Adansonia digitata ameliorates lead-induced memory impairments in rats by reducing glutamate concentration and oxidative stress

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**ABSTRACT**

Lead (Pb) is a neurotoxicant that still remains a health problem despite many efforts to minimize its levels in the environment. The use of medicinal plants in the treatment of many diseases and different toxic agents has become popular due to their effectiveness and lower costs. The *Adansonia digitata* L. (AD) fruit is called a super fruit because of its exotic nature and rich nutrient profile with several medicinal and antioxidants properties. This study was designed to explore the optional protectivity of *Adansonia digitata* L. fruit pulp aqueous extract against lead-induced memory impairment, oxidative stress and brain damage. Thirty adult male Wistar rats were distributed into five groups: control, Pb 30 mg/kg, AD 250 mg/kg plus Pb, AD 500 mg/kg plus Pb and Succimer 10 mg/kg plus Pb. Administrations were through oral gavage once daily for 42 days. Lead administration caused memory impairment, increased concentration of glutamate in brain and induction of oxidative stress. AD-treated groups protected memory impairment, reduced glutamate concentration, prevented oxidative stress and ameliorated histopathological changes in the brain. It was concluded that *Adansonia digitata* ameliorates lead-induced memory impairment in Wistar rats by improving the memory index, controlling glutamate concentration, preventing oxidative stress.

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**Introduction**

An increase in the number of industries as a result of development has led to heavy metals accumulation on land, air and water; the heavy metals include lead, mercury and nickel [1]. Lead (Pb) is one of the heavy metals that constitutes numerous environmental and health hazards despite efforts to minimize its environmental level [2]. The brain is a vital organ mostly affected by lead; the adult brain is less susceptible to lead toxicity compared to children [3–5]. However, some reports showed that lead is a neurodevelopmental toxicant that also affect adults with more burden in pregnant women [6]. Reports from previous studies suggest that exposure to lead disrupts the glutathione system [7,8], causing oxidative stress [9,10] and inducing neuronal cell death [11,12].

Because of its strong affinity to protein kinase C (PKC), Pb displaces Ca$^{2+}$, binds to PKC and activates it under normal physiologic conditions; the binding affects the normal functions of PKC thereby affecting neurotransmitters like glutamate and other second-messenger systems, leading to changes in gene expression as well as protein synthesis [13].

Glutamate is the most abundant excitatory neurotransmitter; it activates more than 50% of the brain synapses and is responsible for learning and memory [14]. Previous studies report that Pb exposure can influence glutamate signaling that plays an important role in neuronal degeneration and cognition [15,16]. In the brain, astrocytes carry glutamate through excitatory amino acid transporter-2 (EAAT-2) and glutamate transporter-1 (GLUT-1) in humans and rodents, respectively [17,18]. Glutamate acts on ionotropic receptors to enhance ion entry into cells that eventually trigger intracellular signaling. N-methyl-d-aspartate (NMDA), an ionotropic receptor, plays an important role in the progress of neurodegenerative diseases. NMDA binds to glutamate and prompts ion influx at post-synaptic membrane, thereby linking pre-synaptic ad post-synaptic activation [19,20]. Lead was reported to affect glutamate by selective blockage of the N-methyl-D-aspartate (NMDA) receptor, responsible for neuronal plasticity and development [13].

The *Adansonia digitata* L. (baobab) fruit is called a super fruit because of its exotic nature and rich nutrient profile including vitamin C, pectin, potassium, calcium, flavonoids, sterols/terpenes, tannins, saponins, coumarins, glycosides, reducing sugar, lignin, carbohydrates and antioxidants [21,22]. The vitamin C content of baobab fruit pulp is about 10 times the values found in oranges, and this confers the antioxidant properties of baobab fruit enabling it to prevent oxidative stress [23,24]. Antioxidant prevents oxidative stress related diseases such as inflammation, cancer, neurodegenerative and cardiovascular diseases [25,26]. The purpose of the present study was to evaluate the role of *Adansonia digitata* L. fruit pulp on lead-induced memory impairment by assessing the memory index, determining glutamate concentration and oxidative stress in rats. In addition, the study also evaluated the histology and histochemistry of rats’ brain through H&E and Bielschowsky stains, respectively.

