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Original Article

Effects of aqueous fruit extracts of *Adansonia digitata* on lead acetate-induced hippocampal toxicity on adult *Rattus Norvegicus*

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ABSTRACT

Background and aim: Baobab *(Adansonia digitata*) is a super fruit acclaimed for its high antioxidant content and associated medicinal benefits. Oxidative stress, a key contributor to various brain disorders—including stroke, traumatic brain injury, and neurodegenerative diseases—underscores the need for effective neuroprotective agents. This study aimed to assess the ameliorative effects of an aqueous baobab fruit extract against lead-induced hippocampal structural and functional toxicity.

Methods: Twenty adult male Wistar rats were randomly divided into four groups (n = 5 per group). Group 1 (control) received distilled water. Group 2 was treated with lead at 233 mg/kg body weight (bwt). Group 3 received 500 mg/kg bwt of baobab extract, while Group 4 received both lead (233 mg/kg bwt) and baobab extract (500 mg/kg bwt) orally for 28 days. Following the treatment period, the rats were sacrificed via partial decapitation. Blood was collected by ventricular puncture for biochemical analyses—specifically, the measurement of malondialdehyde (MDA), and key antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPx], and catalase [CAT]). Brain tissues were harvested for histological examination of the hippocampus.

Results: Lead-treated rats exhibited a significant increase in serum MDA levels (2.00 U/L) compared to the baobab-treated group (1.60 U/L). Furthermore, the levels of antioxidant enzymes (SOD, GPx, and CAT) were significantly higher in both the baobab-only and combination treatment groups than in the lead-only group. Histologically, the lead-treated group showed marked distortion of the hippocampal cellular architecture, a change that was not observed in groups receiving baobab extract.

Conclusion: The aqueous extract of baobab fruit effectively mitigates lead-induced oxidative stress and preserves hippocampal structure, supporting its potential as a neuroprotective agent.

Keywords:
Baobab; lead; neurotoxicity; hippocampus; histology; oxidative stress; antioxidants

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INTRODUCTION

Newer sources of lead are being discovered on daily basis, therefore lead remains a considerable occupational and public health problem (WHO, 2016). Studies have shown that low level lead exposure has strong correlation with some diseases such as hypertension, peripheral artery diseases, kidney dysfunction, neurodegerative diseases and cognitive impairement (Ahamad and Siddiqui, 2007). Numerous bodily systems, including the cardiovascular, renal, and reproductive systems, can be impacted by lead poisoning. Lead, however, most severely and negatively affects the central nervous system. Lead inhibits the N-methyl-D-aspartate receptor in the nervous system, which is a useful receptor for the development of brain plasticity—alterations in the structure of the brain. Long-term potentiation is disrupted when this receptor in the brain is blocked, which therefore reduces the brain's capacity to permanently absorb and retain newly learnt information. Additionally, the blood-brain barrier is compromised by high blood lead levels (BLLs) (Brochin *et al*., 2008). While redox-inactive metals like lead, cadmium, mercury, and others diminish cells' main antioxidants, especially thiol-containing antioxidants and enzymes, redox-active metals like iron, copper, and chromium go through redox cycling. A rise in the formation of reactive oxygen species (ROS) such as hydroxyl radical ($HO.^{-}$), superoxide radical ($O^{2.-}$), or hydrogen peroxide (H_2O_2) can be attributed to either redox-active or redox-inactive metals. "Oxidative stress" is a situation caused by increased production of ROS that surpasses cells' natural antioxidant defenses (Ercal *et al*., 2001). The toxic effects of lead are treated by chelating therapy which is rarely available in this part of the world and it is associated with a lot of side effects such as depletes the body store of essential cations and

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as such there is need to look for alternative therapy to lead poisoning.

MATERIAL AND METHODS

Ethical clearance

All protocols for handling and caring for the animals adhered strictly to the guidelines of the National Health Institute (NIH) and the Institutional Animal Care and Use Committee (IACUC). Ethical approval for the animal care and experimental procedures was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of Bayero University, Kano, Nigeria.

Methodology

Twenty (20) Wistar rats weighing between 160 to 200 grams and between 7-10 weeks old were used for the study. The animals were obtained from the animal house of the Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano. The animals were acclimatized for two weeks before the onset of the experiment during which they were fed with standard animal feeds and water *ad-libitum*, and this continued throughout the experiment period.

Preparation of Aqueous extract of *Adansonia digitata*

The baobab fruit was purchased locally in Kano metropolis and was taken to the Herbarium of Plant Biology for identification and a voucher number of BUKHAN 0036 was assigned to the sample. The pulp was separated from the seed and fibre by sieving after using a mortar and pestle to crush the sample. The baobab powder was placed in a conical flask, distilled water was added to the flask and left to stand for 72 hours. It was then filtered using 850 nm and 150 nm sieve respectively. The third stage of filtration was done using Whatman filter paper No.1 and cotton wool was placed in the filter paper to get a pure solution. It was then frozen and dried using freeze-drying machine (ILSHIN freeze dryer with concentrator, Ilshin Lab. Co. Ltd, Netherlands) in BUK. This protocol for baobab extract production was in line with the protocol used in an earlier study by Ogunleye *et al*. (2019). About 3000 mg of the dry powder was obtained and used for preparation of aqueous standard solution by dissolving aliquots of the powder in distilled water.

Animal Groupings

Twenty Wistar rats of the same strain were divided into four (4) groups of five animals each.

Group one (1) animals were given 0.2 ml of distilled water for 28 days. Group two (2) animals were given 233 mg/kg body weight of lead acetate (5% of the LD_{50}) for 28 day. Group three (3) animals were given 500 mg/kg body weight of aqueous fruit extract of *Adansonia digitata* for 28 days. Group five (4) animals were given 500 mg/kg bwt of aqueous fruit extract and 233 mg/kg bwt of lead acetate for 28 days.

Neurobehavioral Studies

Object recognition test (ORT), or novel object recognition test (NOR)

This is a preclinical test for the different phases of learning and memory in rodents. It was originally described by Ennaceur and Delacour in 1988 (Lueptow, 2017). Novel object recognition test is based on three phases which included: habituation phase, training phase, and test phase.

Habituation phase

The rat was removed from the cage and placed in the middle of an empty arena (empty space). The animal was allowed to freely explore the arena for 5 min and then removed and placed in a holding cage. The empty arena was cleaned, between successive animals, using ethanol (Lueptow, 2017). During habituation, anxiety-like behavior can be assessed by calculating time spent in the center of the empty arena, rather than exploring the entire nooks and crannies of the empty arena. Thus, a highly anxious mice may require a longer time session to achieve any minimal level of exploration of the empty arena.

Training phase

Training simply involves visual exploration of two identical objects, placed in diagonal direction (i.e. one in the NW corner and the other in the SE corner) during a ten minutes assessment period (Lueptow, 2017).

