treatment**

Thirty-six adult male Wistar rats (weighing about 120-160 g) were purchased from the animal house of the College of Medicine, University of Nigeria, Enugu, Nigeria. The animals were bred in the animal facility. They were housed in netted iron cages and had free access to food (rat chow) and water *ad libitum*. The animals were maintained under standard laboratory conditions and allowed for two days of acclimation before the experiment. The sample size was determined using the resource equation [33]:

E = Total number of animals – Total number of groups, where E is the degree of freedom for the analysis of variance.

A total of 36 male adult Wistar rats were used for the study. At the end of the acclimation, 36 animals were weighed, sorted, and randomly divided into six groups of six animals each. They were labelled Groups A-F, of which Group A served as the control group, while Groups B-F served as the experimental groups. The experimental groups were administered 120 mg/kg of LA and various doses of MP extracts (Table 1) orally with an orogastric tube. All doses were compared to the administration of Vitamin E (100 mg/kg/day), a standard drug. The experiment lasted for twenty-four days, with animals' body weights and appetite monitored weekly and daily during the investigation. The extract dosage used was adopted from Fung *et al*. [34], and the dosage of vitamin E used was adopted from Jaarin *et al*. [35]. Since there is no safe level of lead exposure in the environment, the 120 mg/kg of LA administered to experimental animals was adopted from Muhammed *et al*. [36]. The vehicle of transport used for LA was water. The median lethal dose ($LD_{50}$) of the MP extract is 2000 mg/kg [37].

**Table 1: Animal groupings and experimental design**

| Groups | Duration | Dosage (mg/kg/bwt) |
|---|---|---|
| **Group A (Control group)** | 28 days | Normal saline |
| **Group B (Positive control)** | 28 days | LA at 120 mg/kg |
| **Group C (Test group 1)** | 28 days | 120 mg/kg of LA(14 days) +100 mg/kg of MP (14 days) |
| **Group D(Test group 2)** | 28 days | 120 mg/kg of LA (14 days) +150 mg/kg of MP (14 days) |
| **Group E (Test group 3)** | 28 days | 120 mg/kg of LA (14 days) +200 mg/kg of MP (14 days) |
| **Group F (Standard group)** | 28 days | 120 mg/kg of LA (14 days) +100 mg/kg of VE (14 days) |

*LA: Lead acetate, MP: Mucuna puriens, Bwt: body weight, Vit E: Vitamin E.*

**Observation for appetite of experimental animals**

All experimental animals were fed with equal quantities of feed (growers mesh) in each cage every morning. Appetite was observed by weighing and comparing the quantity of leftover feeds between the control and experimental groups. The quantity of food was measured daily for all groups by the same observer in the evening and the morning all through the experiment.

**Animal sacrifice**

At the end of the experiment, the rats were sacrificed under anesthesia with chloroform and decapitated. The skull was carefully dissected to obtain the brain tissue of the experimental animals.

**Biochemical analysis**

Biochemical assays were carried out using brain homogenates.

**Measurement of total lipid peroxidation**

Part of the dissected hippocampal brain tissue was washed in normal saline solution, minced, and homogenized in ice-cooled potassium phosphate buffer (50 mM pH 7.5). The level of lipid peroxidation was determined according to the modification method of Niehaus and Samuelsson [38].

**Determination of reduced glutathione**

Part of the brain was briefly rinsed with normal saline solution, minced, and homogenized in ice-cooled buffer containing: 1.15% KCl, 0.01M sodium phosphate buffer pH 7.4. Concentrations of GSH were determined using the modified method of Ellman [39].

**Histological procedures and photomicrography**

The fresh brain tissues were fixed in a freshly prepared 10% formal saline solution for 48 hours, processed using standard histological tissue processing protocols described by Burnett and Crocker [40]. Brain tissues were processed with an automatic tissue processor. Tissues were dehydrated by passing tissues in 70%, 90%, 95%, and two grades of absolute alcohol changes for 90 minutes each. Tissues were cleared in two xylene changes for 2 hours each and infiltrated in two changes of paraffin wax for 2 hours, after which

they were embedded. Sections were cut at 5 µm with a rotary microtome and stained using Hematoxylin and Eosin (H&E) stain for general tissue architecture [40]. The prepared stained and mounted slides of hippocampal tissues were examined with a Motic™ compound light microscope using 4×, 10×, and 40× objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at 400× magnifications.

