**ORIGINAL ARTICLE**

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# COMPARATIVE STUDY OF THE EFFECT OF GINKGO BILOBA EXTRACT AND VITAMIN C ON LEAD INDUCED HIPPOCAMPAL TOXICITY IN ADULT MALE WISTAR RATS

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## ABSTRACT

Pharmaceutical and industrial preparations contain lead and exposure to it has been associated to be the cause of neurodegenerative diseases. Therefore, in this current study, we investigated the comparative neuroprotective potentials of graded dose of *Ginkgo biloba* and Vitamin C in lead-induced alterations in the hippocampus of Wistar rats. Six groups of six adult Wistar rats each contained 36 animals in total. Group A served as the control and received normal tap water. Group B received 100mg/kg body weight of lead only. Group C received 100mg/kg body weight of lead and 200mg/kg body weight of *Ginkgo biloba*. Group D received 100mg/kg of lead and 250mg/kg of *Ginkgo biloba*. Group E received 100mg/kg of lead and 1192mg/kg of vitamin C, Group F received 200mg/kg of *Ginkgo biloba* only. For twenty-one days, all administrations were done orally. The Data were expressed as mean ± SEM and were analyzed. Statistical significance between the means was analyzed using one-way analysis of variance (ANOVA) followed Tukey post-hoc test. P-value < 0.05 was considered statistically significant. There is statistically significant (P= 0.0004) reduction in the percentage body/weight (0.6217±0.05566) of the Vitamin C treated group when compared to the control. The Biochemical analysis revealed that there is a statistically significant (P<0.0001) reduction in the concentration of superoxide dismutase, SOD (25.00 ± 0.6952) and catalase, CAT (25.00 ± 0.2728) in the lead only group when compared with the control. Additionally, treatment with Vitamin C causes a statistically significant (P<0.0001) increase (30.00±0.8021) in the CAT level when compared to the lead group. The histology of the dendate gyrus in lead only group shows distortion with fragmented hippocampal layer. Groups that were treated with *Ginkgo biloba* and Vitamin C shows gradually normalized cellular assortment of hippocampal layers. However, the group treated with *Ginkgo biloba* has more healthy cells. Also, the histology of the regions of the hippocampus in lead only group reveals several pyknotic changes, including disorganized pyramidal and grandular neurons within severed fragmented layers. Treatment with *Ginkgo biloba* and Vitamin C normalized DG architecture with cellular features that is similar to that of the control groups. Furthermore, the group treated with *Ginkgo biloba* appears healthier. Similarly, treatment with *Ginkgo biloba* and Vitamin C prevents degeneration of pyramidal neurons and shows pyramidal neurons with normal cell bodies with dendritic and axonal processes. This present study shows that *Ginkgo biloba* and Vitamin C have similar neuroprotective effects on lead-induced neurotocity in Wistar rats in a dose-dependent manner, with *Ginkgo biloba* being more effective.

**Keywords:** Hippocampus, histology, cells, *Ginkgo biloba*, lead.

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## INTRODUCTION

Lead is common industrial and environmental toxin and due to its malleability, ductility, and corrosion resistance; it is frequently employed in industrial settings. Both natural and man-

made sources, such as mines, paints, solder, battery acids, water pipes, plastic, and other consumer products, can cause lead contamination (Guilarte and Neal, 2012). The continuous use of lead in industries and its non-biodegradability have made it a common environmental pollutant. Despite being hazardous to multiple organs, lead has the biggest impact on the neurological system, where it impairs cognition and memory and can even cause neuronal diseases. Anatomically, the hippocampus is a part of the brain situated deep in the medial temporal lobe. It performs several cognitive functions, such as learning and memory processes, storage and processing of spatial functions, formation and storage of episodic memory, working memory and spatial memory (Holdstock et al., 2002; Ezzyat and Olson, 2008; Cao et al., 2013; Barkur and Bairy, 2015). Previous studies have reported that exposure to lead triggers hippocampal neurodegeneration, cell death and vacuolization resulting in impaired memory and learning (Owolabi et al., 2014; Oliveira et al., 2020). The concept of selective neuronal vulnerability describes the response of different neuronal populations to stresses that leads to neurodegeneration (Wang and Michaelis, 2010). The hippocampus is known to require a high level of ATP and oxygen due to its enormous mitochondrial activities required to perform its functions. This high level of activities makes its susceptible to oxidative stress. For example, the hippocampal CA1 region are most sensitive to neurodegeneration (Terry et al., 1991).