Testing phase

On the testing day, two copies of identical objects were placed in diagonal position in the cage and the animals were allowed to explore for five minutes. One of the familiar objects was then replaced along with a novel object and the time used in exploring both the novel and familiar objects was recorded using a camera placed above to capture the interior of the cage. Because rodents have an innate preference for novelty, a rodent that remembers the familiar object will spend more time exploring the novel object

Calculations Based on Object Recognition Test

1. E1 = a1 + a2, E1 is the total exploration time during training for 2 identical objects, where a1 and a2 are the identical objects.

2. E2 = a+b, E2 as the total exploration time during testing for the familiar object (a) and the novel object (b)

3. D1 = b-a, D1 is the time spent exploring the novel object minus time spent exploring the familiar object.

The absolute discrimination measure (D1) does not take into account differences in exploration time between mice or treatment groups, though in certain circumstances, it may be a more sensitive measure.

4. Calculate D2 as the time spent exploring the novel object minus the time spent exploring the familiar object divided by total exploration time. The most commonly used measure is a relative discrimination value often referred to as the discrimination index (D2), which is not influenced by differences in exploration time. This means all values will fall between -1 and +1. d2 = d1/e2

5. D3 = b/e2*100, the recognition or preference index (D3). This is the time spent exploring the novel object divided by the total time. This means all values will fall between 0 and 1. It is often multiplied by 100 and used as percentage value (Lueptow, 2017).

This test was be used to assess short and long term memory.

Animal sacrifice

The animals were sacrificed by partial decapitation after intraperitoneal injection of ketamine (50mg per kilogram body weight) and blood was collected with the aid of a 10 mill syringe through cardiac puncture. The blood samples were stored in plain specimen bottles, anticoagulant free and allowed to coagulate and serum harvested for biochemical analysis.

Collection of Brain Tissue

Incisions were made through the skin and muscles of the skull (scalp) and the skull was opened through the mid sagittal suture in order to remove the brain tissues which was immediately fixed in Bouin's fluid before processing the tissue further using routine histological techniques in the histology laboratory of Anatomy Department, Bayero University Kano.

Superoxide Dismutase (SOD)

Total serum SOD (Cu-Zn and Mn) activity was determined based on the method of Sun *et al*., (1988) which is based on the inhibition of Nitro Blue Tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1 ml of ethanol-chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD is defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U/g protein (Kus *et al*., 2013).

Malondialdehyde (MDA)

The tissue malondialdehyde (MDA) level was determined based on reaction with thiobarbituric acid (TBA) at 90–100 °C. In the TBA test reaction, MDA reacts to produce a pink pigment with an absorption maximum of 532 nm. The reaction was performed at pH 2–3 and 90 °C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was centrifuged and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbancewas read at 532 nm and the results were expressed asnmol/g wet tissue, by reference to a standard curve prepared from measurements made with a standard solution (1,1,3,3-tetramethoxypropane) (Kus *et al*., 2013).

Cell counting

Pyknotic cells of the Hippocampus were counted using Digimizer image analysis software. Each field of the hippocampal tissue was captured and studied at 100 and 250 magnifications with a BMS Am microscope digital camera and uploaded into the image area of the software. Specific marker tools were used to click in the image on the cells to mark and count the number of each cell (Iliyas *et al*., 2015). The total number of pyknotic cells was automatically displayed in the statistics window and were subjected to statistical analysis

Histopathological Examination

Hippocampal tissue was viewed under light microscope for any evidence of histopathological changes by an experienced pathologist.

Statistical Analysis

Data were expressed as Mean±SEM (Standard Error of Mean). One Way Analyses of Variance (ANOVA) was employed to compare the mean difference between and within the group P-value less than 0.05 was considered statistically significant. Analysis were performed using Statistical Package for Service Solution (SPSS) version 20.

RESULTS

Neurobehavioral Studies

The result showed decrease in exploration time, E1, between two identical objects in group treated with lead (32.60 ± 14.74 seconds) as compared to the control group (36.00 ± 21.46 seconds). The total exploration time, E1, for all the other treated groups: baobab-only treatment group (68.20 ± 19.25 sec) and baobab plus lead treatment group (68.80 ± 10.80 sec) were higher than that of the lead-only treated group (32.60 ± 14.74 sec) There was a significant difference in the exploration time, between the different groups assessed at $P<0.05$ (Table 1). The familiar and novel object assessment, E2, increased down the group with the control group having the least E2 (14.40 ± 08.23 sec) with a statistically significant difference between the group and baobab plus lead group (53.20 ± 12.99 sec). A similar trend for the assessment f time obtained for the E2 feature was observed for the discrimination time (DT), Relative

discrimination time (RDT) and recognition or preference index (R/P index) with the baobab plus lead group having the highest time when compared to the control (Table 2).

Biochemical analysis

There was rise in the level of MDA (1.98 ± 0.17) U/L in the lead treated group when compared to other treated groups and the control though not statistically significant. Lower MDA level was observed in all the baobab treated groups with the least value in group that received only baobab fruit extract (1.5 ± 0.12) U/L, followed by the control group (1.65 ± 0.19) U/L and then the group that was administered with both lead and baobab fruit extract (1.75 ± 0.06) U/L.

The bar chart represents the levels of malondialdehyde (MDA), a biomarker of oxidative stress, across the four experimental groups. MDA is a byproduct of lipid peroxidation and is commonly used to assess oxidative damage in biological tissues. Group 1 (Control): The MDA level in the control group was relatively low, indicating normal oxidative balance and absence of significant lipid peroxidation. Group 2 (Lead-treated): A notable increase in MDA levels was observed in the lead-treated group, suggesting that lead exposure induced oxidative stress and lipid peroxidation, in agreement with previous reports linking lead toxicity to increased ROS production. Group 3 (Baobab-treated): Rats that received baobab extract alone had MDA levels similar to the control group, indicating the antioxidant properties of baobab in maintaining oxidative balance and preventing lipid peroxidation. Group 4 (Lead + Baobab-treated): The MDA level in this group remained elevated, though slightly lower than in the lead-only group. This suggests that while baobab extract mitigated oxidative stress, it did not completely prevent lead-induced lipid peroxidation.

The SOD level in baobab treated group was 2.38 ± 0.22, followed by a level of 2.2 ± 0.22) U/L among the control group and a level of 2.05 ± 0.13 among the lead treated group. The least SOD level of 1.8 ± 0.08 was observed in the group of rats that received both lead and baobab fruit concurrently as demonstrated in figure 3.

The result of catalase (CAT) level from this study shows no significant difference between all the groups. See figure 3. The least amount of CAT was observed in lead-only group with a mean value of 41.5 U/L, followed by lead and Baoba treatment group with a mean value of 43.5U/L, then the control group with a mean value of 45.75 U/L. The highest value was observed in the baobab-only group with a mean value of 46 Unit per liter (U/L). See figure 3. A similar trend was observed for glutathione peroxidase (GPX) activity, with the least value observed in baobab plus lead group, with a mean value of 44.5 U/L, followed by lead group with a mean value of 46.5 Unit per liter, then the baobab group and the control group with a mean values of 50.75 and 51 U/L respectively (figure 3).