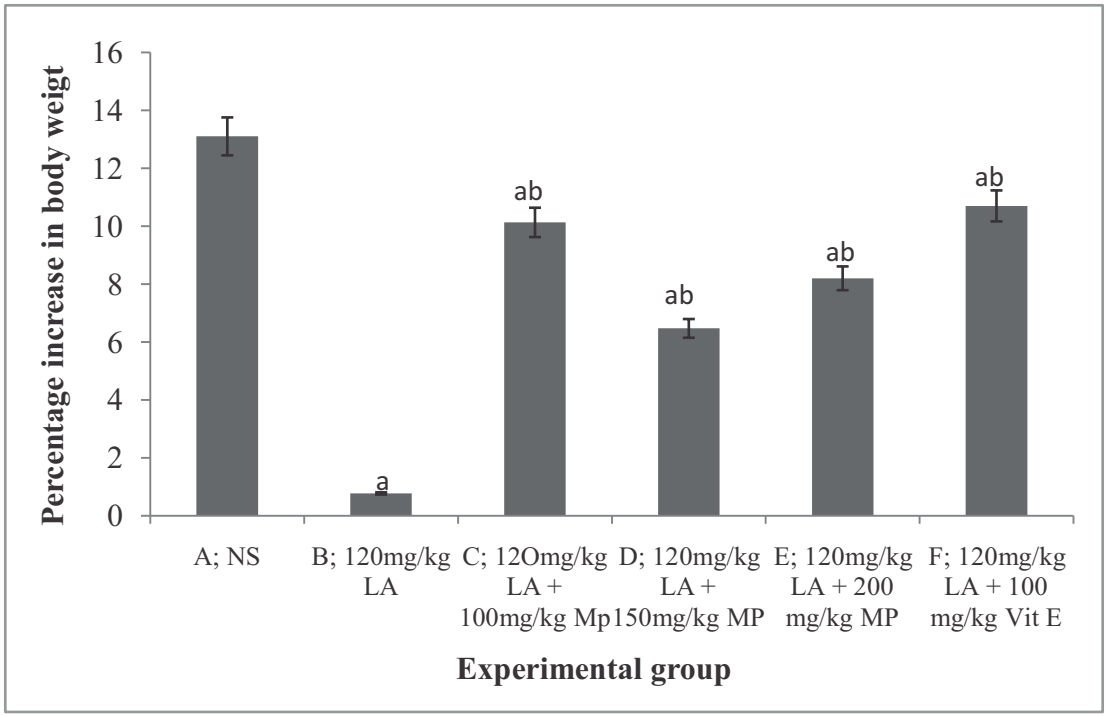
**Cell count estimation**

Viable cells in the hippocampus were estimated using the methods described by Oyem and Odokuma. [41]. ImageJ software version 6 was used for the hippocampal neuronal cell count estimation.

**Statistical analysis**

Data obtained from the neuronal count, body weight, observation of appetite, and biochemical analysis were statistically analyzed using One-way Analysis of Variance (ANOVA). Tukey's *post hoc* test was used to determine significant differences between each group. The results were expressed as Mean ± Standard Deviation (SD), using Statistical Package for Social Sciences, version 23 (SPSS produced by SPSS Inc. Chicago). Mean differences were considered significant at $p < 0.05$.

