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RESEACH ARTICLE

COMPARATIVE STUDIES ON THE PROTECTIVE AND CURATIVE EFFECTS OF ASCORBIC ACID ON LEAD-INDUCED NEURAL TOXICITY ON THE HIPPOCAMPUS OF ADULT WISTAR RATS

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Abstract

Background: Neurological disorders often arise from an imbalance between oxidants and antioxidants. Ascorbic acid (vitamin C) is renowned for its antioxidant and reducing properties, yet its protective versus curative effects remain underexplored. This study compared the protective and curative effects of vitamin C on lead-induced neurotoxicity in the hippocampus of adult Wistar rats. **Methods** Twenty-five male Wistar rats were divided into five groups. Group I served as the control, while Group II was given lead (233 mg). Group III received vitamin C (200 mg), Group IV was administered lead and vitamin C concurrently, and Group V was first exposed to lead for two weeks, followed by vitamin C treatment for another two weeks. Neurobehavioral performance was assessed using the Y-Maze, and biochemical and histological analyses were conducted post-administration. **Results**: Neurobehavioral results showed no significant differences between the control and treated groups ($p = 0.78$). However, biochemical assays revealed increased malondialdehyde (MDA) levels and reduced superoxide dismutase, glutathione, and catalase activities in the lead-treated group. These effects were mitigated in both the curative and protective vitamin C groups, with the protective group showing stronger amelioration. Histological examination of the hippocampus demonstrated reduced pyramidal cells, distorted layers, and increased pyknotic cells in the group II. These changes were more effectively reversed in the protective group than in the curative group. **Conclusion:** Vitamin C exhibits stronger protective effects than curative effects against lead-induced neurotoxicity in the hippocampus, highlighting its potential for preventive applications in oxidative stress-related conditions.

Keywords; Neurotoxicity, hippocampus, lead, protective and curative, vitamin C

INTRODUCTION

Various biochemical properties, such as antioxidant and reducing activities, have been ascribed to ascorbic acid (Vitamin C) (Osifo et al., 2015). The use of vitamin C for chelating lead has been a novel development in science and health. Vitamin C, a water-soluble vitamin, is a strong reducing agent capable of giving electrons to lead and thereby generating soluble complexes that are easily flushed out of the body (Adeniyi *et al.,* 2008).

Ascorbic acid was reported to significantly increase the level of reduced glutathione (GSH) and reduce the elevated level of plasma amino levulinic acid (ALA) in lead-exposed workers (Akinhanmi *et al.,* 2016). A dosage of 100 mg/kg body weight of vitamin C improved protein concentration and lessened the negative effects on serum liver enzymes (alkaline phosphatase (ALP), aspartate transaminase (AST), alanine Transaminase (ALT), total protein and albumin) (Osifo *et al.,* 2015). The highest ascorbate concentrations in the body are found in the brain and neuroendocrine tissues such as the adrenal gland (Harrison & May, 2009). Ever since the initial in-depth exploration of brain AA over 55 years ago (Rajalakshmi & Patel, 1968), two significant

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neurobiological functions of this vitamin have become apparent: neuroprotection (Coverrubias-Pinto *et al.,* 2015; Rice, 2017, Moretti *et al.,* 2017) and neuromodulation (Harrison & May, 2009). These roles have sparked optimism for finding therapeutic uses of pharmacological AA administration. Due to its involvement in the production and regulation of biogenic amines, AA is likely to serve as a supplement to anxiolytic, antidepressant, antipsychotic (Rebec, *et al.,* 1985; Moretti *et al.,* 2017), and antiepileptic medications (González-Ramírez *et al.,* 2010). The antioxidant properties of AA help combat oxidative stress associated with ageing, a contributing factor to neurodegeneration. Therefore, ensuring the brain receives an ample supply of AA is likely to postpone the onset of neurodegeneration (Harrison *et al.,* 2014, Han *et al.*, 2018).

MATERIALS AND METHODS

Chemicals

Lead acetate, normal saline and vitamin C tablets were obtained from reputable a pharmaceutical store in Kano, State Nigeria, they were reconstituted in distilled water prior to daily administration.

Experimental Animals

Adult male Wistar rats were obtained from the animal house of Department of Microbiology, Faculty of Sciences, Bayero University Kano. The animals were taken to the animal house of Anatomy Department Bayero University, Kano for acclimatization and adaptation for two weeks during which they were given food and water ad libidum. After the acclimatization period, the animals were then weighed using a sensitive balance and then divided into five groups of five animals each. Group I, II, III, IV and V were given normal saline, 233mg of lead, 200 mg of vitamin C, 233 mg of lead and 200 mg of vitamin C and then 233 mg of lead for two weeks and then treated with 200 mg of vitamin C for another two weeks respectively. As shown in table I below:

Table 1: Animal grouping, duration and treatment by oral intubation

Groups	No. of animals	Treatment	Duration
Group I	5	0.2mls distilled water	28 days
Group II	5	Vit C	28 days
Group III	5	233 mg of lead acetate	28 days
Group IV	5	233mg lead aceate + Vit C	28 days
Group V	5	Lead acetate 2wks + next Vit. C for 2wks	28 days

mls= millimeters, mg= milligram, wks= weeks, vit. C = vitamin C, route of administration is oral intubation.

