Original Article

Evaluation of Ethanol Extract of *Curcuma longa* in Lead-induced Hippocampal Neurotoxicity

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Abstract

Background: Heavy metals such as lead are ubiquitous elements at exposure causing deleterious effects on the brain and leading to neurodegenerative diseases. **Aim:** In this investigation, the neurotherapeutic effects of ethanol extract of *Curcuma longa* (EECl) against lead-induced hippocampal neurotoxicity in rats were examined. Biochemical examination for antioxidant enzyme activity and lipid peroxide level (malondialdehyde [MDA], superoxide dismutase [SOD], and glutathione [GSH]) was evaluated, the Barnes maze for learning and memory, and histological analysis (H and E stain) for general histoarchitectural features to investigate the neurotherapeutic characteristics of EECl. **Materials and Methods:** Six groups totalling 36 rats were created (n = 6). In the first group, rats received distilled water (2 mg/kg), in the second, lead acetate (LA) (120 mg/kg), in the third, ascorbic acid (100 mg/kg), and the 4th, 5th, and 6th groups, rats received LA (120 mg/kg) and EECl (375 mg/kg, 750 mg/kg, and 1500 mg/kg, respectively) for 14 days. **Results:** A significant learning and memory deficit was seen in the LA-treated group's results, but a significant improvement was seen in the EECl-treated group. Increased oxidative stress was seen in the LA-treated group, as evidenced by an increase in MDA levels and a decrease in antioxidant enzymes (SOD and GSH). A decline in MDA levels and an increase in SOD and GSH activity was the evidence of the ameliorative effects of EECl treatment. Cytoarchitectural distortions relative to the control were observed with the LA-treated group. Mild distortion was however detected with EECl treatment. **Conclusion:** EECl has possible neurotherapeutic properties against LA-induced pathological changes in the hippocampus of Wistar rats. EECl may have neuroprotective effects against degenerative alterations brought on by LA.

Keywords: *Barnes maze, cytoarchitecture, hematoxylin and eosin, learning and memory, oxidative stress*

Introduction

Humans' daily interactions with the environment make them susceptible to pollution. Environmental pollution is the prevalence of pollutants in the soil, air, water, and subsequently in food that can harm environmental organisms.[1,2] In their daily interactions with their surroundings, both humans and animals are exposed to a variety of heavy metals such as lead, mercury, aluminum, and cadmium. These relationships with the environment happen through air, water, and food.[3] Heavy metals become harmful when they accumulate in soft tissues instead of being metabolized by the body.[4]

In underdeveloped and developing nations, lead toxicity is a common concern to public health because of human activities such as mining and farming.[5] Lead is a multi-organ toxin that damages several organs and is linked to several cancers, damage to the kidneys and nervous system, and problems with reproduction in both humans and animals. It can finally result in a child's death.[5,6] Several public health and occupational safety actions have been implemented to minimize lead exposure instances, yet several lead poisoning cases are still reported.

Lead crosses the blood − brain barrier and causes neurotoxicity through oxidative stress molecular mechanism.[7,8] Essential macromolecules such as proteins, lipids, and DNA are oxidized as a result of the increased formation of nitrogen-based radicals and oxygen and associated nonradical products.[9] Reactive oxygen

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species (ROS) are by-products of metabolic activities in aerobic organisms. Under normal circumstances, antioxidant enzyme activity, such as that of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and lipid peroxidase, regulate ROS concentration.[10] The imbalance between the scavenging and generation of ROS, which can harm the detoxification system and promote ROS production, causes oxidative stress.[11] However, during oxidative stress, the excessive free radical formation has detrimental consequences on cells, tissues, inflammatory responses, and apoptosis.[10,11] Neurodegenerative ailments such as Parkinson's and Alzheimer's are the main results of increased production of ROS in the body system.[12]

The hippocampus, a complex brain structure which is deeply ensconced in the temporal lobe of the cerebral cortex, is crucial for spatial navigation, learning and memory, emotional behaviour, and the regulation of hypothalamic functions.[13] Cornu ammoni (CA: CA1, CA2, and CA3) and dentate gyrus are the two major divisions of the hippocampus. These parts curve into one another and are separated by the hippocampal sulcus.[14] The dentate gyrus serves as the starting point for information flow through the hippocampus, which continues through CA3 to CA1 to the subiculum with extra input information at each stage and outputs at the two last stages. Even while CA2 makes up a very small percentage of the hippocampus and is sometimes overlooked in descriptions of hippocampal function, it is noteworthy that this tiny area appears remarkably resistant to situations that typically result in significant cellular damage, such as epilepsies.[14,15]