Histological observation

Histological slide from hippocampus of control group (gp I) shows normal cornu ammonis I (CA) region with normal pyramidal cell (PC), glia cells (GC) and pyramidal cell layers (PCL), blood vessels and neuropiles, using haemathoxyline and eosine stain see plate 1A. However, the hippocampal tissue of group II animals (lead), showed distorted pyramidal cells (PC), pyramidal cellular layer (PCL), degenerating neurons and vacuolations. CA I region of hippocampus of baobab treated group showed normal PC, GC, and glia cells similar to those of control group of animals. CA I section of hippocampus of group 4 animals (receiving lead and Baobab extract) had normal pyramidal cells (PC), glia cells (GC) with few degenerating glia cells (DGC) and distorted pyramidal cell layer (DPCL). Likewise for CA2 & CA3 area, a similar trend were observed with more cavitation, vaculation and pyknosis in the lead treated group when compared to the control group and other treated group. However, for the dentate gyrus more degenerating granular cells were observed in the lead-treated group, followed by the group that was given lead and baobab fruit extract concurrently when compared to the control and the baobab-treated groups (Plates I to IV).

Pyknotic Cell Count across Experimental Groups

The analysis of pyknotic cell count across the different treatment groups revealed significant variations in neuronal degeneration with a p-value ≤ 0.005 across all the regions of the hippocampus: Control group (group 1), showed the lowest number of pyknotic cells, indicating normal cytoarchitecture and minimal neuronal damage. Lead-treated group (group 2), displayed a marked increase in pyknotic cell count, reflecting extensive neuronal degeneration due to oxidative stress and neurotoxicity induced by lead exposure. Baobab-treated group (group 3), demonstrated a lower pyknotic cell count compared to the lead-treated group, suggesting a protective effect of baobab fruit extract against neuronal damage. Baobab Plus Lead-Treated group (group 4): showed a significant reduction in pyknotic cell count compared to the lead-only group, indicating that baobab fruit extract mitigated the neurotoxic effects of lead. Similar trend is observed across all the region of the hippocampus (Figures 4-7).

Table 1: Total exploration time for identical, novel object, discrimination time, relative discrimination time and preference index during short time memory assay in Wistar rats

GROUPS	E1 (s)	E2 (s)	DT (s)	RDT	R/P INDEX
Control	36.00±21.46[a]	14.40±08.23[a]	01.6±04.00[a]	00.10 ± 00.10	55.24±17.60
Lead	32.60±14.74[b]	21.20±03.35[b]	07.60±06.39[b]	00.40 ± 00.38[a]	68.94±19.14[a]

Baobab	68.20±19.25[a,b]	36.00±24.29	11.60±10.71[c]	00.39 ± 00.30[a]	64.28±18.03[a]
Baobab + Lead	68.80±10.80[b]	53.20±12.99[a,b]	25.20±16.51[a,b,c,d]	00.54 ± 00.34[a]	22.97±17.17[a]

E1, exploration time 1 (total time used in exploring identical object), E2, exploration time 2 (total time use in exploring novel and familiar object). DT, discrimination time, Values are presented as mean ± standard deviation (SD),RDT, relative discrimination time, R/P Index, Recognition or Preference index. Values with the same superscript on the same column are significantly different at P<0.05

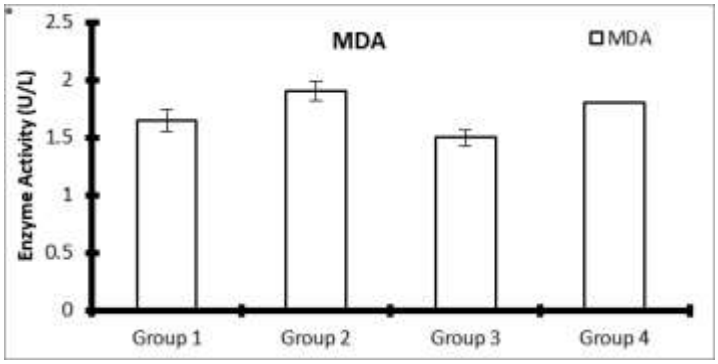

Figure 1; Serum malondialdehyde level in control, lead, baobab and lead co-treated with baobab groups

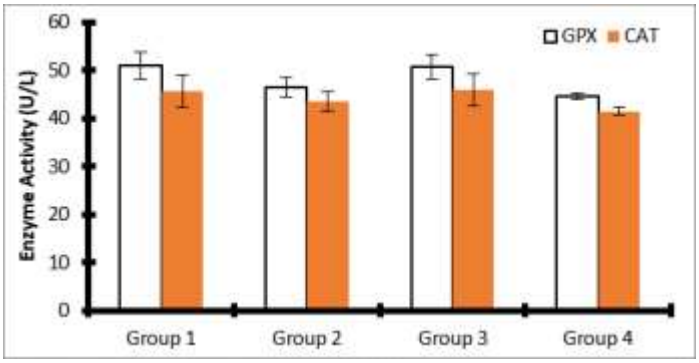

Figure 3: chart showing serum level of glutathione peroxidase (GPX) and catalase (CAT) in all the groups

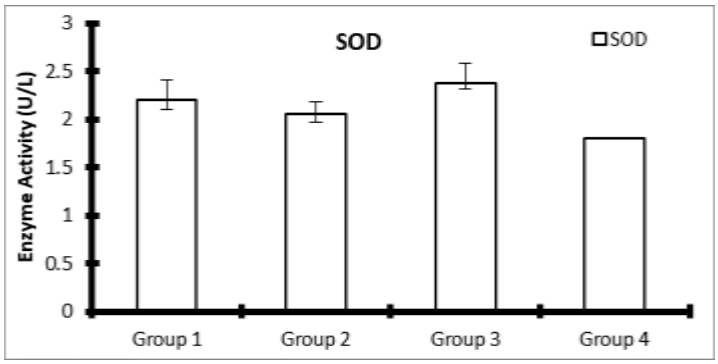

Figure 2: Bar chart demonstrating superoxide dismutase level of activity in the various groups rats treated with baoba extract and/or lead acetate