**Results**

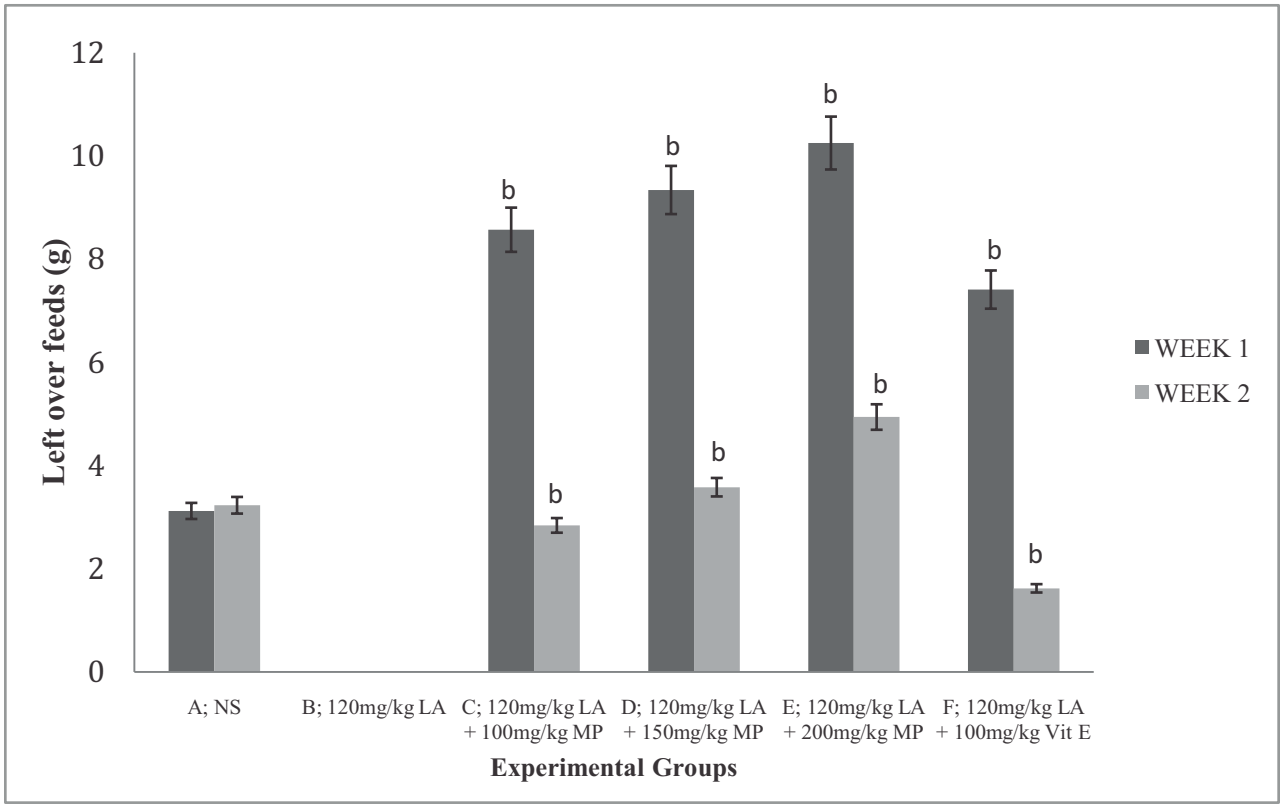



*Data were expressed as mean ± SD. Columns with 'a' are significantly different from the control groups, while columns with 'ab' are significantly different from the control and LA groups.*

**Figure 1: The percentage changes in body weights of the experimental animals during the period of MP, LA, and Vit E administration.**