Curcuma longa (Turmeric) belongs to the *Zingiberaceae* family of ginger. It is extensively cultivated in nations with tropical climates such as India and China,[16] as well as Nigeria, where Hausa speaking Nigerians refer to it as *"Gangamau"* or "*Zabibi"* or "*Magina."* It has been employed in conventional medicine as a home treatment for several illnesses.[17] *C. longa* has numerous pharmacotherapeutic effects including antibacterial, anti-tumor, antioxidant, anti-inflammatory, hepatoprotective, and anti-viral activities.[18,19] *C. longa* offers a wide spectrum of medicinal potentials both *in vivo* and *in vitro*, including antioxidant and anti-inflammatory capabilities,[20] which makes it suitable for reversing the reactions and alterations brought on by lead toxicity in several organs as a result of the formation of ROS and promotion of an inflammatory response during oxidative stress.[21,22]

Materials and Methods

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Materials

Plant

Fresh rhizomes were procured locally in a market in Samaru, Zaria, Nigeria. The rhizomes were taken for the identification and authentication in the Department of Botany's Herbarium, Ahmadu Bello University (ABU), Zaria. Specimen Voucher Number: ABU0551 was provided.

Plant extraction method

The preparation of the ethanol extract of *C. longa* (EECl) took place in the Department of Pharmacognosy and Drug Development, ABU, Zaria. The method for maceration as stated by Brain and Turner,[23] Harborne,[24] and Evans[25] was adopted for the extraction using ethanol.

Experimental animals

Thirty-six healthy male Wistar rats (weighing between 100 and 180 g) were procured from the Faculty of Pharmaceutical Sciences' Animal House. The animals were brought to the Animal Welfare, Department of Human Anatomy, ABU, Zaria, the animals were housed under standard laboratory conditions of 12-hrs dark and light cycles and fed with rat meal and water at will. The animals were allowed to acclimatized for 2 weeks before the commencement of the study.

Drugs

Lead acetate

Lead acetate (LA) was procured and utilized in this work as a neurotoxin. The manufacturer of the item (Product No. 5032) is Hopkin and Williams Chemical Ltd, England.

Ascorbic acid (Vitamin C)

To assess the therapeutic effectiveness of EECl, ascorbic acid (Vitamin C) pills were acquired and utilized as a comparison medication. The item is produced in Lagos, Nigeria, by Emzor Pharmaceutical Industries Ltd.

Ketamine

For anesthesia, ketamine (50 mg/ml Ketamine Hydrochloride injectable USP,) bought from Swiss Parenterals PVT Ltd. in Gujarat, India.

Experimental design

Six groups of six rats each were formed from 36 healthy male Wistar rats. Group I acted as the control, and distilled water (2 mg/kg) was given to the rats. Only LA (120 mg/kg) was given to the rats in Group II. In addition, LA (120 mg/kg) and ascorbic acid (100 mg/kg) were given to rats in Group III. Rats in Groups IV, V, and VI received doses of 120 mg/kg of LA and 375, 750, and 1500 mg/kg, respectively, of EECl. Fourteen days were allotted for administrations.

At the end of the experiment, the rats were anesthetized using 75 mg/kg ketamine intraperitoneally.[26] The brains of all the rats were harvested and cut into two halves, one was preserved in freshly prepared Bouin's fluid immediately for histopathological studies while the second half was homogenized in 0.1 M phosphate buffer solution (pH 7.4) for biochemical analysis.

Barnes maze

The neurobehavioral assessment was conducted using the Barnes maze apparatus (BMA). The Barnes maze test is an apparatus used in assessing the spatial memory and learning. The test was developed by Dr Caro Barnes in 1979. The primary purpose of the Barnes maze is to assess the capacity of a rat to learn and remember the location of a target zone and escape into the safety spot within a set amount of time.[27] BMA consists of a round surface with up to 20 circular holes around it. The diameter of the circular surface is 92 cm with a central circle measuring 10.5 cm in diameter. There are 20 holes around the circular surface and each hole is 5 cm in diameter. Each hole is at a distance of 7.5 cm from one other and also 2 cm from the circular surface. A wooden box is constructed beneath the circular board which is movable serving as the safest spot for the escape.