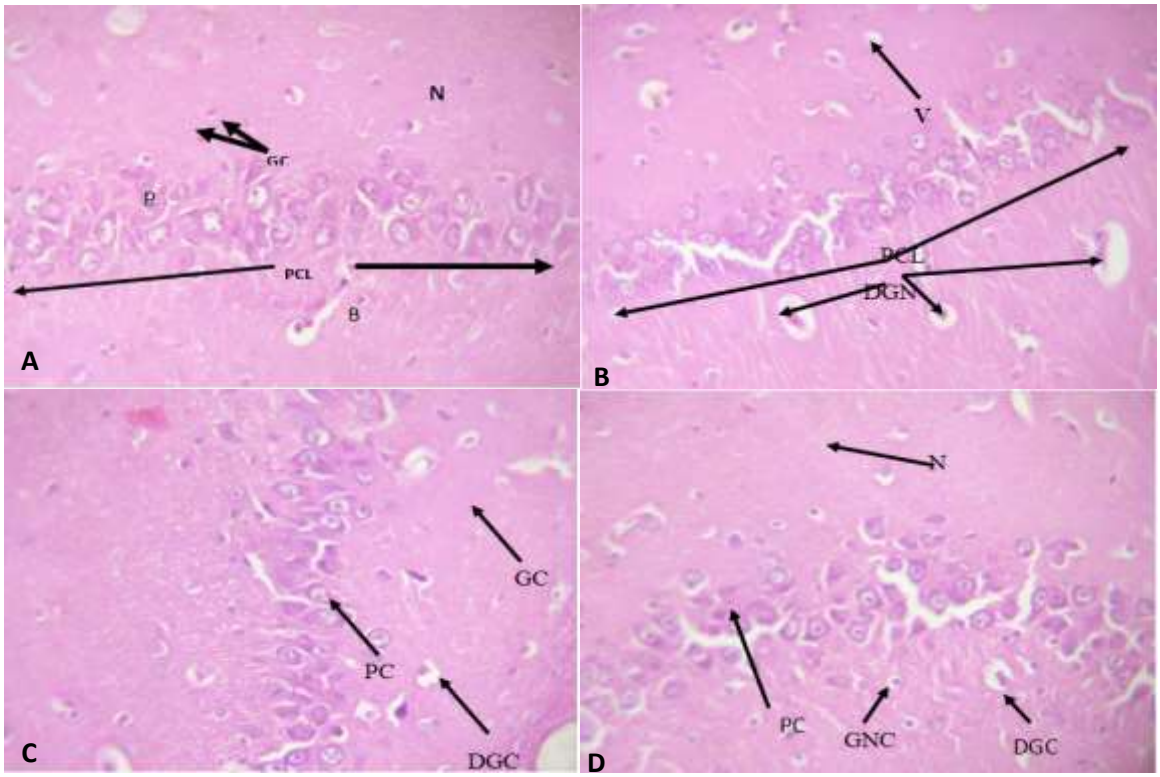

Plate I; showing cornus amminus I area of hippocampus of the Wistar rats; control (A), with normal pyramidal cells, P, Glia cell (GC), Pyramidal cell layer (PCL), Blood vessel, Neuropile (N). Group 2 animals (B), distorted pyramidal cell layer (PCL), degerating neurons (DGN), vacuolation (V). C, group III animal showing normal pyramidal cell (PC), glia cells and some degenerating glia cells (DGC).D,group IV animals, normal pyramidal cells (PC), granular cells, degenerating pyramidal cell and neuropile (H&E; 400x).

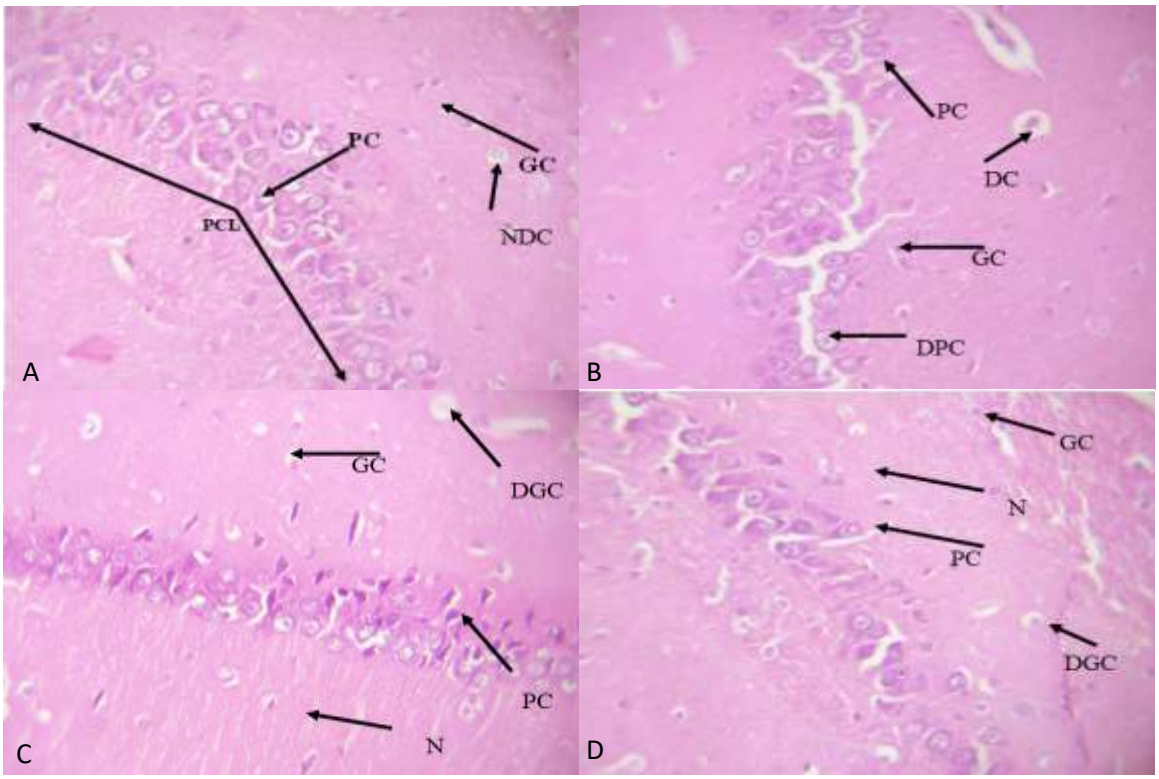

Plate II: CAII area of hippocampus of Wistar rats; showing normal cellular architecture of control group (A) with normal pyramidal cells (PC), pyramidal cellular layer (PCL), granular cells (GC) and neurodegerating cell (NDC). (B)Lead acetate group: animal showing distorted pyramidal cells (DPC), degenerating cell (GC). (C), baobab treated group, showing normal cellular architecture with normal pyramidal cells (PC), neuropile (N). (D) Group IV animals showing ameliorated cellular architecture (H&E; 400x).