The origin of *Ginkgo biloba* is said to be remote mountainous valleys of Zhejiang province of eastern China. Commonly referred to as "EGb 761," the leaf extract of Ginkgo biloba is a popular phytomedicine in many nations. Terpenoids, polyphenols, allyl phenols, organic acids, carbohydrates, fatty acids, lipids, inorganic salts, and amino acids are among the secondary metabolites derived from the extract. Nevertheless, flavonoid glycosides and terpene trilactones are responsible for its pharmacological activity (Kubitzki 1990).

The dried leaves of *Ginkgo biloba* is used to produce standardized *Ginkgo biloba* extract used as therapeutic drug for the treatment of dementia, memory impairment and Alzheimer's disease (Vellas et al., 2012).

Several *in vitro* and *in vivo* models studies have reported the neuroprotective effect of *Ginkgo biloba* extract. *In vitro* studies showed that *Ginkgo biloba* extract protected cultured neurons against death caused by hydrogen peroxide, hypoxia, glutamate, verapamil, amyloid-β, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, , nitric oxide (NO), , and cyanide (Zhu et al., 1997; Ahlemeyer and Krieglstein 1998). Also, it has been documented that neuroprotective effect of *Ginkgo biloba* is dose-dependent with sustain improvement in cognitive and memory function at high dose (Bastianetto et al., 2000).

The possible mechanisms of action of neuroprotective effects of *Ginkgo biloba* include Anti-oxidation, anti-inflammation, anti-apoptosis, defense against mitochondrial dysfunction, amyloidogenesis and Aβ aggregation, modulation of phosphorylation of tau protein, ion homeostasis, and even induction of growth factors (Singh et al., 2019).

Vitamin C, also known as Ascorbic acid is a vital and most powerful water-soluble antioxidant which is involved in numerous cellular functions. Vitamin C is found majorly in citrus fruits, strawberries, and vegetables. With the exception of humans and some animal species, it is also produced by most plants and animals. Vitamin C protects against oxidative stress and induced cellular damage by neutralization of lipid hydroperoxyl radicals (Berger et al., 2003). The neuroprotective role of Vitamin C has been reported by Figueroa-Méndez and Rivas-Arancibia (2015); Kumar et al., (2018), who reported that Vitamin C

directly neutralizes oxidative and nitrosative stress- causing agents and also performs neuromodulatory functions.

## MATERIALS AND METHODS

### Experimental Animals

Thirty-six (36) apparently healthy male Wistar rats (weighing 110g-290g) were obtained from the animal house at the University of Medical Sciences Ondo. Rat was used in this study because it is the largest used animals in drug discovery, efficacy, and toxicity studies. This is because it has known genetic background; short generation time; similarities to human disease conditions; and known microbial status (Clause, 1993). The rats were kept in new wired cages at the same facility for a week to acclimatize before the commencement of the experiment. They weighed within the range of 110 – 290 g. the animals were housed under standard and laboratory conditions, dark and light cycles of 12 hours provided and fed, rat chow and water *ad libitum.*

### Exclusion and inclusion criteria

Only healthy male Wistar rats were selected for this study

### Drugs

### Gingko Biloba

Gingko biloba supplement capsules produced by Nature's Field Nigeria Limited, Lagos was used in this experiment. It was purchased from Uche care pharmacy and supermarket, Ondo city, Nigeria with LOT number 2003175. Each capsule contains 500mg of gingko leaf powder. The aqueous solution was gotten by mixing tap water and the leaf powder. This resulted into a dark greenish solution.