After the rat is being placed in the central area of the BMA, it was allowed to freely roam around it for 90 s. The equipment was cleaned with methylated spirit following each animal trial and allowed to dry before the next animal was tested. Behaviors scores were:

- Wrong Trial (WT): This is the frequency at which the rat tried to escape through the wrong holes
- Time Spent: This is the total time spent by a rat within the given time duration, till it was able to escape into the safety spot.

All of the animals underwent daily training in the BMA (habituation) for 5 days. On the 3rd, 6th, 9th, and 12th days following administration, repetition of this learnt activity was conducted.

Biochemical analysis

A digital weighing scale (Acculab Vicon VIC-511 Precision Balance/Scale, USA, 0.001 g) was used to calculate the weight of the brains. The brain tissues (1 g tissue/4 ml) were homogenized in 0.1 M phosphate buffer at pH 7.4.[28] The homogenate was centrifuged and the supernatant was collected in plain sample bottles for estimation of oxidative stress markers (Malondialdehyde [MDA], GSH, SOD). This was carried out in Human Anatomy Department, ABU, Zaria. According to Ohkawa *et al*.[29] and somewhat modified by Atawodi *et al*.,[30] MDA was identified as a thiobarbituric acid (TBA) reactive compound utilizing 15 percent trichloroacetic acid and 0.67% TBA (TBA). Analysis of SOD activity using the Fridovich method[31] and GSH activity using the Ellman method[32] were used to measure the enzyme-based antioxidant activity.

Histological studies

Histological methods were used to process fixed brain samples by cutting sections that were specifically aimed at the hippocampus (CA1 and CA3) using Rats Brain Atlas as a guide.[33] The Department of Human Anatomy's Histology Unit, ABU, Zaria produced, processed, and stained histological sections using hematoxylin and eosin (H and E) stains to display the histoarchitecture of the CA1 and CA3. The Laboratory for Microscopy and Stereology Researchof the same institution were used to conduct microscopy and micrography utilizing an optical microscope (HM-LUX, Leitz Wetzlar, Germany) and a digital microscopic camera (MA 500 AmScope®, USA).

Data analysis

The results obtained were analyzed using the IBM Statistical Package and Service Solution (version 26.0 Armonk, NY, USA) and the results were expressed as mean ± SEM The presence of significant differences within the means of the groups was analyzed using one-way analysis of variance (ANOVA) while two-way Repeated measures ANOVA was used to compare the mean differences for neurobehavioral studies. *P* values less than ($P < 0.05$) were considered to be statistically significant.

Results

Neurobehavioural study

Barnes maze was utilized to test spatial memory and learning.

The results obtained from the acquisition escape time (ET) test following 90 s of learning and memory test using Barnes Maze revealed a significant ($P < 0.05$) increase in group II treated with only LA (120 mg/kg) when compared to the control, Group IV administered LA (120 mg/kg) + 373 mg EECl revealed a significant decrease when compared to control, also, Group VI treated with 120 mg/kg Pb + 1500 mg EECl showed a significant decrease in ET in relation to Group II treated with only lead [Figure 1a].

The result obtained from the probe test ET following 90 s of learning and memory test using Barnes Maze showed a significant increase in the ET in Group II in relation to the control group. However, there was a substantial decrease in the ET in groups III, IV, and V when compared to groups I and II, there was also a significant decrease in the ET in group VI when compared to group II [Figure 1b].

The result obtained from the frequency of acquisition WT following 90 s of learning and memory test using Barnes Maze showed an increase in WT in group II which is not significant ($P > 0.05$). However, there was a substantial decrease in group VI when compared to the control and group II [Figure 1c].

The result obtained from the frequency of probe WT following 90 s of learning and memory test using Barnes Maze showed a substantial decrease ($P < 0.05$) in the control group when compared to LA-treated group. Also, there was a significant decrease in the frequency of WT in groups V and VI when compared to group II [Figure 1d].

