

Neuroprotective Effect of *Xylopia Aethiopica* Seed on Lead-Induced Injury on the Cerebral Cortex of Male Wistar Rat

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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 *Xylopia aethiopica* against lead -induced neurotoxicity in male Wistar rats. *Methods:* Six groups of six weight-matched animals each, were used for the study. The group 1 (normal control) was treated with distilled water and group 2 (toxic control) with lead acetate at the doses of 50 mg/kg b.wt, whereas group 3 received only *Xylopia aethiopica* 40 mg/kg only, groups 4, 5, and 6 were simultaneously treated with lead (50 mg/kg b.w.), and 40mg/kg, 80mg/kg, and 160mg/kg of *Xylopia aethiopica* 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 *Xylopia aethiopica* 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 *Xylopia aethiopica*. *Conclusion:* Aqueous *Xylopia aethiopica* has a neuroprotective role against lead - induced neurotoxicity probably mediated through its antioxidant properties.

Keywords: Neurotoxicity, *Xylopia aethiopica*, Antioxidant, Lead

1. Introduction

Lead (Pb) is ubiquitous in the environment and toxic. Once introduced into the body, Pb has a wide-ranging of negative effect on many organs, and induces many biochemical, physiological, and behavioral alterations [1]. The brain is particularly susceptible to the deleterious effects of Pb, which has been reported to induce damage to the nervous system through several direct and indirect mechanisms. Oxidative stress has been recognized to be a major indirect mechanism

of Pb neurotoxicity [1]. The induction of oxidative stress is characterized by increased levels of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($\cdot OH$) radicals, hydrogen peroxide (H_2O_2), and lipid peroxide [1].

Medicinal plants are important sources of drug synthesis. Ksouri (2012) [2] has argued, much of the modern pharmaceutical industry is founded on compounds identified from medicinal plants discovered by native peoples and local societies. 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 *Xylopi*a *aethiopica* or African negro pepper enjoys a huge patronage both in nutrition and in ethno medicine [3-5] The plant is thought to be rich in a number of physiologically and pharmacologically active agents, including volatile oils [6, 7]. The seed of *Xylopi*a *aethiopica* have been reported to act as antioxidant, hypolipidemic and hypoglycaemic agent [8]. Flavonoids from *X. aethiopica* have been shown to exhibit both antioxidant and anti-inflammatory properties [9]. Its antioxidant or free radical scavenging activities are considered plausible candidates for the prevention of tissue injury occasioned by lead. Antioxidant enzymes are capable of scavenging free radicals from oxidative stress thereby stabilizing the membrane. It has been found that many neurodegenerative diseases are as a result of oxidative stress which could be ameliorated by antioxidant enzymes. This antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and numerous other antioxidant phytonutrients present in a wide variety of plant foods.

To date, however, there remain a paucity of data on the neuroprotective effects of *Xylopi*a *aethiopica* on Pb-induced neurotoxicity. The current study was therefore planned to utilize a rat model to elucidate whether *Xylopi*a *aethiopica*, when pre-administered before lead acetate (LA) treatment, can ameliorate Pb-induced neurotoxicity which will proffer a solution of designing a drug model as cheap antidote in treatment of lead poisoning.

2. Materials and Methods

2.1. Plant Collection/Sample Identification

Plant material dried seed of *X. aethiopica* were bought from local market in Port Harcourt, Rivers State, Nigeria. It was identified and authenticated in Department of Pharmacognosy, Faculty of Pharmacy, University of Port Harcourt, Rivers State Nigeria.

2.2. Sample Processing and Extraction

The dried *X. aethiopica* seed was milled into fine powder. After weighing the powder, the extract was prepared using cold maceration method thus: In 100ml of distilled water, 1 gram of powder was dissolved. The mixture was strained after 24 hours, pressed, filtered and stored inside in refrigerator at 4°C. Fresh extracts were prepared every three days.