**Material and methods**

**Plant extraction**

*Adansonia digitata* fruit pulp was ground, soaked in distilled water and filtered. The filtrate was oven dried at 40°C. Percentage yield of 5.5% was obtained and was calculated using the formula: % yield = (weight of AD fruit pulp/weight of extract) x 100%

**Animals treatment**

Thirty (30) Wistar rats (120–160 g) were obtained from the Faculty of Pharmacy, Ahmadu Bello University (ABU), Zaria, Nigeria. The rats were acclimatized to the Department of Human Anatomy animal house for 14 days.
They had free access to feed (Grower mash, Grand Cereal, Nigeria) and water. The research was approved by the ABU Zaria Research and Ethical Committee (ABUCAUC/2018/064).

**Experimental design**

The rats were divided into five groups (n = 6). Group I (control rats) received distilled water at 1 ml/kg. Group II received lead acetate at 30 mg/kg. Group III received AD extract at 250 mg/kg + lead acetate (30 mg/kg). Group IV received AD extract at 500 mg/kg + lead acetate (30 mg/kg). Group V received succimer at 10 mg/kg [27] + lead acetate (30 mg/kg). Note that 250 mg/kg and 500 mg/kg represent 5 and 10% LD<sub>50</sub> of the extract, respectively [28]. All administrations were done through oral gavage daily for 6 weeks.

**Behavioral test**

The Morris water maze (MWM) test for spatial memory and learning was carried out as described by [29,30]. Briefly, during the training phase, rats were placed on a platform for 20 sec and then lowered into the pool. The time taken for each rat to locate the platform (escape latency) was recorded. Any rat that could not locate the platform after 60 sec was guided to the platform and 60 sec was allocated to the rat. During the test phase, the pool was filled with water 1 inch above the platform and rats were lowered into the water at one quadrant. The test finishes when the rat finds the platform or after 60 sec. Rats that could not locate the platform within 60 sec were placed on the platform for 15 sec and then removed from the tank. The rats were than tested for three trials per day and the latency of each animal was recorded. Rats were tested every two weeks, escape latency on last day of training (day 5) before treatment was considered as an index of learning.

The probe test was also carried out on the same groups of rats 24 hours after the escape latency test. The test was performed 3 consecutive times the average computed. Briefly, the platform was removed, and the time spent by the rat in the target quadrant, where the platform was previously placed, was recorded. The average time spent by each rat in the target quadrant searching for the hidden platform was considered as an index of memory [31].

**Histological examination**

At the end of the study, all the rats were euthanized with ketamine injection, and the brain of each rat was divided into two halves. One half was fixed in 10% neutral buffered formalin, processed for light microscopy and stained with H&E for general histology and Bielschowsky stain for neuronal processes [32,33].

**Glutamate analysis**

The other halves of the brain were weighed and homogenized in phosphate buffer (pH 7.4) at 1 g:5 ml. The homogenate was centrifuged at 3000 rpm for 20 min, and the supernatant was used for glutamate analysis and biochemical analysis. Glutamate concentration was measured using Rat Glutamate Elisa Kit: CK-bio-20422 (Biotech Limited, Shanghai, China) according to manufacturer’s instruction.

**Biochemical analysis**

Malondialdehyde (MDA) was determined by quantifying the lipid peroxidation (LPO) level from the supernatant using the protocols of [34]. The reaction contained tissue homogenates, 5% (w/v) butylated hydroxytoluene (BHT), 10% TCA and 0.75% TBA in 0.1 mol/L of HCl. Values are expressed in μmol/mg of tissue. Catalase (CAT) activity was analyzed using the method of [31] in a solution containing tissue homogenates, 50 mM phosphate buffer and 19 mM hydrogen peroxide. Glutathione peroxidase (GPx) activity was assayed according to the method described
by [35] in a solution containing tissue homogenates, sodium phosphate buffer, 10 mM sodium azide, 4 mM GSH and 2.5 mM H$_2$O$_2$. Superoxide dismutase (SOD) activity was determined using the protocol of [36, 37]. The solution includes tissue homogenates, 0.05 carbonate buffer and 0.3 mM epinephrine. Values are expressed in IU/mg of tissue.

**Statistical analysis**

Data were analyzed using GraphPad Prism 7. One-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test was used. The results were presented as mean ± standard error of mean (SEM), and p < 0.05 was considered statistically significant.