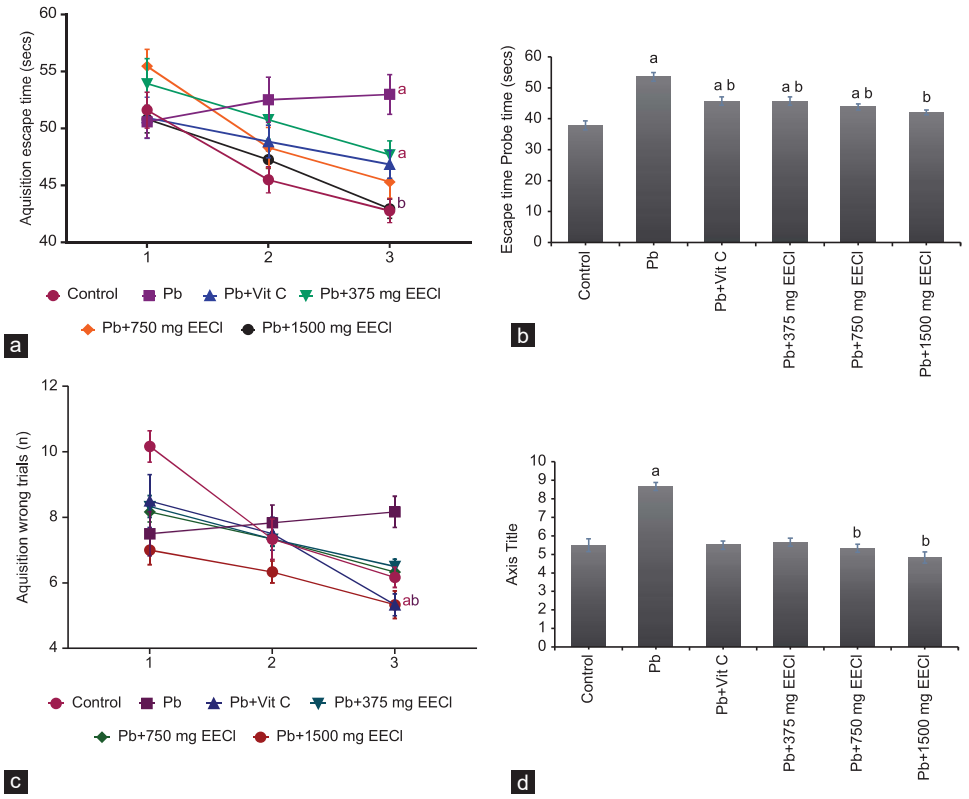

Figure 1: (a) Barnes maze acquisition escape time (s) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, two-ways repeated measures ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (b) Barnes maze probe test escape time (s) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, one-way ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (c) Barnes maze acquisition wrong trials of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, two-ways Repeated measures ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (d) Barnes maze probe test wrong trials (n) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, Kruskal − Wallis test, Dunn's *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. Pb: Lead, Vit C: Vitamin C, EECI: Ethanol extract of turmeric, ANOVA: Analysis of variance

Biochemical studies

MDA, SOD, CAT, and reduced GSH were tested for antioxidant enzyme activity and lipid peroxide levels in the brain tissue homogenate of Wistar rats.

Results on MDA showed a surge in the MDA level of rats administered with only LA (120 mg/kg) when compared to the control group which was not significant. MDA level significantly decreased ($P < 0.05$) in Groups V and VI when compared to the group treated with only LA [Figure 2a].

The level of SOD decreased in Group II treated with only LA 120 mg/kg as observed which was not significant. SOD level increased in Groups III and VI as observed when compared with Group II. However, the changes in the SOD level were not statistically significant [Figure 2b].

A decrease was also observed in the reduced GSH level of rats administered with LA (120 mg/kg) when compared to the control group and an increase in roups III, IV, V, and VI when compared to group II treated with only LA. However, the changes in GSH levels were not statistically significant as observed [Figure 2c].

Histological studies

The hippocampus of Wistar rat is subdivided into four regions, namely: CA1, CA2, CA3, and CA4 (CA stands for *cornu ammonis*), based on the size, density, and branching of the pyramidal cells' axons and dendrites. Each of these pyramidal cell sections is composed of three layers: The stratum molecular (molecular layer), the stratum pyramidale (pyramidal layer), which houses the bodies of the pyramidal cells, and the stratum multiforme (multiform layer). The continuation of CA3 in the concavity of the dentate gyrus (*fascia dentata*) is the CA4. Medium-sized cells are packed closely together in the CA1 region, giant cells are clustered closely together in the CA3 region, and the CA2 region is located between the CA1 and CA3 regions.