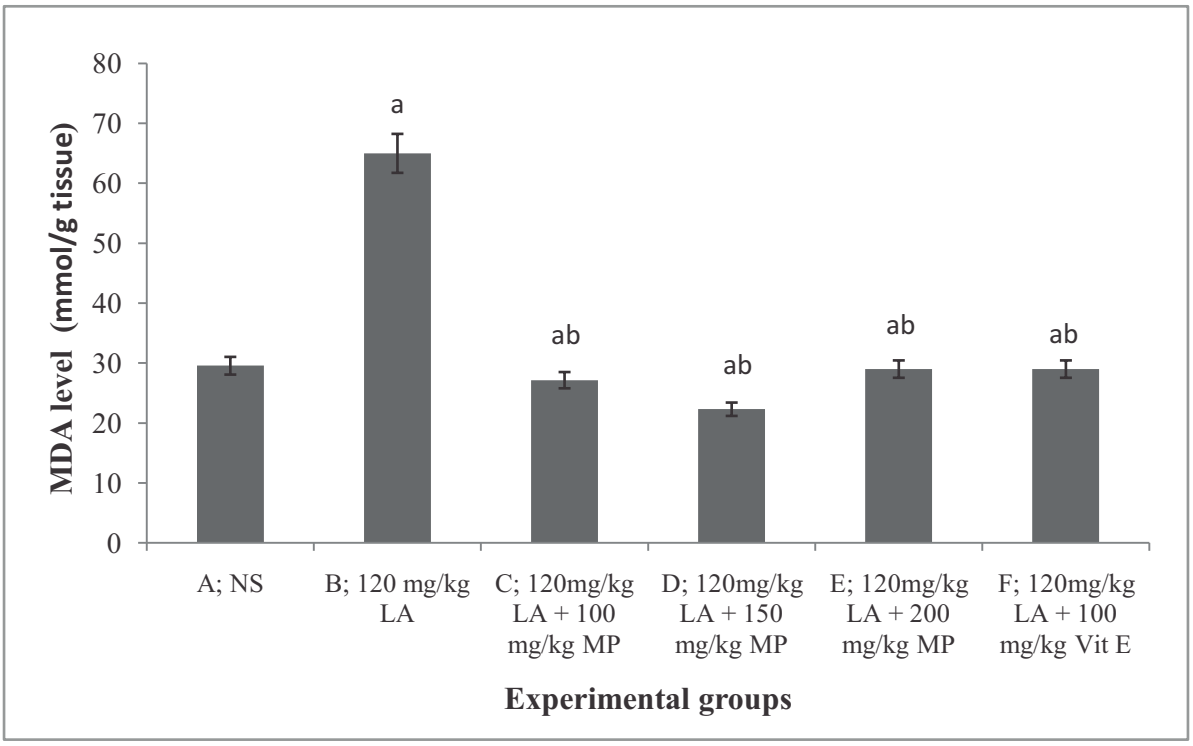
Values in Figure 1 indicated that experimental animals in Group B administered only LA showed a significant decrease ($p < 0.05$) in body weight while animals in Groups C-F that received 120 mg/kg of LA and increasing doses of the ethanolic root extract of MP and Vit E showed a rapid, significant increase in their body weights when compared with Group B animals ($p < 0.05$). The highest recorded percentage change in body weight gain of test animals was recorded in Group C (120 mg/kg LA + 100 mg/kg MP) and Group F (120 mg/kg LA + 100 mg/kg Vit E).

The appetite of animals was measured by measuring the quantity of feed leftover after administering the test compound and compared with the positive and negative control groups. The administration of LA leads to a significant decrease ($p < 0.001$) in the quantity of feed leftover at the end of week one and week 2. However, subsequent co-administration of LA and MP led to a dose-dependent significant increase in the quantity of feed leftover at the end of weeks 1 and 2. A significant decrease was observed in experimental animals administered LA and vitamin E (Vit E).




*Data were expressed as mean±SD. Columns with 'b' are significantly different from the control and LA groups.*