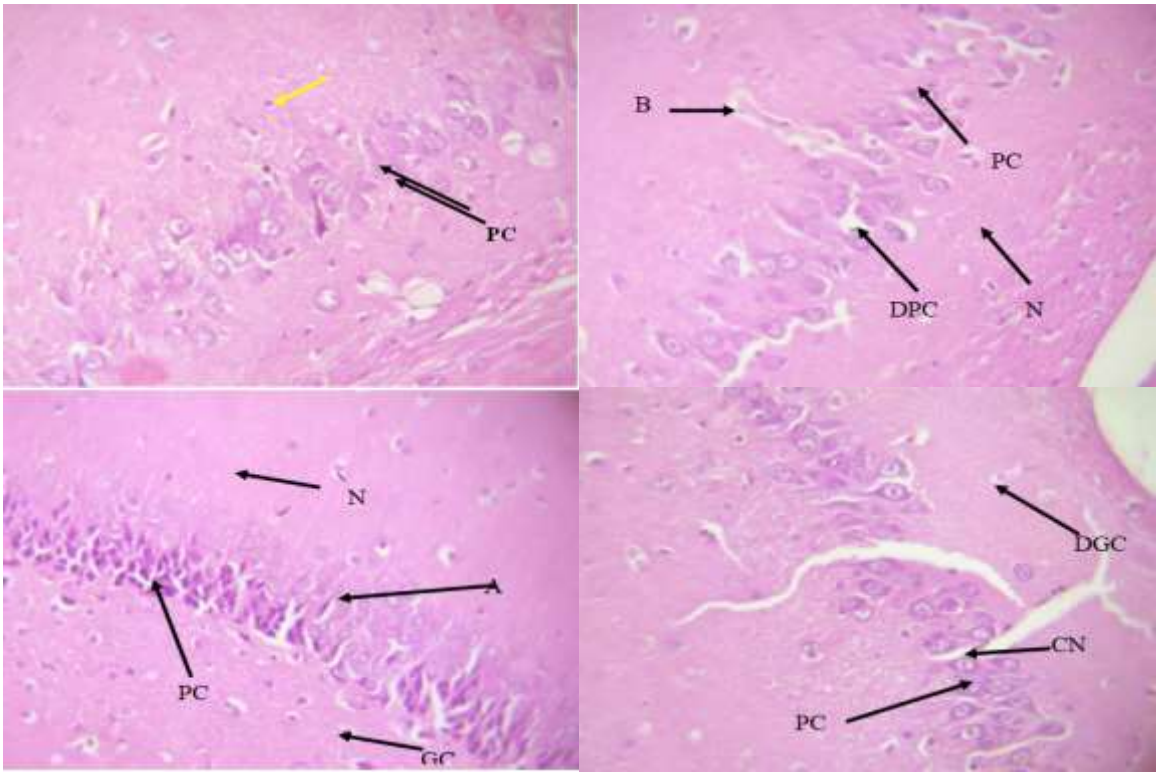

Plate III, CAIII area of the hippocampal tissue of Wistar rats; control group (A), with pyramidal cell (PC). B, Lead treated group hippocampal tissue showing some degenerating pyramidal cells (DPC), C, Baobab treated group showing normal pyramidal cell, (PC), granular cell, (GC), Axon, (A) and D, group treated with lead and baobab concurrently with normal pyramidal cells and some degenerating granular cells (DGC), (H&E; 400x).

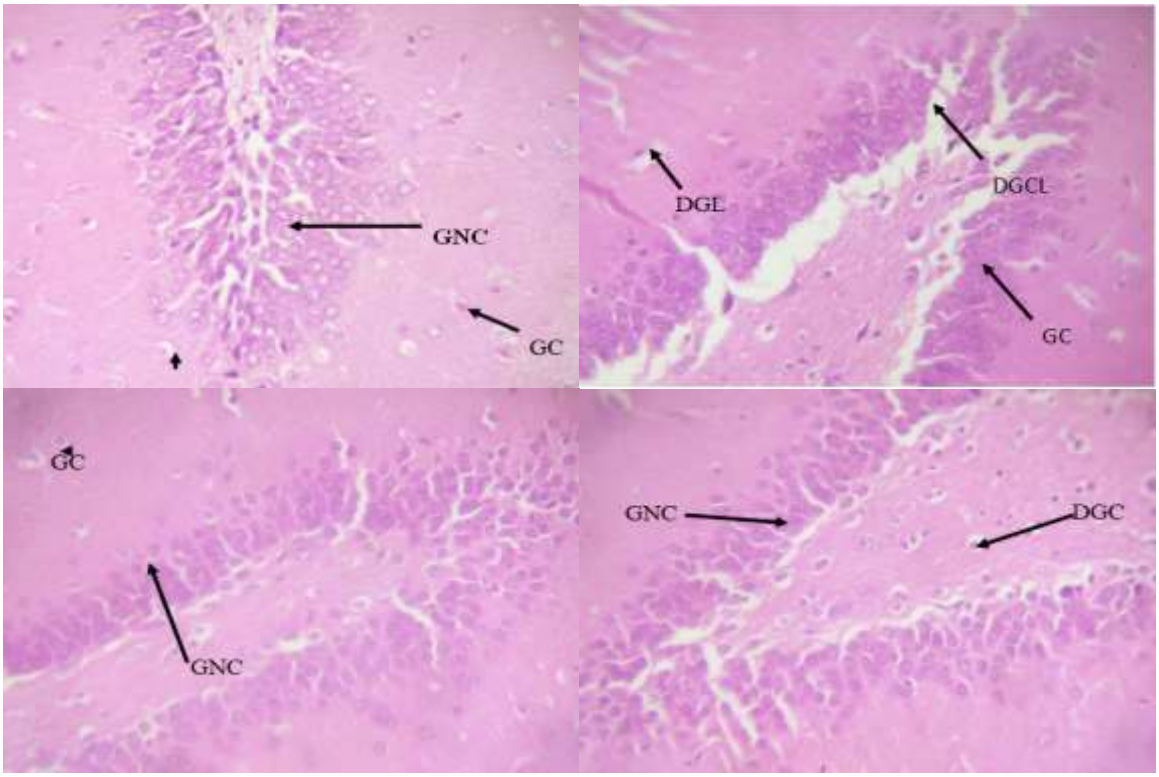

Plate IV: Dentate gyrus (DG), of the Wistar rats; (A) control group, showing normal granular cells, (GNC), B, group II degenerating granular cell (DGCL), C, group III normal granular cells and D, group IV, degenerating granular cell (DGC), (H&E; 400x).