Histological examination of the Wistar rats' hippocampi, particularly the CA1 regions in the control group demonstrated their typical histoarchitecture of these regions which is essentially a sheet of organized large neurons (pyramidal cells). Relative to the control, histological examination of Wistar rats' hippocampal CA1 region exposed to lead demonstrated pathologic

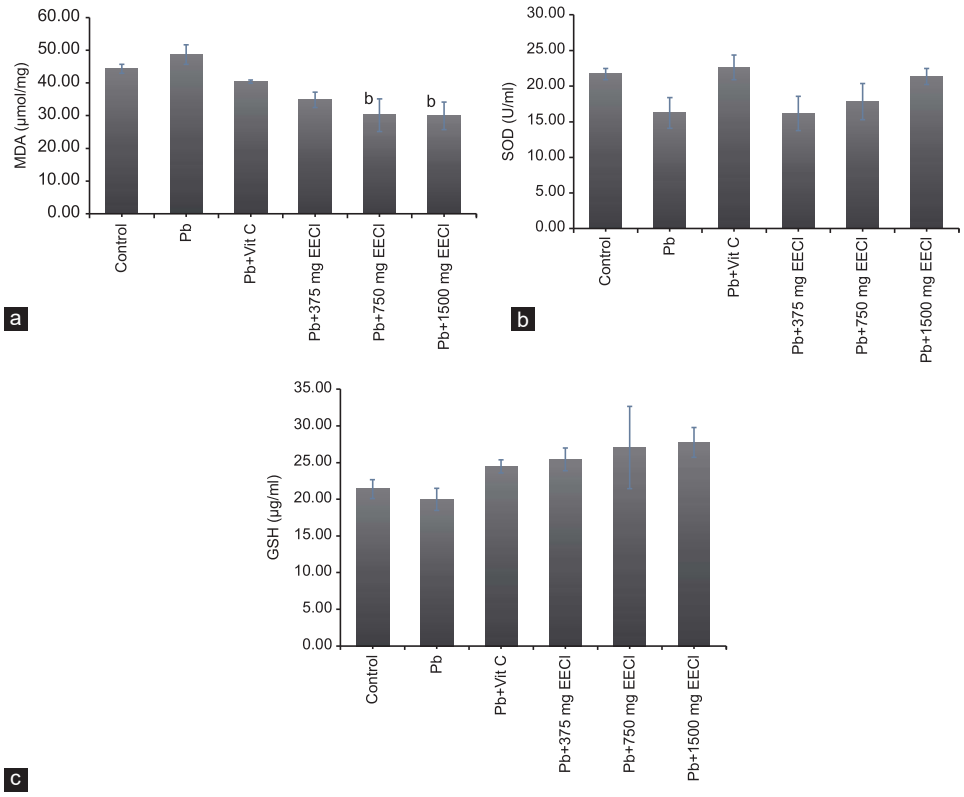

Figure 2: (a) MDA levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, Tukey *post-hoc* test. $^{b}P < 0.05$ when compared to lead $Pb2^{+}$ group. (b) SOD levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, *P* > 0.05 when compared to lead $Pb2^{+}$ group. (c) GSH levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, *P* > 0.05 when compared to lead $Pb2^{+}$ group. Pb: Lead, Vit C: Vitamin C, EECl: Ethanol extract of turmeric, MDA: Malondialdehyde, ANOVA: Analysis of variance, SOD: Superoxide dismutase, GSH: Glutathione

features such as irregular sizes of pyramidal cells of CA1 hippocampal neurons. Some pyramidal cells had a proliferation of glial cells (gliosis), fragmented nuclei (karyorrhexis), and dissolved nuclei (karyolysis). Vitamin C (100 mg/kg) and EECl (375, 750, and 1500 mg/kg)-treated groups revealed some restoration with mild distortions in the histoarchitecture of the pyramidal cells (P) with some pyknosis (Py), karyorrhexis (K), karyolysis (Ky), cytoplasmic vacuolation (cV), and gliosis (g) [Figure 3].

Histological examination of the Wistar rats' hippocampi, CA3 regions in the control group demonstrated typical histoarchitecture of these regions; the basic pattern of large neurons (pyramidal) whose cell bodies are all packed together. The big neurons are the enormous pyramids of CA3 with each neuron having a central vesicular nucleus with a prominent nucleolus. Relative to the control, histological examination of Wistar rats' hippocampal CA3 region exposed to lead demonstrated gross histoarchitectural distortions with karyorrhexis (K) and gliosis (g). Moreover, Ascorbic acid (100 mg/kg), EECl (375, 750, 1500 mg/kg) improved with mild distortion in the pyramidal neurons of the CA3 region of the hippocampus such as cytoplasmic vacuolation (cV), gliosis (g), karyorrhexis (K) [Figure 4].