### Lead(II) Acetate

Lead(II) acetate was purchased from the Histology Laboratory, Department of Anatomy, University of Medical Sciences Ondo City, Ondo State.

In this study, we investigated the comparative neuroprotective effects of graded dose of *Ginkgo biloba* and Vitamin C on lead-induced hippocampal neurotoxicity in adult Wistar rats.

### Vitamin C

Vitamin C tablets (500mg of ascorbic acid) produced by Skg-Pharma Limited (NAFDAC number C1-3098L**)**, was purchased from Uche Care Pharmacy and supermarket,Ondo city, Ondo state.

### Experimental Design

Thirty-six Wistar rats were classified into six groups, with six rats in each group. The control group was administered distilled water; another group received 100 mg/kg of lead only (Pb), another group received 100mg/kg of lead and 200mg/kg of *Ginkgo biloba* (LD Gb+Pb), another group received 100mg/kg of lead and 250mg/kg of *Ginkgo biloba*, (HD Gb+ Pb), while two other groups were administered 100mg/kg of vitamin C (Pb+Vit C) and 200mg/kg of *Ginkgo biloba* only (Gb) respectively. All administration was done via oral route once daily for twenty-one days. This study was conducted according to guidelines of institutional research ethics committee, University of Medical Sciences Animal Research Ethics Committee and obtained ethical clearance under NHREC/TR/UNIMED-HREC-ONDO ST/22/06/21.

### Tissue Processing

12 hours after last administration, rats for histology were euthanized using 20 mg/KgBw of ketamine (intraperitoneal) and subjected to transcardial perfusion in which a flush of 50 ml of 0.1 M PBS (pH 7.4) was followed by 500 ml of 4% paraformaldehyde (PFA). The brain tissues were then excised, rinsed in 0.25 M sucrose 3 times for 5 mins each and then post fixed in 4% PFA for 24 hours after which they were stored in 30 % sucrose at 4 °C until further processing. Rats processed for biochemical studies were sacrificed by cervical dislocation (to eliminate the interference of

ketamine induced change in biochemical redox); brains were then excised, rinsed in 0.25 M sucrose 3 times for 5 mins each and placed in 30% sucrose in which they were stored at 4°C. Sagittal sections were made to expose the cerebellar cortices, following which the sections were processed routinely to obtain paraffin wax embedded blocks for histology and antigen retrieval. The hippocampus was dissected out of the cerebral region of the brain, following which the sections were processed routinely to obtain paraffin wax embedded blocks for histology and a part was fixed in buffered normal saline for biochemical assays.

**Data analysis**

Data were expressed as mean ±S.E.M and analyzed using GraphPad Prism (Version 8.0.1). One–way Analysis of Variance (ANOVA) was used to determine the mean difference among the treatment groups and followed by Bonferroni's post hoc test. The level of significance was accepted at $P < 0.05$.

## RESULTS

In the present study, it was observed that the animals were less active physically, and there was firm, black excrement present. Some of the animals exhibited symptoms of a disease by walking one-sidedly. There was also a decrease in food intake brought on by a lack of appetite. Biochemical analysis revealed that there is a significant reduction in the concentration of superoxide dismutase and catalase in the lead treatment group ($p<0.05$). The histology of the dendate gyrus in lead-treated group shows distortion with fragmented hippocampal layer. Groups that were treated with *Ginkgo biloba* and Vitamin C shows gradually normalized cellular assortment of hippocampal layers. However, the group treated with Vitamin C has more healthy cells. Also, the histology of the regions of the hippocampus in lead treated group reveals several pyknotic changes, including disorganized pyramidal and grandular neurons within severed fragmented layers. Treatment with *Ginkgo biloba* normalized DG architecture with cellular features that is similar to that of the control groups. Also, the group treated with Vitamin C appears healthier. Similarly, treatment with *Ginkgo biloba* and Vitamin C prevents degeneration of pyramidal neurons and shows pyramidal neurons with normal cell bodies with dendritic and axonal processes. Our study shows that *Ginkgo biloba* and Vitamin C have similar neuroprotective effects on lead-induced neurotocity in Wistar rats in a dose-dependent manner