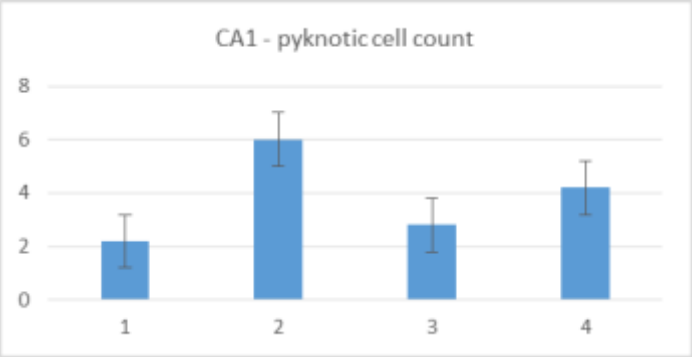

Figure 4; Pyknotic cells count in CA1 region of control, lead, baobab and lead co-administered with baobab treated groups

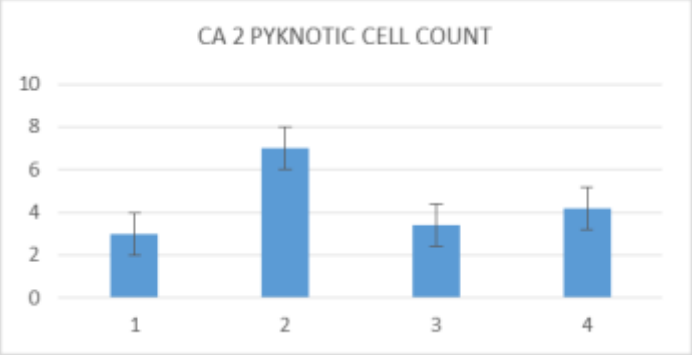

Figure 5; Pyknotic cells count in CA 2 region of hippocampus in control, lead, baobab and lead co-administered with baobab treated groups

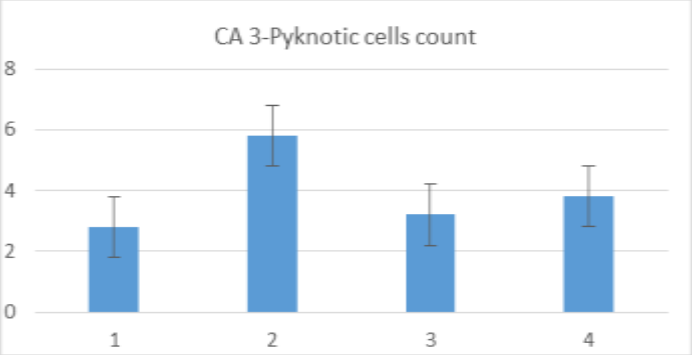

Figure 6; Pyknotic cells count in CA 2 region of hippocampus in control, lead, baobab and lead co-administered with baobab treated groups

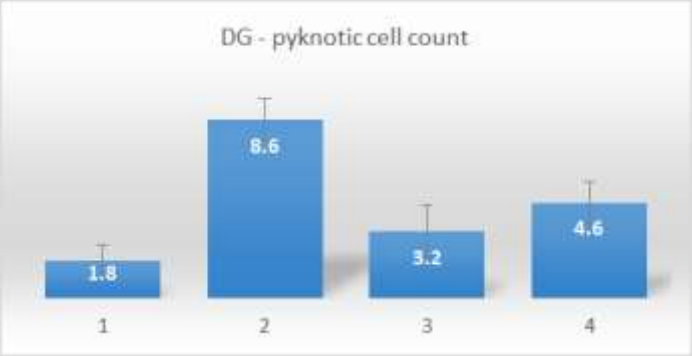

Figure 7; Pyknotic cells count in dentate gyri region (DG) of hippocampus in control, lead, baobab and lead co-administered with baobab treated groups

DISCUSSION

This studies showed an alteration neurobehavioral activities, rise in the level of MDA, with reduction in SOD, CAT, GPx activities and distortion in the cytoarchetecture of the hippocampal tissue of the lead treated rats. The increase in the level of cognitive performance observed in baobab treated group was as a result of it antioxidant activities, earlier report by Cangao (2020) showed that baobab fruit has a high amount of antioxidant. This coincides with the work of Docherty and Haskell-Ramxy (2020) in which baobab fruit powder was reported to improve certain aspects of cognitive performance. The decline in memory of the lead-treated group could be due to anxiety-like behaviour in the group treated with lead as can be seen in the low exploration time for both identical objects and novel objects as an earlier report by Lueptow (2017) linked anxiety to decline exploration time in laboratory animals. This could result from an increase in reactive oxygen species in the lead-treated group due to the direct effect of lead on the tissues and depletion of tissue of it antioxidants (Mousin *et al.*, 2010; Sunusi *et al.*, 2023). The discrimination time was also higher for the group treated with Baobab extract as compared to the lead acetate-treated group. This is in accordance with the work of Silver *et al.* (2023) in which baobab fruit powder was reported to improve certain aspects of cognitive performance in humans. Ascorbic acid, a strong anti-oxidant, was reported to ameliorate some of the behavioural changes induced by: environmental heat stress (Ambali and Ayo 2012), sepsis (Zang *et al.*, 2021), infection, (Ogunleye, *et al.*, 2019). It was reported that deficits in locomotion efficiency, motor strength, righting reflex and excitability score induced by chronic chlorpyrifos (CPF) were mitigated by vitamin C. Rats administered with CPF showed deficits in motor strength, coordinated gaits, neuromuscular coordination, learning and memory, slight decrease in AChE activity and an increase in brain MDA concentration which was ameliorated by vitamin C (Ambali *et al.*, 2010). Single prolonged stress (SPS) was reported to induce a significant increase in the oxidized glutathione levels of the hippocampus which was accompanied by a significant decrease in glutathione peroxidase and catalase enzyme activity, and a significant increase in lipid peroxidation, all these changes were attenuated by vitamin C (Alzoubi *et al.*, 2020). Vitamin C was reported to inhibit impairment in synaptic plasticity and neurobehaviours when beta amyloid deposition occurs in the brain of Wistar rats (Sattari *et al.*, 2021). Animals treated with ascorbic acid (AA) after induction of traumatic brain injury, were found to have improved learning and memory, locomotor function, and decreased anxiety (Bulama *et al.*, 2020). Antioxidants such as black seed oil (Maibindiga *et al.*, 2024), Arbutin (Zhoreh *et al.*, 2019) were reported to improved cognitive performance in laboratory animals. Earlier report also shows that high dose vitamin C improve cognitive impairment in sepsis-induced cognitive impaired rats (Zhang *et al.*, 2021), which was believed

to be as result of reduction in the level of free radical by vitamin C decreased MDA and increased SOD. Otong *et al*. (2022) reported that adansonia digitata fruit extract was able to ameliorate neurobehavioral deficit in Wisrtar rats during Morris Water Mass experiment. This is agreement to our findings in this studies using novel object recognition test and lead as an inducer of neurobehavioral deficit.