Weight Measurement

The weight of the animals was taken every week using a sensitive weighing balance.

Preparation of vitamin C solution

Vitamin tablet was obtained from a reputable pharmaceutical company in Kano, 1000 mg of it was dissolved in 5ml of water to obtain a 200 mg/ml solution. The volume to be administered to the animals was calculated using the following formula:

$$\text{Volume} = \frac{\text{weight of animal (Kg) x dosage (mg/kg)}}{\text{Concentration of Stock solution}}$$

Toxicological Studies

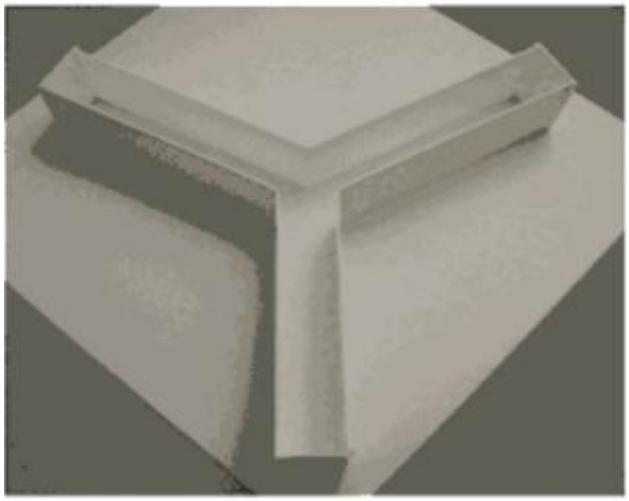
The Lead ii acetate was tested for acute toxic effects using the method described by Lorke (1983). The test was conducted in two phases. In phase one, nine rats weighing between 100 and 150 g were randomly selected and used for the experiment. The nine rats were divided into three groups of three animals each. Groups 1, 2 and 3 were given 10, 100, and 1000 mg/kg of lead acetate respectively. All the animals were observed for 24 hours for any sign of toxicity or death. In phase two of the trial, which depended on the outcome of the first trial, three healthy rats were grouped into three groups containing one animal each. Rats in groups 1, 2 and 3 were orally given 1600, 2900 and 5000 mg/kg of the lead ii acetate respectively in accordance to the method of adapted by Ogunleye *et al.,* (2019).

Neurobehavioral Studies

Y-Maze spontaneous alteration test: this behavioral test used to assay the willingness of rodents to explore new environment. This test was based on the principle that rodents generally preferred to investigate new arm of the maze rather than returning to previously visited arm.

Procedures: the rat is placed on the center of Y-Maze which consisted of three arms at 120^0 from each other and allowed to explore the arms of the Y-Maze for five minutes. The number of times each arm of the Y-Maze is visited is counted as the alteration and converted into

percentage i.e. percentage alteration. As illustrated in figure 3.3 below:



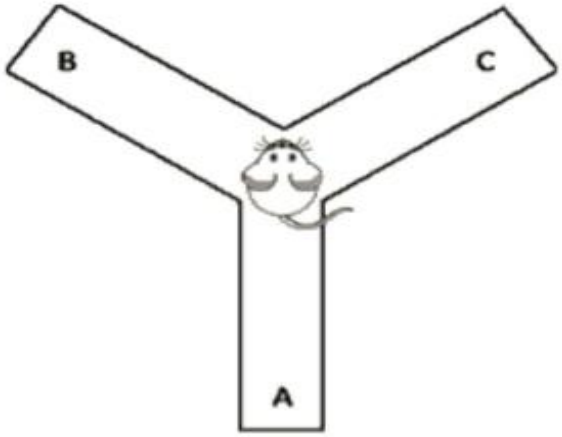


Figure 1: Y-Maze (Behavioral and functional neuroscience laboratory).

Animal Sacrifice and blood collection

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 ten (10) mill syringe through ventricular puncture. The blood samples were stored in plain specimen bottles, anticoagulant free and allowed to coagulate and serum was harvested for biochemical analysis.

Superoxide dismutase activities measurement

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 Measurement

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).

Measurement of catalase activities

Catalase Activity Assay Kit (Colorimetric/Fluorometric) Ab83464 Abcam USA: was used to determine the catalase activities according to the manufacturer's protocols.

Serum Calcium

Serum calcium was determine using automated method using automated biochemistry analyzer (Seamaty, SMT-120, China).

COLLECTION OF BRAIN TISSUE

Incisions were made through the skin and muscles of the skull and the skull was opened through the mid-sagittal suture in order to remove the brain tissues which were fixed in Bouin's fluid before being taken to the histology laboratory of the Anatomy Department, Bayero University Kano, for tissue processing.