Discussion

This work used neurobehavioral, pharmacological, and histological evaluation to examine the neurotherapeutic potential of an ethanol extract of *C. longa* on lead-induced hippocampal neurotoxicity in Wistar rats. The importance of the hippocampus in learning and memory cannot be overemphasized. Memory is known as the mental ability of an organism to store, retain and recall information.[34,35] Barnes Maze is a neurobehavioral task that is used in behavioural science and psychology, it serves as a behavioral model to evaluate learning and memory in Wistar rats.[27,36] Learning and memory were reflected by the rat's ability to find the safety spot with a reduced number of WT of treatment session compared to pretreatment session. Increased WT and ET as detected in the lead-treated group are indicative of learning and memory impairment. Results are in line with Naqi,[37] who stated that lead intake could induce cognitive hippocampal damage in adult Wistar rats. LA intake according to report causes mental retardation and reduce learning and memory.[38] Encephalopathy, a response to extremely high concentrations of lead, causes the development of irritability, mental dullness, headache, tremors, attention trouble, memory loss, and hallucinations within weeks of exposure, and it is one of the main neurological effects of lead exposure.[39]

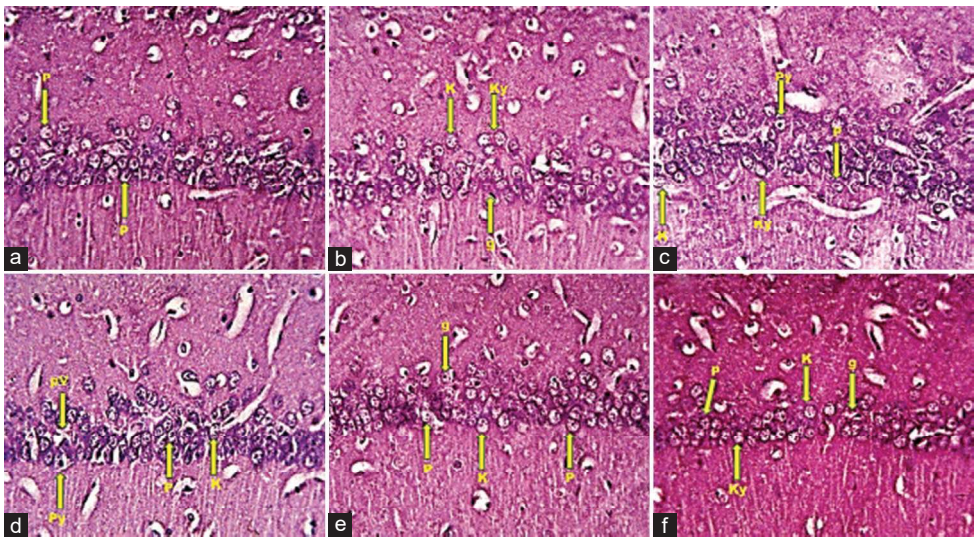

Figure 3: Micrograph of Hippocampus (CA1) of Wistar rats (H and E, × 250). (a) Control (distilled water 2 ml/kg) with normal histoarchitecture of the pyramidal cells (P). (b) Lead acetate (120 mg/kg) treated group with distortions in the histoarchitecture of the cells such as karyorrhexis (K), Ky and gliosis (g). (c) Lead acetate (120 mg/kg) and Ascorbic acid (100 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. Py, karyorrhexis (K), Ky, cV, and gliosis (g). (d) Lead acetate (120 mg/kg) and EECL (375 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. pV, Py, karyorrhexis (k), and some restoration of the histoarchitecture of the pyramidal cells (p). (e) Lead acetate (120 mg/kg) and EECL (750 mg/kg) treated group with some improvement in the histoarchitecture of the hippocampus. Karyorrhexis (k) and gliosis (g). (f) Lead acetate (120 mg/kg) and EECL (1500 mg/kg) treated group with marked improvement in the histoarchitecture of the hippocampus. Gliosis (g), karyorrhexis (K), Ky. pV: Perineuronal vacuolation, Py: Pyknosis, Ky: Karyolysis