**Figure 2: Changes in the appetite of experimental animals administered MP, LA, and Vit E.**

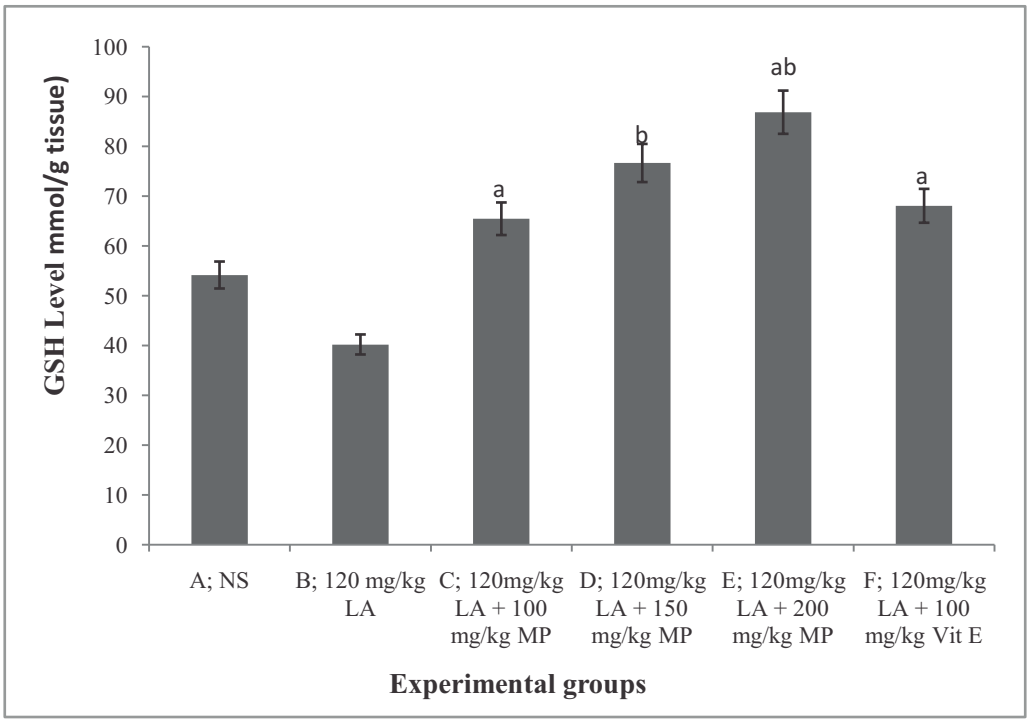



*Data were expressed as mean ± SD. Columns with 'a' are significantly different from the control groups, while columns with 'ab' are significantly different from the control and LA groups*

**Figure 3: Malondialdehyde activities in experimental animals administered MP, LA and Vit E**

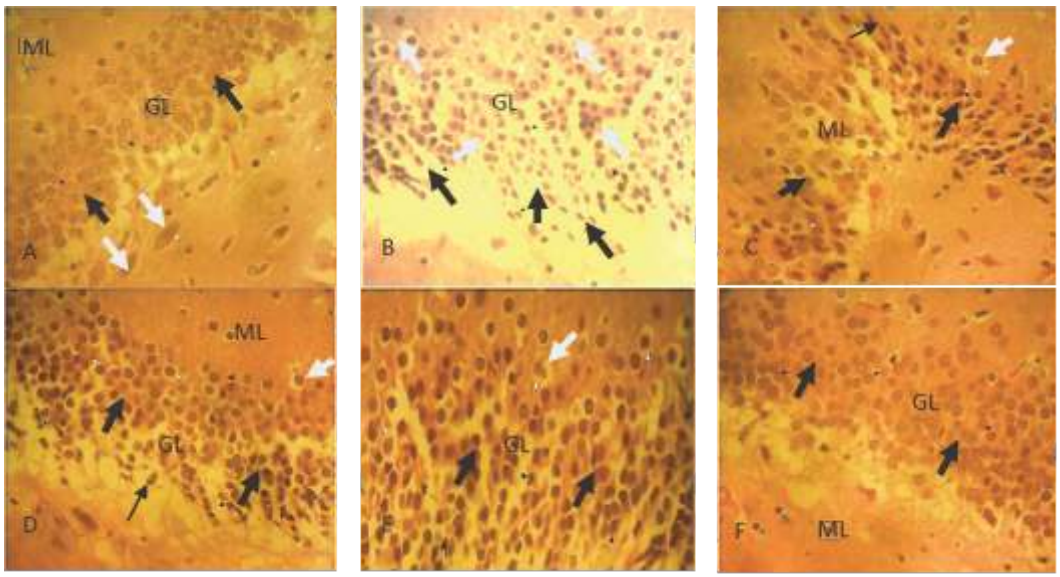
The bar chart above indicates a significant decrease in MDA activities in Groups C, D, and E compared to Group B animals. The maximal significant elevated response ($p < 0.05$) in the activity of MDA (65.00 ± 6.55) was recorded in Group B animals that received LA only. Consequently, experimental animals in Groups C and D showed a statistically significant difference ($p < 0.05$) in MDA levels compared to Group F, the standard group.

The chart above showed the mean response of experimental animals to glutathione. Experimental animals in Groups C-F administered lead acetate, and varying doses of MP or Vit E showed a rapid, significant increase ($p < 0.05$) in GSH concentrations. The highest mean significant concentrations of GSH ($p < 0.05$) was seen in group E and D with a mean GSH value of 86.85 ± 4.03 and 76.65 ± 2.76 compared to the reference group (Group F) with a mean GSH concentration of 68.05 ± 1.90.