2.3. Experimental Design

Six groups of five weight-matched animals each, were used for the study. Group 1 was treated with distilled water and Group 2 animals received only 50mg/kg b.w. lead acetate, group 3 received 40 mg of *Xylopi*a *aethiopica* only Group 4-6 received 40mg/kg, 80mg/kg and 160mg/kg of *Xylopi*a *aethiopica* p.o. and 50 mg/kg b.w of lead acetate orally respectively for 42days. Animals were sacrificed 24 h

after the last treatment. Rats were sacrificed under diethyl ether anesthesia; cerebrum was excised, rinsed clean in saline, weighed and preserved in 10% formalin for histopathological study. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals, norms and approved by the Institutional Animal Ethical Committee with clearance number UPH/R&D/REC/04

2.4. Antioxidant Assay

The cerebrum was 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) [10] Superoxide dismutate (SOD) was assayed by the method described by (Misra and Fridovich 1972) [11]. Glutathione-S-transferase (GST) was assayed by the method of Habig *et al.* 1974 [12]. Reduced glutathione (*GSH*) was determined at 412 nm using the method described by Sedlak and Lindsay 1996. [13] Lipid peroxidation was also determined as malondialdehyde (MDA) according to the method describe by Ohkawa *et al.* 1978 [14] and the MDA level was calculated according to the method of Todorova *et al.* [2005 [15] and expressed as $\mu\text{mol MDA/mg protein}$.

2.5. Histopathological Studies

Cerebrum from all the experimental groups were 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. The sections were examined for the pathological findings.

2.6. Statistical Analysis

The data obtained from the experiment was statistically analyzed and expressed as Mean \pm Standard deviation (SD) using SPSS (Statistical package for social sciences) and one-way analysis of variance (ANOVA) followed by post-hoc Turkey test. Values of $*p < 0.05$ were considered statistically significant

3. Results

The neuroprotective effect of *X. aethiopica* on lead induced injury on the cerebrum in male Wistar rats is shown in Figures 1 (a-f).

The control group (Figure 1a) showed intact neuronal cells with no degeneration whereas in toxic Pb only treated group, there was neuronal degeneration evidenced by cytoplasmic vacuolation (Figure 1b). Group treated with *X. aethiopica* only, the neuronal cells in various layers appear intact with increase regeneration (Figure 1c). The concurrent treatment with the aqueous leaf extract of *Xylopi*a *aethiopica* dose

dependently showed neuronal regeneration in cerebral vacuolation. histology (Figure d, e and f) evidenced by reduced cell