**Results**

**Cognitive ability**

Lead treated rats showed significant increase in latency when compared to the control (P < 0.05) with respect to the pre-treatment period. Rats treated with AD extract plus lead and succimer plus lead showed significant reduction in latency when compared to the lead-treated group at P < 0.05 (Figure 1a). For the probe test, lead-treated rats showed significant decrease in memory index when compared to the control group (P < 0.05). Groups treated with AD extract plus lead and succimer plus lead showed significant increase in memory index when compared to the lead-treated group at P < 0.05 (Figure 1b).

**Histopathological studies**

Hippocampal architecture CA1 and CA3 areas of the control showed closely packed, linearly arranged pyramidal cells with a well-defined shape (Figure 2). By contrast, architectural distortion especially in the pyramidal layer and cell loss with cellular disarray were observed in the Pb-treated group; the group also showed cytoplasmic features suggestive of cell death (Figure 2). Distortions were markedly reduced in the AD250mg + Pb, AD500mg + Pb, and DMSA + Pb groups compared to the control group. These groups showed a nearly similar arrangement of cells with an intact nucleus and well-defined boundaries with occasional areas having few randomly arranged cells exhibiting mild reactive neurodegenerative changes such as necrosis and some levels of neuronal losses (Figure 2).

Bielschowsky stain showed normal neuronal organization and well-organized neuronal processes in the control group (Figure 3). By contrast, architectural distortion and neuronal processes degeneration were observed in Pb-treated rats (Figure 3). Mild distortion and loss of the neuronal processes were noticed in the CA1 region of AD250mg + Pb, AD500mg + Pb and DMSA + Pb-treated rats (Figure 3) when compared with the control group. Degeneration and severe neuronal loss were

![Figure 1](image1.png)

**Figure 1.** Latency (a) and probe test (B) of Wistar rats treated with Pb, AD + Pb and DMSA + Pb. # indicates significant difference with the control group at P < 0.05, (n = 4).
Figure 2. Composite photomicrograph of CA1 and CA3 hippocampal subfields of the brain of rats treated with Pb, AD + Pb and DSMA + Pb showing normal pyramidal cells in A and F, mild distortion in C, E and H and necrosis and neuronal loss in B, D, G, I and J. H and E × 400.

Figure 3. Composite photomicrograph of CA1 and CA3 hippocampal subfields of the brain of rats treated with Pb, AD + Pb and DSMA + Pb showing normal pyramidal cells in A and F, mild distortion in C, D and E and degeneration and neuronal loss in B, G, H, I and J. Bielschowsky x 400.

Figure 4. Glutamate concentration in the brain tissue of rats treated with Pb, AD + Pb and DMSA + Pb. # indicates significant difference with the control group at P < 0.05, (n = 4).
observed in the CA3 region of Pb, AD250mg + Pb, AD500mg + Pb and DMSA + Pb treated (Figure 3).

**Glutamate concentration**

Glutamate concentration increased significantly in lead-treated rats when compared with the control (p < 0.05) group. There was a significant decrease in the glutamate concentration of rats treated with AD250 + Pb, AD500 + Pb and DMSA10 + Pb when compared with that of lead-treated rats at p < 0.05 (Figure 4).

**Malondialdehyde level**

MDA levels increased significantly in all the treated groups when compared to the control group (P < 0.05). A significant decrease in the MDA was observed in rats treated with AD + Pb, and DMSA + Pb when compared with rats treated with lead only (Figure 5).

**Antioxidant enzymes**

**Catalase activity**

CAT activity decreased significantly (P < 0.05) in all the treated groups when compared to the control group. A significant increase in tissue CAT activity was observed in AD + Pb and DMSA + Pb treated groups when compared with that of lead only group at P < 0.05 (Figure 6a).

**Glutathione peroxidase activity**

GPx activity decreased significantly in all the treated groups when compared to the control (P < 0.05). More so, there was also a significant (P < 0.05) increase in tissue GPx activity in AD + Pb and DMSA + Pb treated groups when compared with that of the lead only group (Figure 6b).

**Superoxide dismutase activity**

SOD activity decreased significantly in all the treated groups when compared to the control group (P < 0.05). There was a significant increase in SOD activity in AD + Pb and DMSA + Pb treated groups when compared with the lead only group at P < 0.05 (Figure 6c).