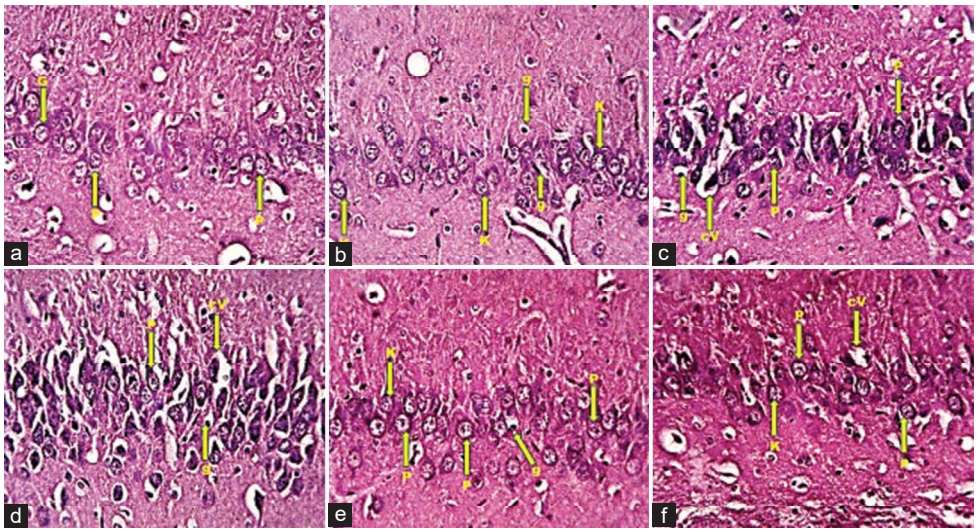

Figure 4: Micrograph of Hippocampus (CA2) of Wistar rats (H and E, × 250). (a) Control (distilled water 2 ml/kg) group with normal histoarchitecture of the pyramidal cells (P). (b) Lead acetate (120 mg/kg) treated group with distortions in the histoarchitecture of the cells such as karyorrhexis (K) and gliosis (g). (c) Lead acetate (120 mg/kg) and Ascorbic acid (100 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. pyramidal cells (P), cV and gliosis (g). (d) Lead acetate (120 mg/kg) and EECL (375 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. Gliosis (g), cV and some restoration of the histoarchitecture of the pyramidal cells (P). (e) Lead acetate (120 mg/kg) and EECL (750 mg/kg) treated group with some improvement in the histoarchitecture of the hippocampus. Karyorrhexis (k) and gliosis (g). (f) Lead acetate (120 mg/kg) and EECL (1500 mg/kg) treated group with marked improvement in the histoarchitecture of the hippocampus cV. cV: Cytoplasmic vacuolation

The Vitamin C-treated group showed decreased WT to the control. Furthermore, administration of EECl after day 14 of treatment had decreased WT in a dose-dependent manner when compared to the control and LA-treated group. This could be due to the ameliorative conclusion of Vitamin C and plant extract on the hippocampus which is in line with the previous studies.[40] Vitamin C is also very rich in biological properties which enhance memory through its bioactive constituents.[41,42] The administration of the plant extracts of turmeric has been reported to chelate the deleterious effects of lead-induced memory deficits and improve memory.[43] These findings imply that turmeric ethanol extracts may help to reduce lead-induced cognitive impairment.

In this study, light microscope examinations of routinely (Hematoxylin and Eosin, H and E) stained

histological sections of hippocampi (CA1 and CA3 regions) were conducted. Neurodegeneration is a process involved in both morphological and brain conditions.[35,44] Observed histoarchitectural distortions of the hippocampal CA1 and CA3 regions, such as an unequal neuronal arrangement of the CA1 and CA3 hippocampal neurons, and changes such as karyorrhexis, karyolysis, clumping of neuronal arrangement of both CA1 and CA3 neurons, also known as gliosis was observed which could be indicative of lead-induced neurodegenerative changes. The deranged CA1 nerve cells in the study imply treatment-related degeneration. This is similar to the work of Barkur and Bairy[45] who reported lead exposure significantly damaged neurons in the hippocampus, cerebellum, and amygdala regions in all lead-exposed groups. Mild degenerative changes in the CA1 and CA3 regions such as cytoplasmic vacuolation, karyorrhexis, karyolysis, pyknosis, and gliosis in the group treated with lead acetated followed by Vitamin C compared to control suggests lead toxicity. Once in the brain, lead may result in morphological changes in the brain that may persevere even after lead levels have decreased because it has an impact on numerous biological processes at the molecular, cellular, and intracellular levels.[46]

Oxidative stress is the difference between the amount of free radicals produced and the biological system's ability to quickly detoxify reactive intermediates or repair the damage they cause.[5] Another sign of oxidative stress is lipid peroxidation, which is also one of the effects of ROS on lipid membranes that have received the most attention. The created free radical harms the cell by stealing electrons from the lipids in the membrane.[47] MDA, a secondary outcome of lipid peroxidation is a decent biomarker of free radical-mediated damage and oxidative stress. In this study, it was observed that rats exposed to only 120 mg/kg LA had an increased concentration of MDA when compared to the control group and rats treated with vitamin C and varying doses of EECl also showed a not significant increase. This finding is not consistent with similar studies by Sudjarwo and Sudjarwo which reported an increase in MDA levels of lead-exposed rats when compared to groups treated with curcumin.[48] However, there was a decrease in the concentration of MDA in groups treated with 750 mg/kg EECl and 1500 mg/kg EECl, respectively, after exposure to LA.