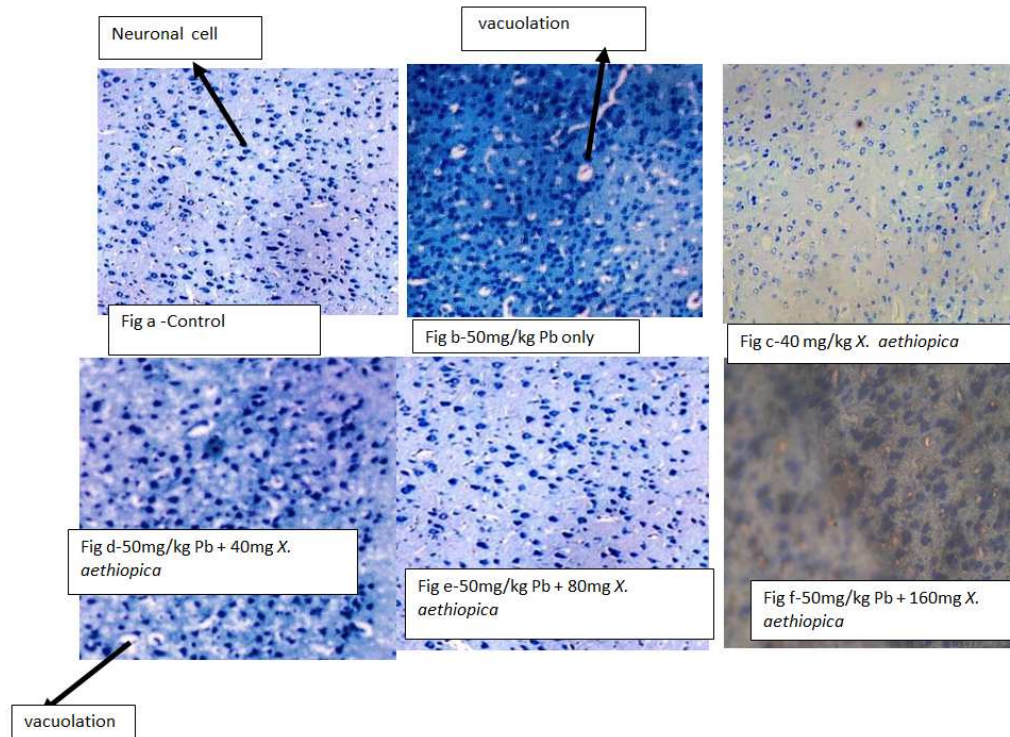


Figure 1. Effect of *X. aethiopica* on lead induced injury on the cerebrum in male Wistar rat.

The effect of *Xylopiia aethiopica* on the feed intake of lead treated Wistar albino rats is shown in Figure 2. There was decrease in feed intake in Lead treated groups when compared with control

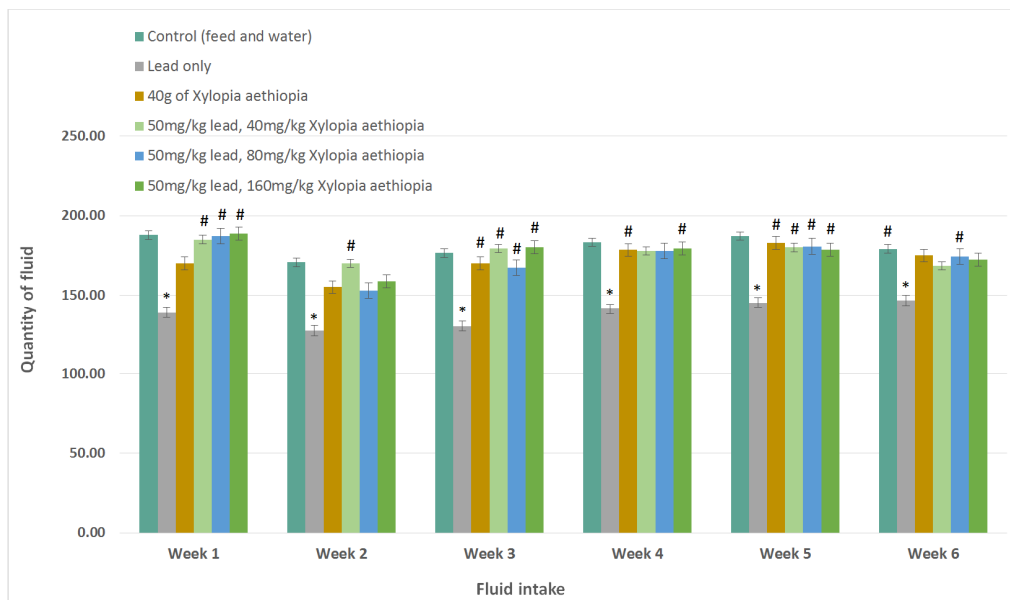


Figure 2. Effect of *Xylopiia aethiopica* on the fluid intake of albino Wistar rats Each value represents mean \pm SD, Values marked with asterisk (*) differ significantly from control value (* $p < 0.05$) while those marked with (#) differ significantly from lead only group (# $p < 0.05$).

Figure 3: shows the effect of *Xylopiia aethiopica* on the fluid intake of lead treated Wistar albino rats. There was a significant decrease in fluid intake in Lead only treated (Group 2) when compared with the control group., Groups 4-6 that received both lead and *Xylopiia aethiopica* showed significant increase in fluid intake when compared with control.