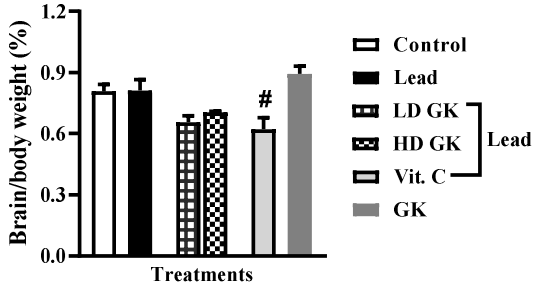


**Figure 1:** Comparative effects of *Gingko Biloba* and Vitamin C on percentage weight change of Wistar rats. n=6; mean ± SEM , P=0.0004 when compared with control. One-way ANOVA followed by Tukey post-hoc test.

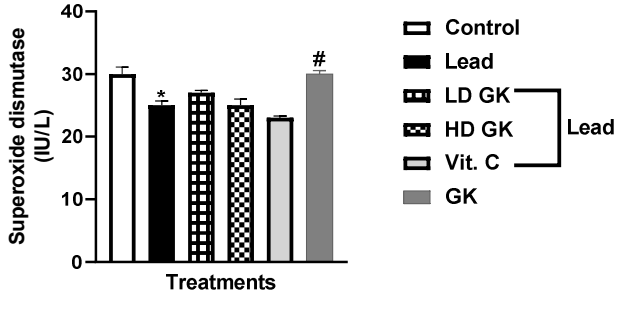


**Figure 2:** Comparative effects of *Gingko Biloba* and Vitamin C on superoxide dismutase (SOD) activity in the hippocampus of rats. n=6; mean ± SEM. (*) denote significant difference ($p< 0.0001$) when compared with control group, (#) denotes significant difference ( $p< 0.05$) when compared toVitamin C treated group (one-way ANOVA followed by Tukey post-hoc test).

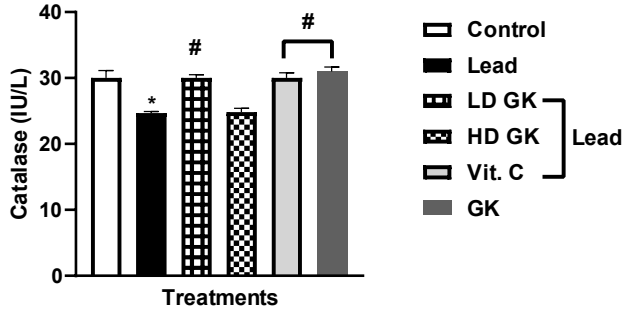


**Figure 3:** Comparative effects of *Gingko Biloba* and Vitamin C on Catalase (CAT) activity in the hippocampus of rats. n=6; mean ± SEM. (*) denotes significant difference (P<0.0001) when compared to control. (#) denote significant difference (P<0.05) when compared to lead only group (one-way ANOVA followed by Tukey post-hoc test).