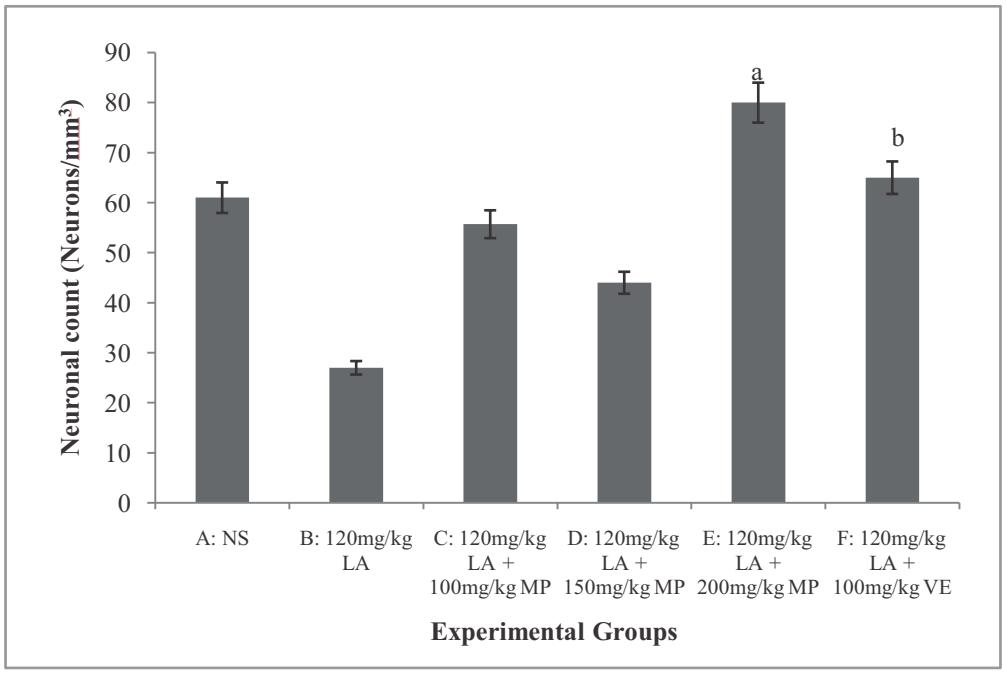

*Data were expressed as mean ± SD. Columns with 'a' are significantly different from the control groups, columns with 'b' are significantly different from the LA group, while columns with 'ab' are significantly different from control and LA groups*

**Figure 4: Glutathione concentration in experimental animals administered MP, lead acetate and Vit E**




*A: -ve control, B: LA 120 mg/kg, C: LA+ 100 mg/kg MP, D: LA + 150 mg/kg MP, E: LA + 200 mg/kg MP, F: LA + Vit E, ML: molecular layer, GL: granular layer. H and E x400.*

**Figure 5: Histological findings on the effect of MP root extract in experimental animals administered lead acetate**




*Data were expressed as mean ± SD. Columns with 'b' are significantly different from the LA group with p < 0.0,5, while columns with 'a' are significantly different from LA with p < 0.01.*

**Figure 6: Neuronal count in experimental animals administered MP, lead acetate and Vit E**

Figure 5 A – F shows the histoarchitectural details of the hippocampus of experimental animals administered with LA only, LA and root extract of MP or Vitamin E. Group A showed normal architecture of the hippocampus with a normal distribution of round to small oval diameter granular cells (black arrow) and large diameter pyramidal cells interspersed within the tissue stroma (white arrow). Group B showed small diameter granular cells round to oval, spindle, and slender in morphology (black arrow), indicating granular cell necrosis characterized by nuclei pyknosis, neuronal edema and chromatolysis (white arrow). The observed features in- Group B reveals marked degeneration of the hippocampal neurons. The microscopic section of Groups C-F revealed a proliferating hippocampal tissue with few too many pyramidal cells (C) and abundant granular cells (D, E, and F) in the tissue stroma (black arrow). Also seen are sparsely degenerating neurons with a pyknosis (thin arrow) and cytoplasmic vacuolations (white arrow) surrounding the granular cells. Features observed in Groups C-F are in keeping with restoring hippocampal tissue architecture.