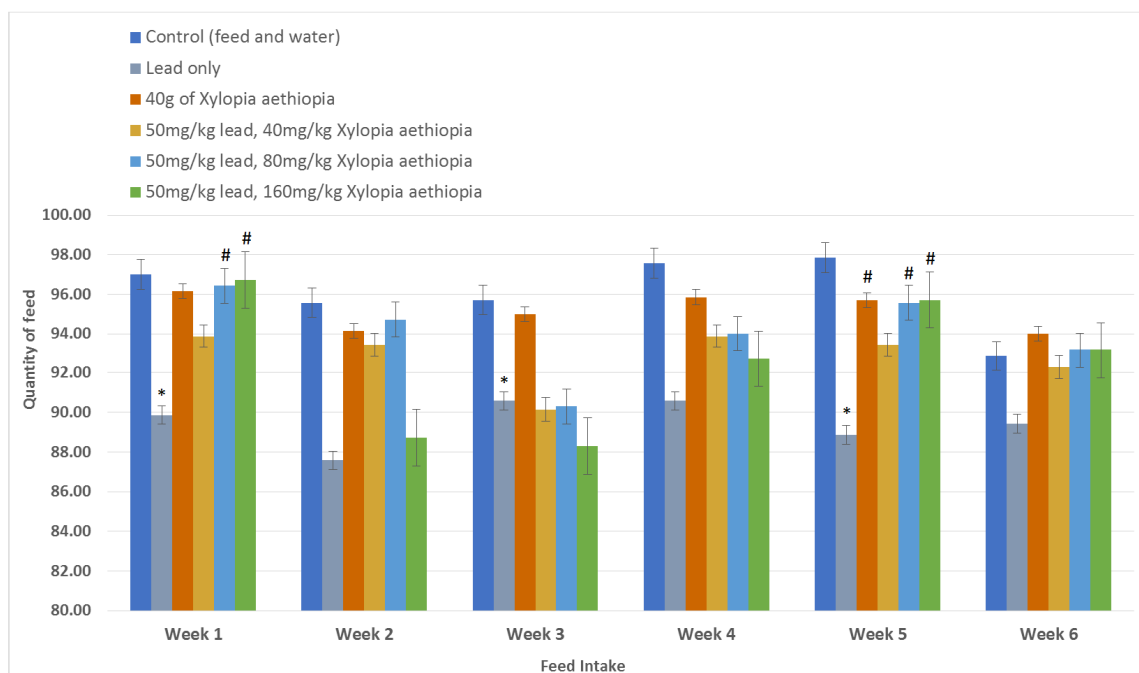


Figure 3. Bar chart showing the effect of *Xylopiia aethiopica* on the feed intake of albino Wistar rats. Each value represents mean \pm SD. Values marked with asterisk (*) differ significantly from control value (* $p < 0.05$) while those marked with (#) differ significantly from lead only group (# $p < 0.05$).

The effect of *X. aethiopica* 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 Groups 4-6 that received both lead and *X. aethiopica* had 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 Groups 4-6 that received both lead and *X. aethiopica* had decrease in malondialdehyde (MDA) when compared with control.