Tissue preparation

The hippocampus tissue specimens were fixed in a Buin's fluid (10%). After dehydration procedures, the tissue specimens were embedded in paraffin wax and sectioned (thickness, 5 mm). Levels of sections and CAl, CA2 and CA3 areas in the hippocampus were found with the help of the stereotaxic atlas and were stained with Hematoxylin-Eosine. Measurements were obtained from 4 images per rat for the left hemispheres (coordinates were adjusted to 2.64–3.00 mm posterior from the bregma, according to (Paxinos & Watson 2007).

In stained with Hematoxylin-Eosine areas, the numbers of pyknotic neurons in the pyramidal cell layer were counted using light microscopy (Olympus BX 50) under a 40-fold magnification objective with the help of the eyepieces graticules.

RESULTS

Result of LD50 of Lead II Acetate

The result of the toxicity studies showed that at 1000, 1650 and 2900 mg/ per kilogram body weight of lead acetate oral administration no death was observed among the rats. Death was observed in the rat that received 5000 mg/kg bwt of lead acetate within 24 hours of administration. The result of the Lead II acetate dosage in relation to mortality recorded are shown in the table 2:

TABLE 2: RESULT OF LETHAL DOSE FIFTY PERCENT (LD50) OF LEAD II ACETATE

s/n	Conc. mg/kg Bwt	No. of rats (n)	No. rats alive after 24 hours (%) of lead admin	No. of rats dead after 24 hours (%)of lead admin
1	1000	3	3 (100)	0(0)
2	1650	3	3(100)	0(0)
3	2900	3	3(100)	0(0)
4	5000	3	0 (0)	3(100)

Note: conc= concentration; No.= number;

Increased in body weight was observed across the weeks in control group, from 214.00 ± 55.05, 219.20 ± 25.17, 220.40 ± 23.03 and 246.60 ± 51.93. group two animals which received lead acetate there was a slight decrease in body weight in the first two weeks followed by slight increase, in single digits, 194.00 ± 32.86, 195.60 ± 31.03, 194.60 ± 39.68 and 198.80 ± 39.87 in the fourth week..

Again group four animals, which received lead and vitamin C concurrently showed a gain in weight initially followed by a decrease in weight and gain in weight again in the subsequent weeks. While group three animals which received vitamin C only shows gain in body weight throughout the experimental duration. Finally group five animals which received lead for two weeks followed by treatment with vitamin C for another two weeks show a loss in weight initially, followed by a gain in weight during the treatment duration as shown in figure 3 below:

TABLE 3: WEIGHT CHANGES ACROSS THE WEEKS DURING ANIMALS TREATMENTS

Groups	week 1	week 2	week 3	week 4
Control	214.00 ± 55.05	219.20 ± 25.17	220.40 ± 23.03	246.60 ± 51.93
Lead	194.00 ± 32.86	195.60 ± 31.03	194.60 ± 39.68	198.80 ± 39.87
Lead + VitC	187.00 ± 14.83	189.40 ± 18.75	178.80 ± 44.33	197.80 ± 32.27
VitC	181.25 ± 11.82	194.50 ± 19.02	209.75 ± 16.86	216.75 ± 21.47
Lead 2wks VitC 2wks	190.00 ± 21.51	213.20 ± 39.98	188.60 ± 08.82	195.60 ± 13.03
F-value	2.34	1.65	2.14	3.09
p-value	0.06	0.173	0.082	0.02

Results are presented as mean ± standard deviation, the same alphabet in the same column signifies significance at a p-value less than 0.05

RESULT OF SPATIAL WORKING MEMORY USING SPONTANEOUS ALTERNATION Y-MAZE

The result of the spatial memory using Y-maze showed no significant difference across the group across all parameters measured with a p-value greater than 0.05, however the highest number of arm visitation was observed in the control group and vitamin C treated groups followed by lead group, lead plus vitamin C group and finally lead two weeks as shown in table 4 below:

TABLE 4: RESULT OF SPATIAL WORKING MEMORY USING SPONTANEOUS ALTERNATION Y-MAZE

Group	no of ani.	Mean ± SD	Mean num arms	% Alternation
Control	5	01.40 ± 0.89	05.40 ± 2.07	24.67 ± 5.45
Pb (233 mg)	5	01.40 ± 0.55	06.40 ± 1.52	22.17 ± 7.67
Pb+Vit. C	5	01.60 ± 0.55	07.80 ± 3.11	21.12 ± 5.58
Vit. C (200 mg)	5	01.80 ± 0.45	07.80 ± 311	24.46 ± 7.04
Pb 2wks + vit. C 2wks	5	01.80 ± 0.84	08.40 ± 2.97	20.97 ±4.12
F		1.083	0.435	0.433
P		0.391	0.782	0.783

Values are presented as mean ± standard deviation (SD). Values with the same superscript on the same column are significantly different at P<0.05.