Lead was observed to increase the level of serum MDA but decrease SOD, CAT and GPX activities, this concurred to the work of Otong *et al*. (2022) in which homogenized neural tissue of animals treated with lead showed a rise in MDA and a reduction in the activities SOD and GPX. Reactive oxygen species (ROS) causes oxidative damage in major cell macromolecules, such as lipids, proteins and nucleic acids, and are said to be the major cause of tissue injury. ROS are scavenged by SOD, GSH-Px, and CAT. MDA is the byproduct of the major chain reactions leading to the oxidation of polyunsaturated fatty acids and thus serves as a marker of oxidative stress-mediated lipid peroxidation (Jelodar *et al*., 2014). The result of this study showed a rise in both CAT and SOD in group treated with baobab fruit extract and a reduction in the level of MDA aggravated by lead II acetate toxicity. This is due to high scavenging activities of adansonia digitata fruit extract whose major component is vitamin C (Althwab *et al*., 2019). Studies have shown that, Ascorbic Acid decreases malondialdehyde level but increases activities of SOD, CAT, and GPx, when compared to the control group in traumatic brain injured- animals (Bulama *et al*., 2020). This is also in agreement with the work of Jelodar *et al*. (2014), in which vitamin C was able to ameliorate both decreased in antioxidant enzymes (Gpx, SOD and CAT) and rise in MDA induced by radiofrequency wave induced oxidative stress. A combination of Chitosan and vitamin C was reported to elevate the activities of antioxidant enzymes altered by lead II acetate (Marianti and Mahatmanti, 2018). Positive correlation was observed between catalase level and the level of vitamin C in milk cell (Kazak and Coskun, 2022). Zhang *et al*. (2021) observed a reduction in the level of SOD and a rise in the level of MDA in sepsis induced rats which were ameliorated by high dose of vitamin C. Reduction in the level of glutathione was reported by Akinhunmi *et al.*, (2016) in artisans with high blood lead level which were ameliorated by daily intake of 400 mg vitamin C. Its homeostatic mechanism and recycling that sustains vitamin C concentrations in the brain and neuronal tissues in relation to other body organs and tissues are indicative of its critical significance in the brain (Spector, 2013). The concentration of vitamin C in cerebrospinal fluid (CSF) is significantly higher than that in plasma in a healthy brain (Harrison *et al*., 2009). While intracellular neuronal concentrations of ascorbic acid can reach up to 10 mM, the total brain has been found to contain just 1 to 2 mM (Harrison *et al.*, 2010). The reason for these elevated levels is that astrocytes, which are made up of glutathione, recycle DHAA into ascorbate (May, 2012). Increase concentrations of glycogen and MDA evoked by chronic CPF were ameliorated by vitamin C which is the major antioxidant in baobab fruit extract (Ambali *et al*., 2010).

The apparently normal cytoarchetecture of cornu ammonis I (CA I) area of hippocampal tissue observed in the control group in this study is as a result of the absence of oxidative stress, since oxidative stress was reported to altered cytoarchitecture of laboratory animals (Mousin *et al*., 2010; Ilyas *et al*., 2015; Otong *et al*., 2022). This is in support of earlier works in which control rats had normal cytoarchitecture of brain sections including the hippocampus (Yogesh *et al*., 2015; Bulama *et al*., 2020). The abnormalities in the cellular architecture seen in CA I area of group II animals is likely due to oxidative stress induced by lead II acetate especially because lead is known to alter the influx of bivalent cations such as calcium and zinc, probably due to their similarities in outermost electrons (Garza *et al*., 2006). The result of the increased number of pyknotic cells in the current study among the rats treated with lead was in accordance with an earlier report among rats with traumatic brain injuries (Yogesh *et al*., 2014). Vacuolation, cavitation, and degeneration of glia and pyramidal cells, observed in the rats treated with lead acetate was following earlier work where hippocampal tissues of rats exposed to radiofrequency of electromagnetic field from mobile phone showed lesions in the hippocampal tissue (Hussein and Muhammad, 2015; Otong *et al*., 2022) with lead-induced alteration in the cytoarchitecture of hippocampal tissues in laboratory animals. Because neurons have ten times the oxidative metabolism of supporting glia, they are particularly vulnerable to antioxidant deficiency (Hediger, 2002). It has been demonstrated that ascorbate efficiently scavenges superoxide at the levels found in CSF and neurons in vivo (Jackson, *et al*., 1998). Ascorbate catalyzes the conversion of superoxide radical to H_2O_2 in the neuron's mitochondria, where it is oxidized to produce DHAA and an ascorbate free radical. Other antioxidants like glutathione and vitamin E are also supported in their regeneration by ascorbate (Jackson, *et al*., 1998).

The current reduction of degenerative features in the cytoarchitecture of the rats treated with baobab fruit and lead could be as a result of the fruits' high antioxidant contents which mitigates the impact of the free radicals (such as those from lead) on the tissues. This is in agreement with the work of Yogesh *et al*. (2014) and Zhorhe *et al*. (2019) where animals given Glycyrrhizic acid and Arbutin respectively showed general improvement in cytoarchitecture of both gray and white matter of the cerebral cortex. This is also in congruent to the work of Echuro *et al*. (2018), where neuropsychotic plant, Khat, was reported to alter histological features of prefrontal cortex such as cavitation, necrosis, apoptosis, and reduced pyramidal cells number. Hence increase pyknotic cells as observed in the lead treated group of the current study has already been associated with both structural and physiological alterations of the nervous tissue. The purported mechanism may be associated with a reduction in cell to cell contact as well as a great reduction in synaptic transmission which can lead to the functional manifestation of deficit in neurobehavior and neural coordination (Ilyas *et al.*, 2015; Echuro *et al*., 2018).

In conclusion, this study demonstrated that lead exposure induced significant neurobehavioral alterations, oxidative stress,

and structural damage in the hippocampus. Lead-treated rats exhibited increased malondialdehyde (MDA) levels, reduced antioxidant enzyme activity (SOD, CAT, and GPx), and cellular distortions in the hippocampus, leading to cognitive and memory impairments. However, baobab fruit extract mitigated these toxic effects, likely due to its high antioxidant content, particularly vitamin C. Rats treated with baobab extract showed improved cognitive performance, reduced oxidative stress markers, and preserved hippocampal architecture. These findings align with previous studies highlighting the neuroprotective properties of baobab and other antioxidants, such as vitamin C and black seed oil, in ameliorating oxidative damage and cognitive deficits. The observed restoration of hippocampal structure and function in baobab-treated rats further supports its potential as a natural therapeutic agent against lead-induced neurotoxicity. Future studies should explore the molecular mechanisms underlying baobab's neuroprotective effects and its potential application in human neurodegenerative conditions.

REFERENCES

Ahmed, M. and Siddiqui, M.K.J. (2007). Low level lead exposure and oxidative stress: Current opinions. *Clinica Chimica Acta*, 138(1): 57-64.

Althwab, S. A., Alsattame, S. M., Al-mundarij, T. I., Hamad, E. M., & Mousa, H. M. (2019). Protective effect of baobab fruit pulp (Adansonia digitata L.) from oxidative stress induced in rats by high-fat diet. *Life Science Journal*, *16*(1), 63-71.

Alzoubi, K. H., Shatnawi, A. F., Al-Qudah, M. A., & Alfaqih, M. A. (2020). Vitamin C attenuates memory loss induced by post-traumatic stress like behavior in a rat model. *Behavioural Brain Research*, *379*, 112350.