## HISTOCHEMICAL EXAMINATIONS

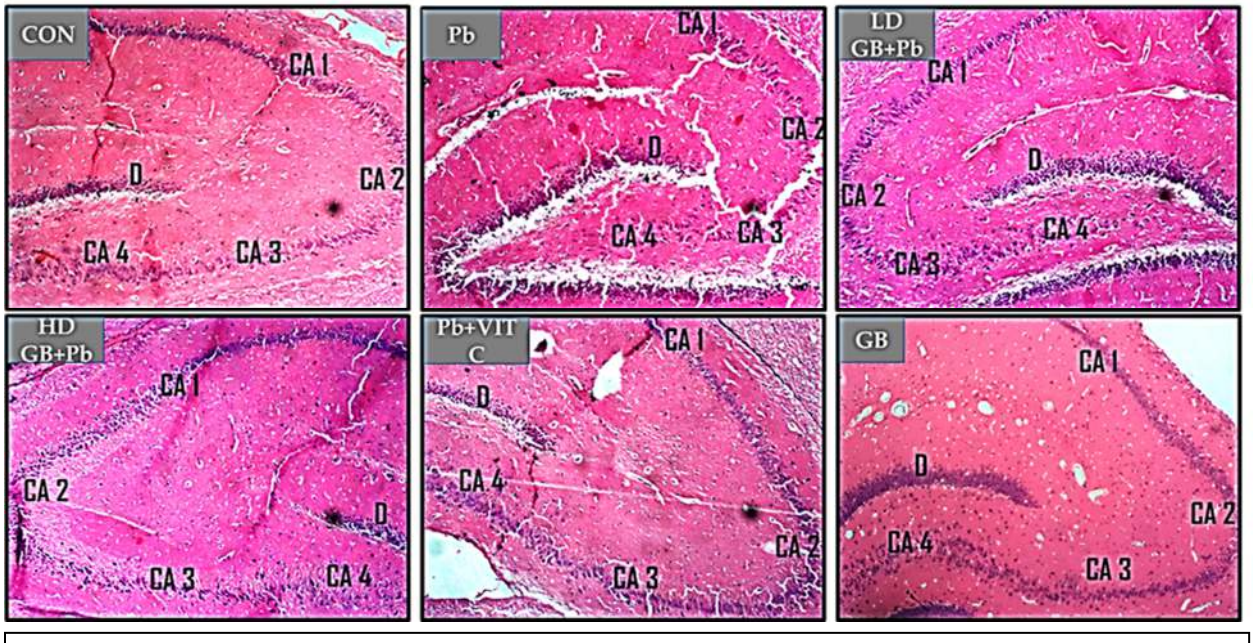


**Figure 4**: Photomicrograph showing the Dentate gyrus (DG), Cornu Ammonis 1 to 4 (CA1-CA4) regions of the hippocampus at low magnification stained with H&E across experimental groups. The cellular assortment in the lead treated group appears to be distorted with fragmented hippocampal layer, however interventional treatment with *Ginkgo biloba* and Vitamin C normalized the cellular assortment and hippocampal layers. However, the group treated with *Ginkgo biloba* show better recovery in a dose-dependent manner.