The result of serum calcium level showed a highly significant value with a p-value of 0.000, the significance was observed between groups I and IV and II and IV. MDA also showed significant differences with a P-value of 0.001. Likewise, GPX and CAT also showed a significant level with a p-value of 002 and 0.023 respectively, as can be seen in the table below:

Table 5: Result of Serum Biochemical Assay among Animal

GROUPS	Calcium	MDA	SOD	GPX	CAT
I	2.25 ± 0.08a	1.65 ± 0.166a	2.20 ± 0.187	51 .00± 2.44a	45.75± 2.86
II	2.25 ± 0..07b	1.90 ±0.122	2.10 ± 0.112	46.50 ± 1.66a	43.50 ± 1.80
II	2.12 ± 0.06	1.87 ± 0.167	2.20 ± 0.163	46.30 ± 2.05a	42.00 ± 2.16a
IV	2.00± 0.01ab	2.00 ± 0.000ab	2.00 ± 0.00	50.25 ± 0.96	46.75 ± 0.50a
V	2.13 ± 0.04	1.63 ± 0.047b	2.13 ± 0.047	49.67 ± 0.12	45.00 ± 1.41
F-VALUE	13.289	7.269	2.155	6.662	3.760
P-VALUE	.000	.001	.118	.002	.023

Data are expressed as mean ± standard error of the mean, the same alphabet within the same column signifies significance. P-value ≤ ± 0.005 is considered significant

Histopathological findings

Histological observation for the CA1 area of hippocampus showed normal pyramidal cells in the control group, with normal pyramidal cellular layer. Few granulocytes pyknosis. However for the lead treated group, there is normal pyramidal cells with distorted pyramidal cell layer and more pyknosis of the granular cells. While for the intervention groups both protective and curative groups' normal pyramidal cellular layer is observed with normal pyramidal cell layer and granulocyte, as shown in plate I below.

While plate II: showed the cornus aminus II (CAII) area of the hippocampal tissue of control rats, showing normal pyramidal cells (PC) and granular cells (GC). B1, Hippocampal tissue of lead treated group showing pyramidal cells (PC), Blood vessels, (BV) and Pyknotic granulocyte (PG). C1; hippocampal tissue of Vitamin C treated animals pyramidal cell (PC) and pyramidal cellular layer (PCL). E1; hippocampal tissue of lead plus vitamin C group showing pyramidal cells (PC) and pyramidal cellular layer (PCL), F1, hippocampal tissue of curative group; showing degenerating granular cells (DGC).

Hippocampal tissue of cornus amminus (III) showed normal pyramidal cells (PC) and granular cells (GC) for the control group A2. B2, hippocampal tissue of lead treated group showing pyramidal cells (PC), Blood vessels, (BV) and Pynotic granulocyte (PG). C2; hippocampal tissue of Vitamin C treated animals

pyramidal cell (PC) and pyramidal cellular layer (PCL). E2; hippocampal tissue of lead plus vitamin C group showing pyramidal cells (PC) and pyramidal cellular layer (PCL), F2, hippocampal tissue of curative group; showing degenerating granular cells (DGC) as can be seen in plate III below:

DISCUSSION

From the result of this study it was found that the LD50 of lead in Wistar rats was 4286, lead causes a decrease in body weight of the experimental animals across the weeks, lead increases the level of malondialdehyde, but reduces the level of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities with distortion in the cytoarchitecture of hippocampal tissues which were ameliorated by both protective and curative forms of vitamin C.

The high LD_{50} of lead obtained in Wistar rat from this study was due to the natural antioxidant of the body which were able to annulled the effects of the reactive oxygen species release by lead, as earlier reports (Mousin et al 2010; Heruye et al., 2022) showed that body natural antioxidants protect it from free radicals released from the body metabolism and other external sources such as lead. This agrees with the work of Fihri *et al.,* 2016 where an LD 50 of lead was reported to be 4.665 g/kg bwt. This contradicted the work of Iliyasu *et al.,* 2015; Sunusi *et al.,* 2023 where they both reported an LD50 of 600 mg/kg bwt as adapted from Sujha *et al.,* 2011. However, most toxicity signs such as excessive salivation, diarrhea, restlessness, loss of appetite, general weakness were in agreement with earlier reports by; Bulus *et al.,* 2011 & Iliyas *et al.,* 2015 where animals administered with lead at lower doses records no mortality but showed signs of toxicity. Other factors that may affect the LD50 include age and sex of the animals, strain of the animal and genetic makeup. Though the previous reports adapted an LD50 of 600 mg/kg of rats from Sujatha et al., 2011 for both sexes of animals, however we used only male Wistar rats. Our work also agrees with most data sheet or toxicity sheets of most leads seen from google search. This showed that despite the fact that lead may be toxic, the effect of it toxicity is as a result of either high dose or long term accumulation.