Ambali, S.F. & Ayo, J.O. (2012). Vitamin C Attenuates Chronic Chlorpyrifos-induced Alteration of Neurobehavioral Parameters in Wistar Rats. *Toxicology Int.* 19(2):144–152.

Ambali, S.F., Idris, S.B., Onukak, C., Shittu, M. and Ayo, S.O. (2010). Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats. *Sage Journal, Toxicology and Industrial health* 26,(9): 547-558.

Brochin, R., Leone, S., Phillips, D., Shepard, N., Zisa, D., & Angerio, A. (2008). The cellular effect of lead poisoning and its clinical picture. *GUJHS*, *5*(2), 1-8.

Bulama, I., Nasiru, S., Bilbis, S. L., Abbas, A. Y., Nasiru, J. I., Saidu, Y., ... & Chiroma, M. S. (2020). Ascorbic acid treatment modulated traumatic brain injury-induced oxidative stress and neuropathic pain in rats. *Journal of Cellular Neuroscience & Oxidative Stress*, *12*(1), 922-936

Cangao, C.A (2013). Baobab; an Undertilize Superfruit of Africa and Central Africa. *International Tropical Fruit Network*; 1-5

Docherty, S., & Haskell-Ramsay, C. (2020). The acute effect of baobab fruit on cognitive performance, cerebral blood flow and blood glucose levels. *Proceedings of the Nutrition Society*, *79*(OCE2), E329.

Echoru, I., Bukenya, E.M. Masilili, G. Owembabazi, E., Lemuel, A.M. and Ahimbisibwe, J. (2018). Commonly consumed Khat (Catha Edulis) distorts the prefrontal cortex histology and function in adult Wistar rats. *Anat. J. Africa*. 7(1): 1121-1132

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current topics in medicinal chemistry*, *1*(6), 529–539.

Garza, A., Vega, R. and Soto, E. (2006). Cellular mechanisms of lead neurotoxicity. *Medical Sciences Monitor*, 12(3), 57-65

Hediger, M. A. (2002). New view at C. *Nature medicine*, *8*(5), 445-446.

Hussein, A. H.H and Muhammad A.M.H. (2015). Histological changes in albino rathippocampus following postnatal exposure to radiofrequency electromagnetic field emitted from mobile phones. *The Egyp. J. of Hist*, 38(2), 253-265.

Iliyasu, M.o., Ibegbu, A.O., Sambo, J.S., Musa, S.A. & Akpulu, P.S. (2015). Histological changes on the hippocampus of adult Wistar rats exposed to lead acetate and aqueous extract of Psidium guajava leaves. *African J. Cell. Path.*, 5(1), 26-31

Jackson, T. S., Xu, A., Vita, J. A., & Keaney Jr, J. F. (1998). Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. *Circulation research*, *83*(9), 916-922.

Jelodar, G.A., Akbari, A. and Nazifi S (2014). Effects of Vitamin C on Oxidative Stress in Erythrocytes Following Exposure to Radiofrequency Waves Generated by a BTS Antenna Model. *Zahedan J. of Res. in Med. Sci.*, 16(12): 48-52.

Kus, M. A, Sarsilmaz, M., Karaca, O., Acar, T., Gülcen, B, Adnan Adil Hismiogullari, A.A. , Ogeturk, M. and Kus, I. (2013). Effects of melatonin hormone on hippocampus in pinealectomized rats: An immunohistochemical and biochemical study. *N. Let.s*, 34(5): 418-425

Lueptow, L.M (2017). Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. *JVE.* (126): 557158

Maibindiga, M.M., Fage R.I , , Sunusi, A , Jibril, A.N , Tela, I.A and , Ammani, T. (2024). Ameliorative Potentials of Black Seed Oil (Nigella Sativa) on Short-Term Memory Impairments and Histological Changes on Ethanol Treated Rats. *J. Bio. & App. Sci. FUD* 3(1): 1-10

May J.M. (2012).Water Soluble Vitamins. Vitamin c transport and its role in the central nervous system. *Springer;* Dordrecht, Netherlands, 85–103.

Missoun, F., Slimani, M., & Aoues, A. (2010). Toxic effect of lead on kidney function in rat Wistar. *African Journal of Biochemistry Research*, 4(2), 21–27.

Ogunleye. O.O, Jatau I.D., Natala, A.J., Idehen, C.O., Salami, O. and Mammam, S.A (2019). In vivo effect of the aqueous extract of Adansonia digitata (Linn) fruit pulp on Trypanosoma brucei brucei infection in Wistar rats. *SJVS* 17(4): 1-8

Otong, E.S., Musa, S.A,. Danbornob, B., Sambo, S.J and Dibal, N.I (2022). Adansonia digitata ameliorates lead-induced memory impairments in rats by reducing glutamate concentration and oxidative stress. *Eg. J. of basic and app sci.*, 9(1); 1–10

Sattari, S. Vaezi, G., Shahidi, S., Hojati, V. and Komak, A (2021). Protective Effects of Oral Vitamin C on Memory and Learning Impairment and Attenuation of SynapticPlasticity induced by Intracerebroventricular Injection of Beta-amyloid Peptide in Male Rats. *Res. Square*, 1-23,

Silva. M.L Rita, K., Bernardo,M.A. Mesquita,M.F., Pintão, A.M and Moncada, M. (2023). *Adansonia digitata* L. (Baobab) Bioactive Compounds, Biological Activities, and the Potential Effect on Glycemia: A Narrative Review. *Nutrient*, 15(9): 2170

Spector R., and Johanson C.E. (2013) Sustained choroid plexus function in human elderly and alzheimer's disease patients. *Fluids Barriers CNS*. ;10-19.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. *Cl. Chem.,* 34: 497–500.

Sunusi, A, Hamman, O.W., Musa, S.A & Adamu, L.H. (2023). The effects of sub-acute oral lead administration on the histology of the kidney and some renal parameters in adult Wistar rats. *The J.A.S*, 2(14): 71-77

World Health Organisation (WHO), (2016). Lead Poisoning and Health. Fact Sheet Review, Geneva, *World Health Organisation*.

Yogesh, K.S., Pradeen, K.N. and Kallyappan, K. (2014). Restorative of Glycyrrhizic Acid on Neurodegeration and Cognitive Decline in Chronic Cerebral Hypoperfusion Model of Vascular Dementia in Rats. *Intern. Journal Anat. Sci.*, 5(2): 57-65

Zhang, N., Zhao, W., Hu, Z. J., Ge, S. M., Huo, Y., Liu, L. X., & Gao, B. L. (2021). Protective effects and mechanisms of high-dose vitamin C on sepsis-associated cognitive impairment in rats. *Scientific Reports*, *11*(1), 14511.

Zohreh, D, Mahdi, P. and Maryam, G.K, (2019). Arbutin reduces cognitive deficit and oxidative stress in animal model of Alzheimer's disease. *Inter. J. Neuro.* 00207454.2019.1638376.