In this study, it was also observed that the levels of SOD in rats administered with only 120 mg/kg LA were lower than that of the control group. Lead-induced oxidative stress is caused by both the generation of ROS and the depletion of antioxidant reserves. A decrease in antioxidant enzyme production may result in alterations in membrane integrity, thereby increasing the vulnerability of the membrane to lead exposure.[46] SOD requires calcium and zinc for their activities. These enzymes can be inactivated by lead by substituting the important co-factors for these enzymes, which are the calcium and zinc ions.[49] Administration of 750 mg/kg and 1500 mg/kg EECl after exposure to 120 mg/kg LA herein study was seen to be ameliorative. The SOD level of rats treated EECl was higher than that of rats administered with only LA although the increase was not significant. This study's findings are congruent with those of Abubakar *et al.* who reported an increase in SOD levels in rats treated with 100 mg/kg and 200 mg/kg curcumin after exposure to lead.[50] Ethanol extracts of EECl have been previously stated to possess very high antioxidant action[51,52] and therefore administration of EECl could have led to the recovery from oxidative stress initially caused by LA.

GSH is a key antioxidant that is present in cells. Reduced GSH is essential for metal scavenging and a precursor to phytochelatins because metals have a significant affinity for their thiol group.[53] In this study, there was a decrease in the concentration of GSH in rats administered with only 120 mg/kg LA when compared to the control group. Administration of 375 mg/kg EECl, 750 mg/kg EECL, 1500 mg/kg EECl, and 100 mg/kg Vitamin C after lead exposure resulted in a surge in the level of GSH when associated with the level observed in rats administered only LA. The finding of this present study could be due to the high antioxidant activity of the ethanolic extract of *C. longa* (turmeric) as earlier reported by Yuliani *et al*.[52] The finding of this study also agrees with other reports by Elsayed on the result of curcumin on the homogenate of mice brain where it was observed to enhance both nonenzymatic and enzymatic cellular antioxidants like CAT, SOD, GSH, and GSH peroxidase (GPx).[54] This result also agrees with prior research by Sidhu and Nehru, who found that GSH levels in the brains of Sprague − Dawley rats exposed to 50 mg/kg LA for 8 weeks were significantly decreased.[46] The finding of this study is also consistent with those of Olayinka *et al.* and Aigbiremolen *et al.* who also reported decreased GSH levels in Wistar rats exposed to LA.[55,56] Lead binds to the sulfhydryl groups of GSH, deactivating it. This causes the-glutamyl cycle, which is often ineffective at replenishing the supply of GSH, to synthesize GSH from cysteine. Lead binds to the sulfhydryl groups of GSH, deactivating it. This causes the-glutamyl cycle, which is often ineffective at replenishing the supply of GSH, to synthesize GSH from cysteine. Similarly, lead inactivates enzymes such as GSH reductase, δ-amino levulinic acid dehydratase, GSH peroxidase, and GSH S-transferase, which lowers the GSH levels even more.[5] The results of this study are also in line with those of a study by Shukla *et al*., who discovered that curcumin greatly decreased lead-induced damage while increasing GSH levels and antioxidant enzymes SOD and CAT in the brains of lead-poisoned rats.[57]

Conclusion

In conclusion, ethanol extract of turmeric possesses possible neuroprotective properties against Wistar rats' hippocampal

lead-induced neurotoxicity. The neuroprotective potentials could be attributed to many phytochemical contents of turmeric such as alkaloids, tannins, steroids, saponins, and flavonoids, among others. To enable the development of therapeutic formulations, more research can be done to determine the efficacy of turmeric as a treatment for physiological changes, oxidative stress-related biochemical disorders, and neuropathologies.

Patient informed consent

There is no need for patient informed consent

Ethics committee approval

Ethical clearance for this study was provided by the Ethics Committee on Animal Use and Care, Ahmadu Bello University (ABU), Zaria: ABUCAUC/16.08.2021/100.

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Conflicts of interest

There are no conflicts of interest to declare.

Author contribution subject and rate

- Rimamnde Usman Elisha (40%): Design the research, data collection and analyses.
- Murdakai Tanko (30%): Research organization and supervision.
- Abubakar Adamu Sadeeq (30%): Research organization and supervision.

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