Ascorbic acid was reported to ameliorate some of the behavioral changes induced by environmental heat stress. Ambali and Ayo (2012) reported that deficits in locomotion efficiency, motor strength, righting reflex and excitability score induced by chronic chlorpyrifo (CPF) were mitigated by vitamin C. Vitamin C was reported to inhibit impairment in synaptic plasticity and neurobehavior as result of beta amyloid deposition 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). Though our studies showed no significant difference in percentage alteration in the experimental groups using Y-maze, there is still better performance in the group treated with as ascorbic acid particularly for the ameliorative group ie the group given lead and ascorbic acid concurrently. This is in accordance to earlier report by Heruye et al., 2022 who stated that ascorbate once in the cerebrospinal fluid get to the brain by diffusion and exhibit it antioxidant property of the brain because of the high metabolic activities in the brain, large amount of this antioxidant is store in the brain. Antioxidants such as Arbutin (Azhoreh et al., 2019), Baobab, (Otong et al., 2022), glycyrrhizic, (Azhoreh, et al., 2019) & vitamin C (Bulama et al., 2018) were reported to mitigate the neurobehavioral changes induced by lead and other stress inducers. The pupated mechanism of action includes enhancement of the antioxidant mechanism of the neural tissues there by reducing the impact of free radicals such as those from lead on the nervous tissues and reducing the level ROS from the nervous tissues (Yousef et al., 2019). Ascorbic acid was also reported to enhance synaptic plasticity and neurobehaviours (Sattari et al., 2021), by improving cell to cell contact and release of neurotransmitters (Spector, 2013). It was reported that 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).