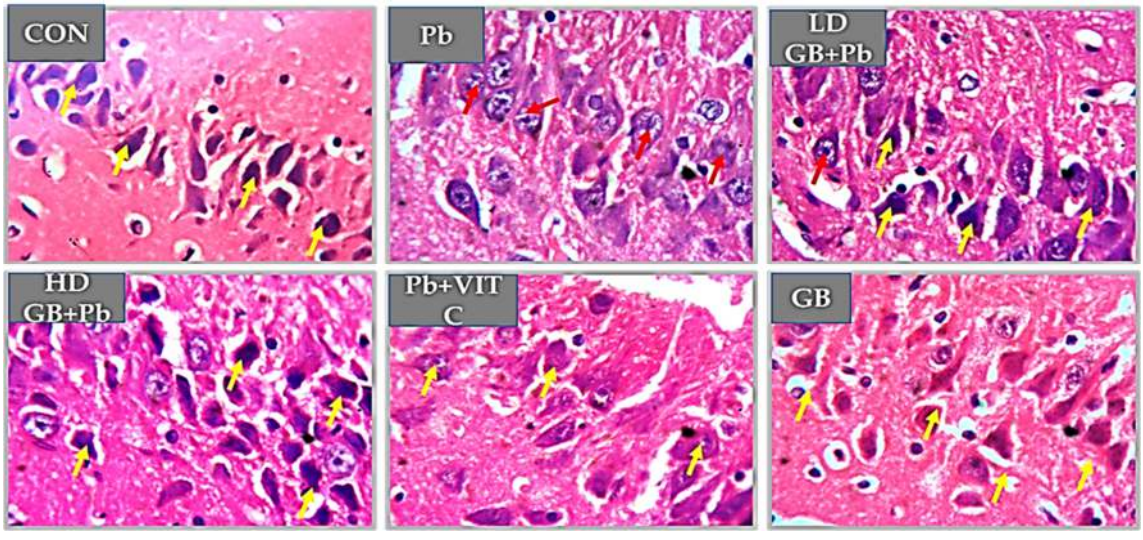


**Figure 5**: **Photomicrograph showing the CA1 region of the hippocampus across experimental groups**. Lead toxicity results to the hippocampus of experimental animals in group 5 and 6 shows several pyknotic changes, including disorganized pyramidal and granular neurons (red arrows) within the severed fragmented layers. In addition, pyramidal neurons and neuroglia in CA1-CA2 exhibit degenerative changes such as clustered cell bodies extruded cytoplasmic contents, indistinct nuclear demarcation, and short projections (red arrows). However, treatment with *Ginkgo biloba* and Vitamin C prevents degeneration of Pyramidal neurons and present pyramidal neurons with normal cell bodies with dendritic and axonal processes that are well expressed across hippocampal

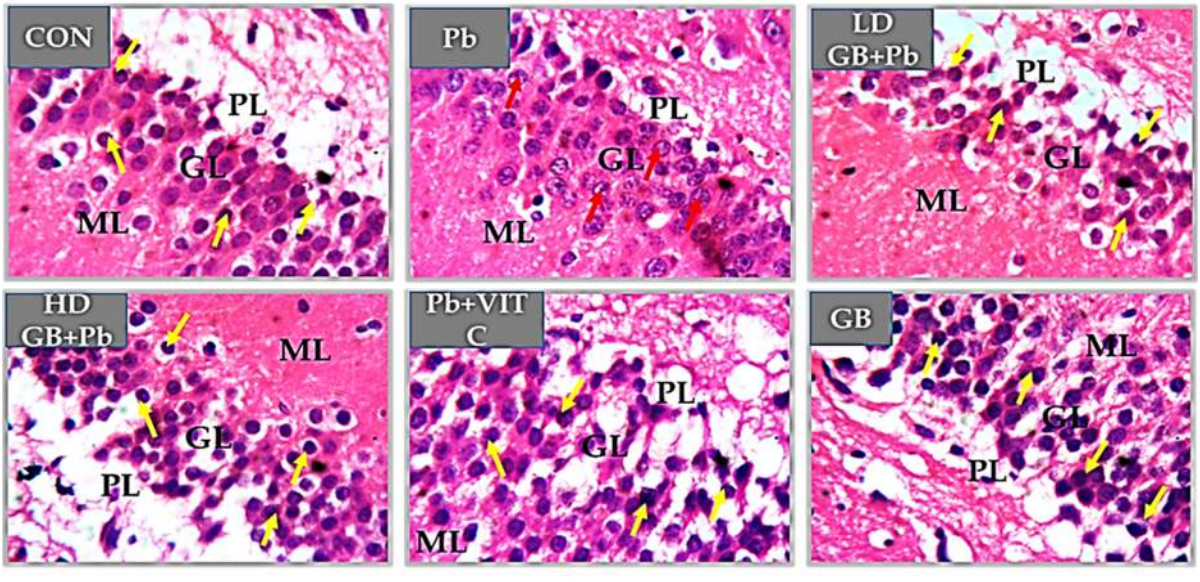


**Figure 6**: **Photomicrograph showing the layers and granule cells in Dentate Gyrus of the hippocampus across experimental groups.** Results show the polymorphic layer (PL), Molecular layer (ML) and Granule cell layer (GL). The layers and distribution of granules cells are normal in the control groups (CON and GB). Furthermore, the granule cells in these groups are nucleated and deeply stained with appropriate shape and morphology (yellow arrows). Granule cells arrangements in the lead group are kind of scatter with series of vacuoles within their neuropil with the cells undergoing pyknotic and necrotic process (red arrows). Treatment with *Ginkgo biloba* and vitamin C normalized DG architecture with cellular features that is similar to that of the control groups.