The graph above shows the neuronal count in the hippocampus of the control and experimental animals. The highest number of neurons was observed in the group that received the highest dose of MP extract. Groups E and F (##p<0.01, #p<0.05*)* increase compared to group B. Other groups showed an increase in the neuronal count compared with Groups A and B but were not significant.

**Discussion**

This study aimed to examine the antioxidative properties of MP on LA-induced toxicity in the hippocampus of adult Wistar rats. Specifically, the key finding from this study is; MP extract displayed a beneficial effect on the hippocampus by preserving the hippocampal neurons, lowering the concentrations of MDA, and increasing the concentration of glutathione. The study also demonstrated the nutritional potential of this plant in the maintenance of body weight.

The harmful effects of lead exposure on body weights have been previously studied in detail over the years [42-43]. A study by Nabil *et al.* reported lead-induced a significant decrease in body weight gain and feeding efficiency [42]. Other studies have reported that reduction in body weights of experimental models and humans is a consequence of stress and malnutrition [43-44]. Our study revealed a significant decrease in body weight in animals' administered LA only. However, a two-week co-administration with the root extract of MP resulted in a significant increase in the weight of experimental animals despite changes in their feeding habit. This significant percentage increase in body weight indicates that the root extract of MP has no adverse effect on feed conversion rates in the animals. This observation may be ascribed to presence of saponin, a phytochemical compound present in MP, which, when converted to aglycon sapogenin, may have stimulatory effects on the feeding centres of animals [45].

Nevertheless, MP has been reported as an essential feed resource due to its high protein, total ash, and phosphorus [46]. The proximate nutritional composition, total protein content, and in vitro protein digestibility of MP seeds are analogous to other edible legumes, so they are applied as a dietary supplement in fish, poultry, and pigs [20, 47]. Moreover, changes in body weight could be a result of growth alterations, mainly if the phytochemical components of MP regulates the secretion of somatostatin or growth hormone, or modifies sex steroids secretion that alters the maturation patterns, or if they cause the release of neurochemicals that affects food consumption by acting on dopamine and serotonin [48]. One of the likely mechanisms for this observation could be that L-DOPA and serotonin, a constituent of MP, acts on dopamine and serotonin in the brain to release neurochemicals that affect food consumption.

It is well known that heavy metals exposure induces polyphagia and increases food intake [49]. However, since the data on the effect of LA on appetite is discordant, it was hypothesized that MP root extract would avert LA-induced polyphagia in experimental animals. In the current study, animals were exposed to LA and combination therapy of LA and different doses of MP for 28 days and the quantity of leftover feeds was measured. Finding revealed a significant dose decrease in experimental animals' appetite administered LA compared with other experimental groups. Subsequent treatment with MP root extracts resulted in a significant increase in the quantity of feed leftover. This implies that MP root extract may play a role in appetite regulation. The mechanisms of controlling one's eating ability are targets for producing weight loss

medications [50]. MP comprises 35-40% crude fibres that enhance metabolism and reduce hunger [51-52]. They have the potential to absorb cholesterol and elevate the secretion of bile, thereby causing the breakdown of fats, which is essential in weight reduction [51-52].

In addition, phytochemicals such as phenols act on adipocytogenesis by inhibiting precursor cell proliferation, improving apoptosis of adipocytes, and interrupting triglyceride absorption by decreasing pancreatic lipase formation [53]. According to Chandrasekaran *et al*. [54], plants possess anti-obesity mechanisms by reducing lipids' absorption, intake of energy, and lipogenesis. Another likely mechanism of action for this finding may be that MP root extract regulates leptin activities, which stimulates the feeling of satiety. Overproduction of leptin in the body causes leptin resistance in the hypothalamus, leading to obesity [50, 55]. Moreover, considering the global epidemic of obesity in the western world and its increasing proportions in developing countries, the appetite regulatory roles of this plant, should be investigated.