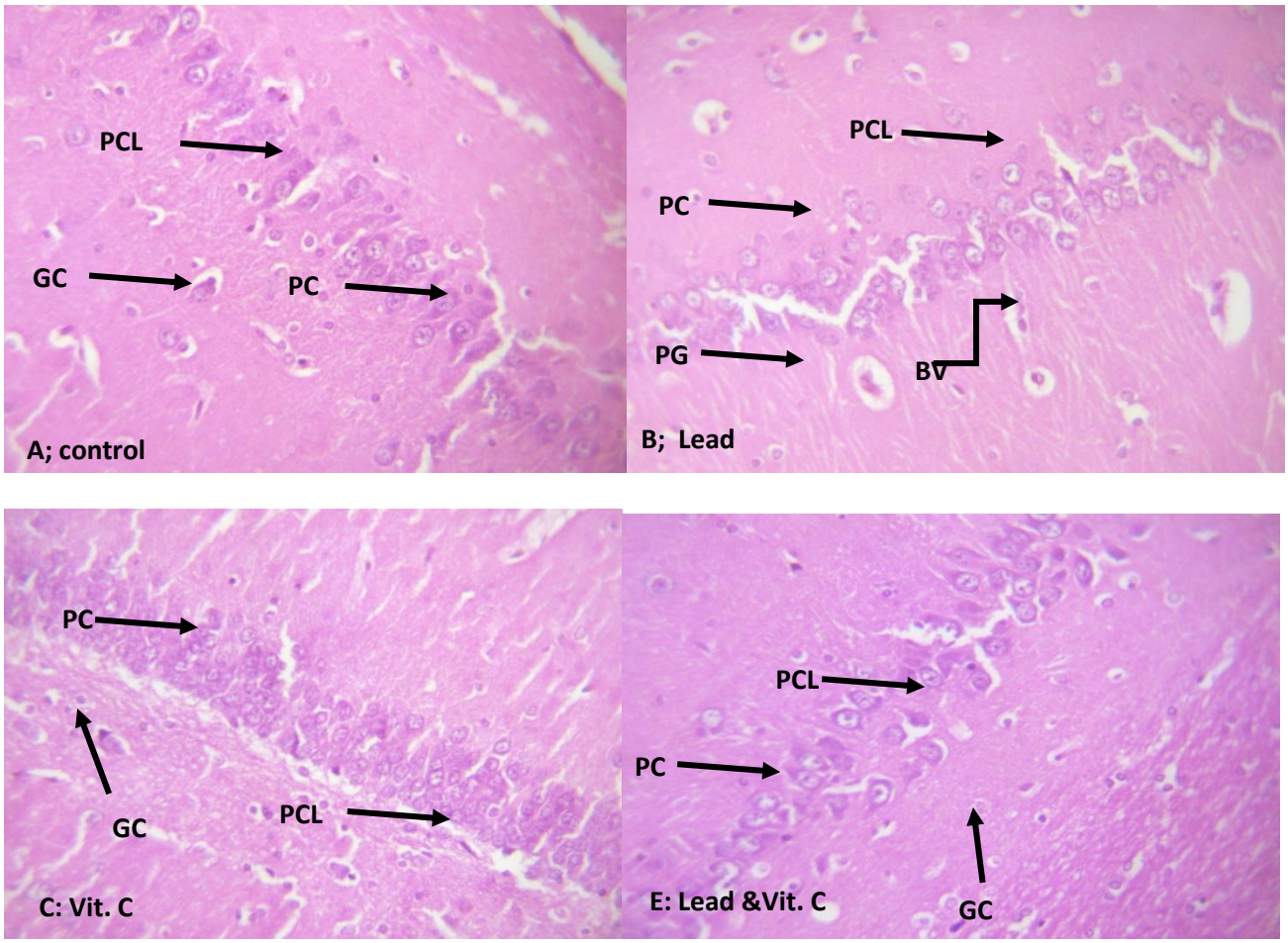


Plate I: A; CA 1 area of hippocampal tissue of control group showing normal pyramidal cells (PC), pyramidal cellular layer, and granular cells (GC). B, hippocampal tissue of the lead treated group showing pyramidal cells (PC), pyramidal cellular layer (PCL), Pyknotic granular cell (PG), blood vessels (BV). C, hippocampal tissue of the vitamin C treated group, pyramidal cells (PC), pyramidal cellular layer (PCL), and granular cells (GC). E, hippocampal tissue of lead plus vitamin C group, pyramidal cells (PC), pyramidal cellular layer, and granular cells (GC). F, hippocampal tissue of animal treated lead for two weeks and then vitamin C for the next two weeks