![Figure 5. MDA level in the brain tissue of rats treated with Pb, AD + Pb and DMSA + Pb. # indicates significant difference with the control group at P < 0.05, (n = 4).](image-url)
Discussion

The present study reported memory impairment, oxidative stress and increased glutamate concentration in brain of lead treated rats. Lead was reported as a poisonous heavy metal that can cross the blood–brain barrier and induces several neurological damages because of its ability to displace calcium ions [2]. The present study suggests that Adansonia digitata L. fruit pulp aqueous extract ameliorates memory impairment caused by lead and prevents oxidative stress due to its antioxidant properties. Previous studies have shown that Adansonia digitata contains several bioactive constituents with strong antioxidant capabilities [22,24]. Increased oxidative stress due to Pb toxicity observed in this study supports the other finding that reported memory impairment and oxidative stress as a result of lead toxicity [38]. The beneficial effect of Adansonia digitata on memory improvement is believed to be mediated through its action as a potent antioxidant that results from its direct scavenging activity of free radicals, thereby preventing oxidative stress [23]. Previous work has shown the use of arbutin extract as an antioxidant in stabilizing the antioxidant system in the cells and was crucial for the protection of the brain from chemical-induced damage as such enhancing learning and memory performance in MWM [39]. Numerous studies in animals and humans have confirmed the involvement of oxidative damage resulting in the production of reactive oxygen species (ROS), leading to peroxidation of cell membrane lipids, disrupting the integrity and function of the cell membrane and causing cell death due to lead-induced neurotoxicity [40]. Some studies have supported the ROS-mediated damage to the cell membrane and the depletion of antioxidant reserves as the possible mechanism of lead-induced oxidative stress, and other studies believed that direct formation of ROS including singlet oxygen, H₂O₂, and hydroperoxides is another possible mechanism for lead-induced oxidative damage [41].

Our study showed that groups treated with AD significantly reduced the level of MDA, but increased the activities of SOD, CAT and GPx in the brain and was similar to the DMSA-treated group when compared with the Pb-treated group. This antioxidant property effect could be attributed to the presence of some bioactive compounds such as flavonoids, phenols and ascorbic acid. Research has shown that flavonoids and ascorbic acid have antioxidant properties such as scavenging free radicals, preventing lipid peroxidation and neuroprotective role [42].

Glutamate is the most abundant excitatory neurotransmitter in the CNS involved in neuronal transmission, development, differentiation and plasticity [43]. However, excess accumulation of glutamate leads to abnormal depolarization of neurons, resulting in excitotoxicity and neuronal cell death [44]. The significant increase in glutamate concentration of Pb-treated rats
was attributed to the fact that Pb can inhibit glutamate uptake by astrocyte at the synaptic cleft. Lead toxicity was reported to affect astrocytes functions by depolarizing the cell membrane, resulting in calcium in entry into post-synaptic cell and affecting normal neurotransmission [45,46]. Notably, our study has showed that AD and DMSA modulated glutamate concentration in lead-treated rats. *Adansonia digitata* L. modulates glutamate concentration by increasing cellular uptake to maintain low extracellular glutamate. Low levels of extracellular glutamate increases neurotransmission, and glutamate has no extracellular metabolism. Therefore, a low level of glutamate can be achieved by improving cellular uptake [47]. *Adansonia digitata* L. protects neurons by preventing cell membrane depolarization while the antioxidant role chelate Pb and prevents its excess accumulation in the brain to allow astrocytes function effectively. The vitamin C content of AD might also prevent cell membrane depolarization. Similarly, some plants with antioxidant potentials like Lion’s Mane were reported to control glutamate concentration and prevent excitotoxicity [48]. It is evident that lead toxicity distorts the histological architecture of brain, resulting in several neurodegenerative changes and hence affecting its function [2]. The ameliorative role of AD in brain architecture could be linked to its antioxidant property and the presence of phenolic compounds. Plants with antioxidant properties have been shown to protect the brain structure against lead toxicity [38].

**Conclusion**

*Adansonia digitata* L. fruit pulp at 250 mg/kg and 500 mg/kg was shown to ameliorate lead-induced brain damage by preventing neuronal cell membrane depolarization to maintain low extracellular glutamate and chelating Pb. These promote neurotransmission, thereby improving the memory index and preventing neurodegeneration. Therefore, *Adansonia digitata* L. fruit pulp could be used in the treatment and prevention of neurodegenerative diseases.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Ethics declaration**

The research was approved by ABU Zaria Research and Ethical Committee (ABUCAUC/2018/064).

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