## DISCUSSION

According to studies, rats exposed to heavy metals like mercury, cadmium, arsenic, and lead typically experience weight loss (Akinyemi et al., 2016). This study investigated how Ginkgo biloba and Vitamin C affected lead-induced alterations in the hippocampus of adult Wistar rats. The outcome showed that, as compared to rats in the control group, rats in the lead-treated group had a lower mean body weight. According to research cited by Okechukwu et al., (2019) the decrease in the mean body weight may be due to loss of appetite or oxidative stress brought on by heavy metals.

The results showed that rats administered in the ginkgo-only group and the *Ginkgo biloba* (200mg/kg)-treated group significantly increased in body weight when compared with the control. The antioxidant capabilities of Ginkgo biloba, which may have sucked up the free radicals produced by the injection of lead acetate to the rats, may be responsible for this gain in body weight in these groups. These findings are in line with the observation reported by Augustine *et al.,* (2021). Additionally, the rats administered to the vitamin C group compared to the control group, exhibited a considerable drop in body weight. This could suggest that vitamin C does not affect the oxidative stress caused by lead.

According to several studies, lead exposure causes neurological damage and behavioral alterations in experimental animals that could impair learning and memory (Yeh et al., 2016; Oliveira et al., 2020).

Biochemical analysis revealed that lead induces oxidative stress indicated by the significant reduction of superoxide dismutase concentration in the lead-treated group. Reduction of the concentration of superoxide dismutase leads to an increase in oxidative stress which implies that lead-induced oxidative stress and ginkgo reduce the production of reactive oxygen species. It was also observed that when the other groups were compared with the lead group, it shows that ginkgo has neuroprotective effects on lead-induced neurotoxicity.

The histoarchitecture of the hippocampus of control group is normal with healthy and adequate cell morphology and population. Histology of the hippocampus of lead only group shows severe disruption and disorintation of hippocampus with extensive vacuolization. This indicates damage to the pyramidal neurons and glia cells. This agrees with the study reported by Owolabi et al. (2014) and Augustine et al. (2021). Histology of hippocampus of animals in Group C (low dose of *Ginkgo biloba*) reveals mild disruption and lesser vacuolization compared to lead only group. This could suggest a minimal level of healing process. Histoarchitecture of hippocampus of animals in Group D (high dose of *Ginkgo biloba*) shows regeneration and distinct healing process in the hippocampal layer. This suggests that *Ginkgo biloba* has healing and regenerative effects on neurotoxicity in a dose-dependent manner. Group E animals that were treated with Vitamin C show mild disorientation and vacuolization with relatively sparse pyramidal cells. The group treated with *Ginkgo biloba* appears healthier with higher cell population. Similarly, treatment with *Ginkgo biloba* prevents degeneration of pyramidal neurons and shows pyramidal neurons with normal cell bodies with dendritic and axonal processes. Histoarchitecture of animals in Group F that receives only *Ginkgo biloba* shows normal cell population and histology with no sign of neuronal damage, similar to the control group. This suggests that *Ginkgo biloba* has no deleterious effects on the hippocampus. Our study compares the neuroprotective effects of *Ginkgo biloba* and Vitamin C in lead-induced neurotoxicity. Our result shows that *Ginkgo biloba* has more neuroprotective potential and capacity than Vitamin C. This suggests that *Ginkgo biloba* could be more

effective than Vitamin C in a dose dependent manner.

## CONCLUSION

The findings of this comparative study demonstrated that lead acetate altered the body weight of adult Wistar rats and caused histological changes in the lead-treated groups, including chromatolysis of nerve cells and disorientation of the cellular layers of the hippocampus. In contrast, the administration of *Ginkgo biloba* ameliorated the effects of lead toxicity in the treated groups in a dose-dependent manner. Also, Vitamin C has mild effects with disoriented histoarchitecture. This could suggest that *Ginkgo biloba* has neuroprotective and neuroameliorative effects on neurodegenation. Further studies should be done using the Golgi stain to study the effect of lead and *Ginkgo biloba* and Vitamin C on the axons and dendrites of the neurons in the hippocampus of Wistar rats.

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