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BV

PG

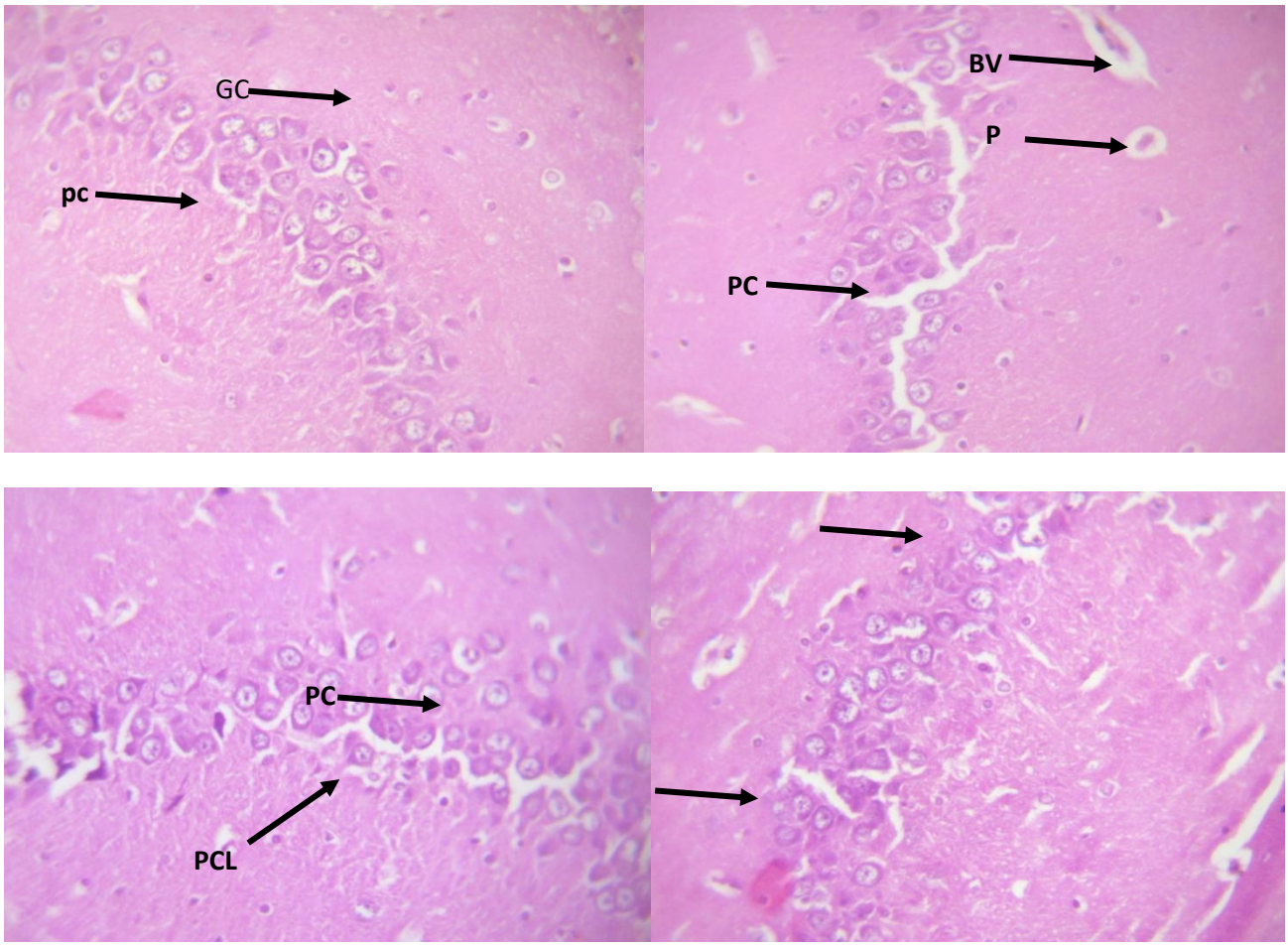

Plate II: A1; hippocampal tissue of control group showing normal pyramidal cells (PC) and granular cells (GC). B1, hippocampal tissue of lead treated group showing pyramidal cells (PC), Blood vessels, (BV) and Pynotic granulocyte (PG). C1; hippocampal tissue of Vitamin C treated animals pyramidal cell (PC) and pyramidal cellular layer (PCL). E1; hippocampal tissue of lead plus vitamin C group showing pyramidal cells (PC) and pyramidal cellular layer (PCL), F1, hippocampal tissue of curative group; showing degenerating granular cells (DGC).

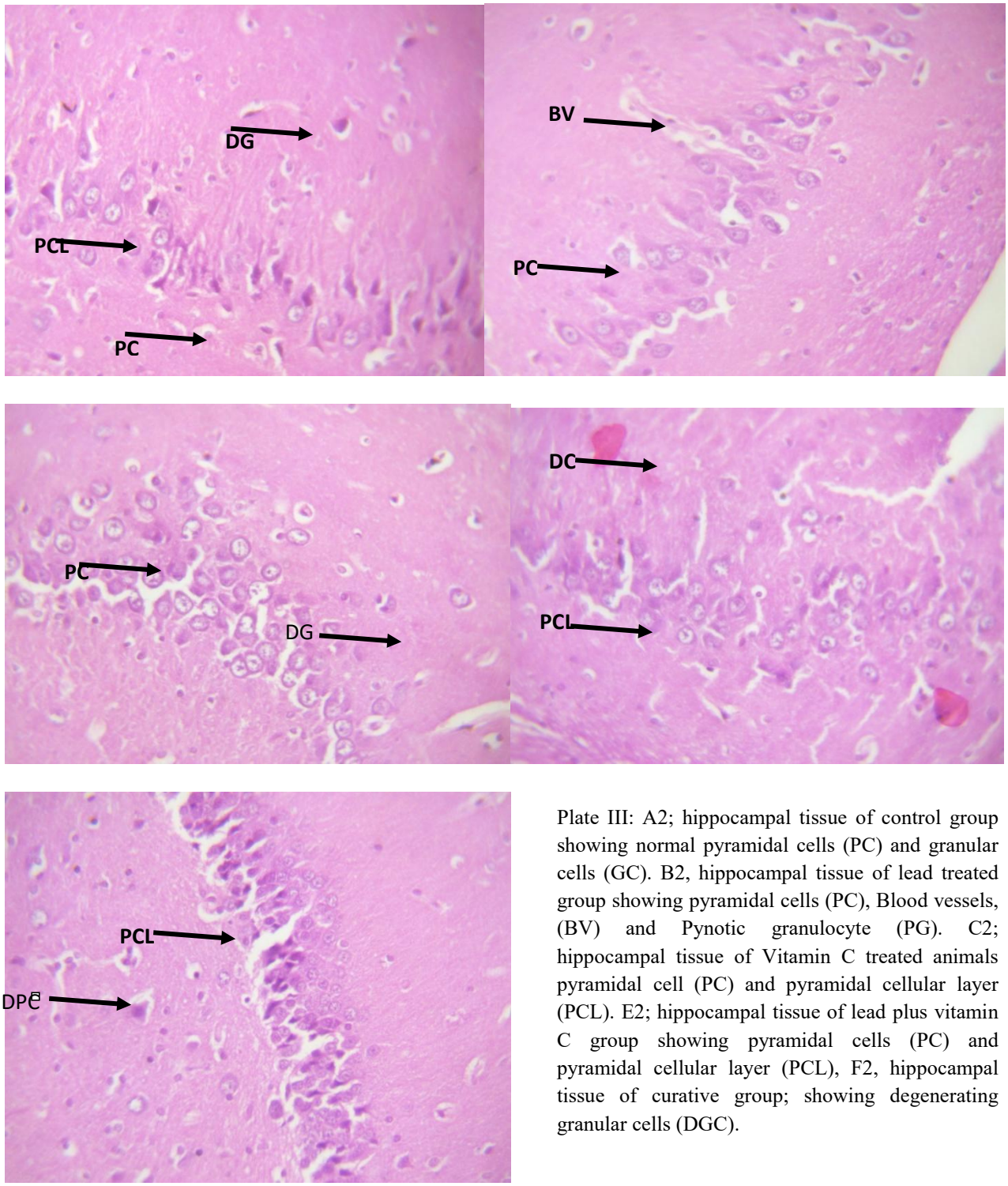

Plate III: A2; hippocampal tissue of control group showing normal pyramidal cells (PC) and granular cells (GC). B2, hippocampal tissue of lead treated group showing pyramidal cells (PC), Blood vessels, (BV) and Pynotic granulocyte (PG). C2; hippocampal tissue of Vitamin C treated animals pyramidal cell (PC) and pyramidal cellular layer (PCL). E2; hippocampal tissue of lead plus vitamin C group showing pyramidal cells (PC) and pyramidal cellular layer (PCL), F2, hippocampal tissue of curative group; showing degenerating granular cells (DGC).