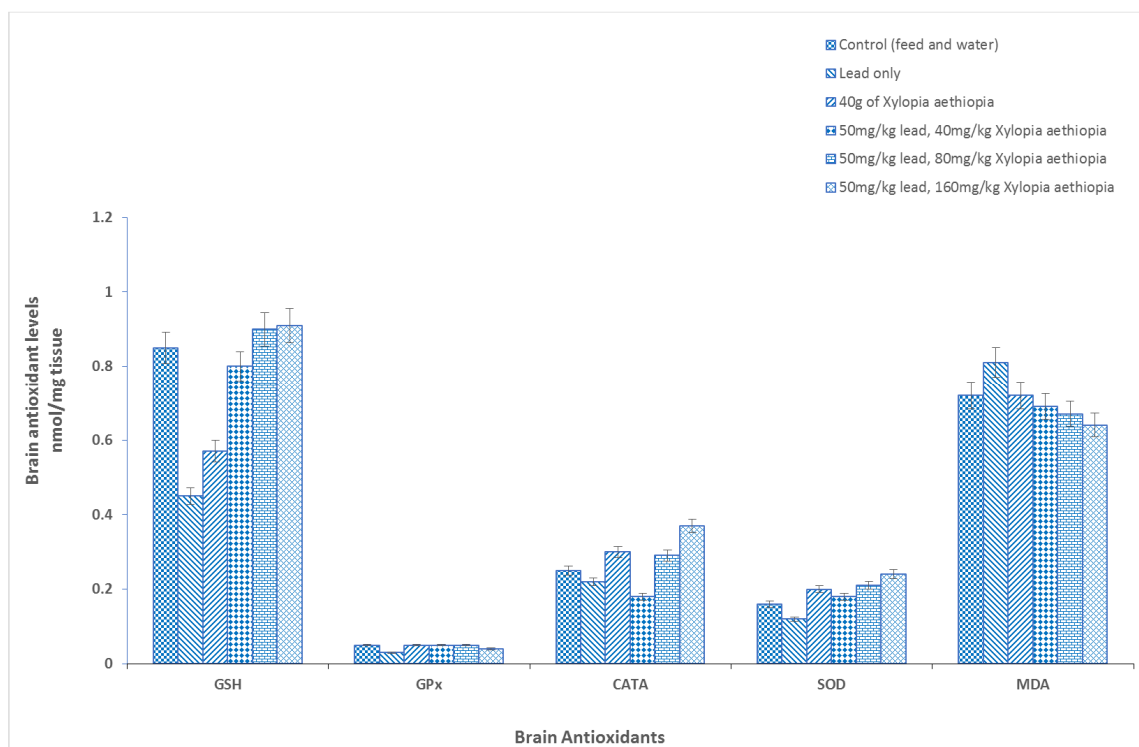


Figure 4. Effects of *Xylopiia aethiopica* on the oxidative stress markers of Wistar rat's cerebral cortex.

4. 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 physicochemical properties, fluidity, and integrity of cell membranes, thereby increasing the cell vulnerability to lipid peroxidation and cell death [16]. Both animal and human studies have suggested that exposure to Pb is associated with increased oxidative stress and a heightened incidence of neurotoxicity. 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 O₂. Antioxidants exert their effects through mechanism that initiate peroxidation, chelating metals, breaking free radical chain reactions and reducing concentration of O₂. As strong free radical scavengers, they can act as very effective neuroprotective agents against lead-induced oxidative stress. The presence of these phytochemicals' tannins and flavonoids in the leaf and fruit extracts of *X. aethiopica* have accounted for the antioxidant activity [17] *X aethiopica* may be stimulating glutathione synthesis, thereby maintaining intracellular glutathione levels and scavenging reactive oxygen species [18]. In addition, it has a lot of micronutrients and can also act as metal chelators. Nutritional factors are often mentioned as important modifiers of lead metabolism and lead toxicity [19]. This intrinsic quality has attracted widespread interest in clinical nutrition and medicinal research.

Our observation of increased aldehydic by-products like 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 [1, 20]. Lead treatment of the rats resulted in an increase in cerebral tissue (L-MDA) levels as compared with the *X aethiopica* extract treated groups ($p < 0.05$) and decrease in antioxidant glutathione, glutathione peroxidase, catalase and superoxide dismutase activity which had been reported by others [21-22]. Similarly, 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 rats treated with *X aethiopica* observed in this study agree with research work of Adaramoye *et al* 2010 [23]. on effects of *X aethiopica* (Annonaceae) fruit methanol extract on γ -radiation-induced oxidative stressing brain of adult male Wistar rats. There were marked neuronal vacuolation and neurodegeneration in lead treated rats. This inflammatory phenomenon was ameliorated by *X aethiopica* in a dose dependent manner. *X. aethiopica* is a rich source of flavonoids which have been shown to exhibit both antioxidant and anti-inflammatory properties [9]

There is sparsity of information on neuroprotective effect of *Xylopia aethiopica* on lead induced injury of cerebral cortex, however according to research by Bolaji and Orhue 2015 [24]. on protective effect of *X. aethiopica* fruits extracts on carbon tetrachloride induced nephrotoxicity in rats, they found that *X. aethiopica* protects kidney against CCl₄ - induced damage via antioxidant potential of the plant. This is in accordance with this study which reported that *Xylopia aethiopica* has neuroprotective effect against lead induced injury. Adaramoye *et al* 2010 [25]. also reported that *Xylopia aethiopica* has a protective effect on testis via its antioxidant property. This agrees with this present study too. In another study, Adaramoye *et al.* 2011 [26] reported that *X aethiopica* protected Wistar albino rats from adverse effects of whole-body radiation. This is in line with this study which showed that *X aethiopica* has neuroprotective effect

5. Conclusion

It can be concluded that *X aethiopica* have a protective role against Lead (50mg/kg) -induced neurotoxicity probably mediated through its antioxidant properties at minimal dose of 40mg /kg.

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