Several studies have demonstrated oxidative stress as one of the likely mechanisms of lead-induced neurotoxicity [10, 12, 55]. Lead poisoning causes lipid peroxidation, DNA damage, interruptions in physiological phenomena, and disruption of intracellular cell signaling [13]. In addition, a study reported that elevated blood lead levels altered the antioxidant defense system in Nigerian children, thus making them vulnerable to chronic diseases [56]. Oxidative damage caused by progressive GSH deficiency is considered one of the earliest biochemical indicators of neuronal degeneration [57-58]. A decrease in GSH concentrations results in deleterious effects in the CNS due to superoxide and hydrogen peroxide accumulation in the brain [59]. Our study revealed a significant decrease in GSH concentrations of animals administered LA only and a significant increase in GSH concentrations in animals administered LA and MP. This implies that root extract of MP averted antioxidative stress induced by LA administration by eliminating reactive oxygen species in the pretreated groups. This may be explained by the rapid, significant increase of GSH concentration in the pretreated groups. Another notable finding was a significant decrease in MDA concentrations in the pretreated groups compared to the positive control animals that received LA, which increased MDA concentration. The rapid drop in MDA activities in the pretreated groups is explained by an increase in the cell defense mechanisms, which resulted in a decline in the formation of free radicals and the alteration of antioxidant defence system of the body. In addition, the rapid rise in MDA activities results in damage to the neuronal membrane's polyunsaturated sites, leading to lipid peroxidation [60]. These result patterns indicated that the antioxidative properties of MP root extract protected the hippocampus from LA-induced neurotoxicity by reducing MDA activities and increasing GSH concentrations. Similarly, other studies have suggested that the phytochemical constituents of MP possess high levels of antioxidants and free radical scavenging activities [23-24, 27, 29-30]. Our previously published studies demonstrated that antioxidative properties of plants attenuated lead-induced alterations via inhibition of oxidative stress [12, 61].

Neurodegeneration is the loss of neuronal structure and functions, leading to progressive loss of cognition, memory, decision-making, and learning [62]. Neurodegeneration is a significant hallmark in the pathogenesis of Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease [62]. It could be very severe, depending on the nature of the disease and the human physiological activities that it affects. Histopathological observation of the brain tissues in our study showed that varying doses of MP were more potent in preserving the hippocampal neurons against neuronal loss and degeneration induced by LA than vitamin E. Interestingly, our observation from the hippocampal cell estimation revealed a dose-dependent significant increase in the neuronal count of experimental animals administered with the highest dose of MP compared to the control and standard groups. The dose-dependent significant increase observed in cell count analysis supports our histopathological results that MP preserves the hippocampal neuron against neuronal loss and degeneration. Thus, it is suggested that MP root extract may possess neuroprotective potentials in the hippocampus. This could be linked to the presence of phenolic acids, flavonoids and tannins that have been reported to possess potent antioxidant properties in combating neurotoxicity induced by LA [15, 16, 21-23]. Our findings concur with a study that reported that ethanol MP seed extract produces the highest neuroprotective potentials in the growth and survival of DA neurons in cell culture [17].

Although the exact neuroprotective mechanisms of MP on LA-induced neurotoxicity have not been elucidated, our result proposes that MP root extract averts LA-induced oxidative damage, neuronal degeneration, and polyphagia by decreasing MDA activities and increasing GSH concentration, decreasing polyphagia and protecting the hippocampal cells from degeneration.

## Conclusion

It demonstrated that oxidative stress, neuronal degeneration, and polyphagia are implicated in LA-mediated neurotoxicity. The protective effects of MP root extract alleviated LA-induced neuronal damage by reducing oxidative stress. The biological findings supported evidence obtained from the histological and cell count analysis and, therefore, it confirms that the root extract of MP exerts beneficial effects against LA-induced neurotoxicity. Based on the findings from this study, our results indicate that MP is exceptional against neurotoxicity. Further studies should investigate the exact mechanism of MP in averting LA-mediated neurotoxicity.

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***Author for Correspondence:***

*John Chukwuma Oyem, Department of Anatomy, College of Health Sciences, Novena University Ogume, Delta State, Nigeria Email: Johnoyem2018@gmail.com Phone: +2348067157749*



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