Single prolong stress (SPS) induced 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 also memory impairment.

The increased level of MDA observed in this study was a result of an increase in ROS by lead as a result of the release of free radicals. Studies have shown that Ascorbic Acid decreases malondialdehyde levels 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 a 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). A positive correlation was observed between catalase level and the level of vitamin C in milk cells (Kazak and Coskun, 2022). Zhang *et al.,* (2020) observed a reduction in the level of SOD and a rise in the level of MDA in sepsis-induced rats which were ameliorated by a 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). In the brain, the amygdala, hippocampus, and cerebral cortex were reported to have the highest levels of vitamin C (Mefford, 1998; Spectrum, 1977). 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).

Vitamin C is a potent antioxidant that neutralizes reactive oxygen species (ROS). Elevated oxidative stress is a common feature in various neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Studies have shown that vitamin C can reduce oxidative damage in neuronal cells, thereby preserving cellular integrity and function (Halliwell & Gutteridge, 2015). Vitamin C plays a role in the synthesis of neurotransmitters, particularly norepinephrine and serotonin. It is involved in the enzymatic conversion of dopamine to norepinephrine, which is critical for mood regulation and cognitive function (González-Marrero et al., 2017). This suggests that adequate vitamin C levels may support neurotransmitter balance and cognitive health. Inflammation in the nervous system is implicated in numerous neurological disorders. Vitamin C has been shown to modulate inflammatory responses by inhibiting pro-inflammatory cytokines and promoting anti-inflammatory pathways (Zhang et al., 2019). This property may contribute to its protective effects against neuroinflammatory conditions.

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 (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. The result of increased number of pyknotic cells in the current study among the rats treated with lead was in accordance to an earlier report among rats with traumatic brain injuries. Vacuolation, cavitation, degeneration of glia and pyramidal cells, observed in the rats treated with lead acetate was in accordance with earlier work where hippocampal tissues of rats exposed to radiofrequency of electromagnetic field from mobile phone showed lesion in the hippocampal tissue Hussein and Muhammad (2015) and Otong et al., (2022) who reported that lead induce 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 a superoxide radical to H2O2 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.

The current reduction of degenerative features in the cytoarchitecture of the rats treated with lead and vtamin C could be as a result of the high antioxidant property of vitamin C which mitigates the impact of the free radicals (such as those from lead) on the tissues. This is in agreement with the work of Li et al., (2024) 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). Several epidemiological studies have suggested that higher dietary intake of vitamin C is associated with a reduced risk of developing Alzheimer's disease (Morris et al., 2018). Experimental models indicate that vitamin C supplementation can attenuate amyloid-beta accumulation and tau hyperphosphorylation, key pathological features of the disease (Zhang et al., 2020). Research indicates that vitamin C may also be protective against Parkinson's disease. It has been observed that patients with Parkinson's often exhibit lower levels of vitamin C. Animal studies show that vitamin C supplementation can improve motor function and reduce dopaminergic neuronal loss (Shin et al., 2021). Vitamin C's role in stroke recovery has garnered attention, as it is believed to protect against ischemic damage. Clinical trials have reported that vitamin C administration can improve outcomes following cerebral ischemia by reducing oxidative stress and promoting neuroprotection (Duan et al., 2022).

However, most of regenerative feature observed in the group that were given lead and vitamin C concurrently were less pronounce in the group given lead for two weeks followed by treatment for the next two weeks. This is because lead induced neural degeneration by altering the body antioxidant system and by the release of free radicals in the form of reactive oxygen species (Andersen, 2004; Ahmad & Siddiqui, 2007; Ashour et al; 2007; El shawakh et al., 2016, Otong et al., 2022& Sunusi et al., 2023). One of the major feature of nervous tissue is the absent of centrosome which make it difficult to regenerate when damage. Hence in the curative group more impact of lead was observed when compared to the protective group.

In conlusion, lead causes neurobehavioral changes, rise in the level of MDA, reduction in the activities of serum GPX, SOD, CAT and $Ca^{2+,}$, and changes in the cytoarchetecture of the hippocampal tissues which were ameliorated by

both protective and curative forms of vitamin Cs however vitamin C has more of protective effects on lead-induce neurotoxicity.

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Conflict of Interest

The authors have declared no competing interest.

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Authors' Contribution

SA; conceptualized, design the research and carryout the research FRI; proof read and correct the work JNA; partake in the laboratory experiment AT; records the data BMI; Corrected both the research design and the manuscript.

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