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## The effect of *Ginkgo biloba* extract and ascorbic acid on mercury-induced changes on the hippocampus of adult Wistar rat

\*<sup>1</sup>Auza M. I, <sup>1</sup>Elijah S. O, <sup>1</sup>Oguntoyinbo F. C, <sup>2</sup>Musa S. A, <sup>1</sup>Angela D. M, <sup>1</sup>Oguche E. E

<sup>1</sup>Department of Human Anatomy, Faculty of Basic Medical Sciences, Bingham University, Karu; <sup>2</sup>Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University Zaria, Nigeria.

### \*Corresponding Author:

Email: [auza.moses@binghamuni.edu.ng](mailto:auza.moses@binghamuni.edu.ng); Tel: +2348032820410

## ABSTRACT

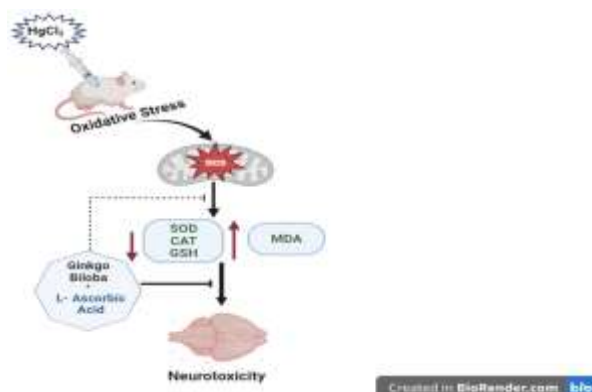
Mercury, a highly toxic heavy metal, poses a risk to humans, animals, and plants. *Ginkgo biloba* has been used to treat various disorders including memory-related conditions such as Alzheimer's disease. Ascorbic acid, which is known for its antioxidant properties, protects cells from free radicals. This study aimed to examine the protective effects of *Ginkgo biloba* extract and ascorbic acid on the hippocampi of adult Wistar rats exposed to mercury. Forty-two rats weighing between 180 to 200 g were divided into seven groups. Group I served as the control, while Group II received mercury (5 mg/kg), group III received mercury (5 mg/kg) + ascorbic acid (100 mg/kg), group IV received mercury (5 mg/kg) + ascorbic acid (500 mg/kg), group V received mercury (5 mg/kg) + *Ginkgo biloba* (100 mg/kg), group VI received mercury (5 mg/kg) + *Ginkgo biloba* (500 mg/kg), group VII received mercury (5 mg/kg) + ascorbic acid (100 mg/kg) + *Ginkgo biloba* (100 mg/kg). Treatment was orally for 21 consecutive days. Results indicated that Group II rats experienced altered feeding patterns, decreased body weight and decreased antioxidant activity levels of glutathione, catalase and superoxide dismutase; increased the brain weight and malondialdehyde level. In contrast, Groups III to VII, displayed positive outcomes, indicating protective effects against mercury-induced changes. Mercury had detrimental effects on the hippocampus in adult Wistar rats. However, the administration of *Ginkgo biloba* extract and ascorbic acid showed potential in mitigating these effects.

**Keywords:** antioxidant, neurodegeneration, neurotoxicity

## INTRODUCTION

Humans and animals interact with their environment and tend to be exposed to a range of chemicals as well as heavy metals. Such include; mercury, lead, thallium, aluminum and cadmium<sup>1</sup>. These exposures and interactions can occur through food, air and water<sup>2</sup>. The toxic effects of these compounds (i.e. protein inhibition, disruption of mitochondrial function, disruption of neurotransmitter and

destruction of the structural framework of neuron) is variable and diffuse, involving different parts of the nervous system<sup>3</sup>. Mercury is a well-known metal contaminant that has significant effects on living organisms and the environment. It is of major concern due to its toxicity and ability to bio-accumulate and bio-magnify in food webs<sup>4</sup>. Mercury contamination is often associated with anthropogenic activities such as small-scale gold mining, which releases mercury into the environment<sup>5</sup>. Mercury chloride is known to induce oxidative tissue damage, cognitive disorders in experimental animals.



**Figure 1:** Schematic representation of the mitigating effects of *Ginkgo Biloba* extract and Ascorbic acid on mercury induced Neurotoxicity.

*Ginkgo biloba* is a plant that has been extensively studied for its potential therapeutic effects. It has also been explored for its potential in the prevention and treatment of cognitive disorders. It has been found to be effective in improving blood circulation in the brain and preventing the progression of Alzheimer's disease<sup>6</sup>. *Ginkgo biloba* extracts have been reported to have antioxidant, anti-inflammatory, anti-allergic, and antibacterial activities. These effects are attributed to the presence of active metabolites such as vitamin C, vitamin E, flavonoids, alkaloids, and other compounds in the extract<sup>7</sup>. In terms of neurological disorders, *Ginkgo biloba* extracts have been found to slow down the progression to dementia and improve cognitive scores in individuals with mild cognitive impairment. They have also been widely used in treating neuropsychiatric disorders<sup>8</sup>. Additionally, *Ginkgo biloba* extract has shown promising results in the treatment of tinnitus, with its vasoregulatory effect, antioxidant activity, and enhancement of neuroplasticity.

Ascorbic acid is an essential nutrient in feeds, and an indispensable nutrient required to maintain the physiological processes of different animals<sup>9</sup>. Small amount of this vitamin is sufficient to prevent and cure scurvy; however, larger amount may be essential to maintain good health during environmental adversities, situation of physiological stress and conditions of infectious and parasitic diseases<sup>10, 11</sup>. Ascorbic acid is an anti-oxidant that prevents the production of free radicals induced by oxidative damage to lipids and lipoproteins in various compartments of cells and tissues. Anti-oxidants are regarded as first-line protective agents. Free radicals are molecules produced when the body breaks down foods or by exposure to tobacco smoke or radiation<sup>12</sup>.

Ascorbic acid plays a vital role in brain function and has been shown to have various effects on the brain, particularly in the hippocampus which is a region of

the brain involved in learning, memory, and cognitive function. One important aspect of ascorbic acid in the brain is its role as an antioxidant. Neurons in the brain are particularly sensitive to ascorbate deficiency due to their high rates of oxidative metabolism<sup>13</sup>. However, there has been little or no scientific evidence on the effects of *Ginkgo biloba* and ascorbic acid on the brain. Thus, we evaluated the neuroprotective effect of *Ginkgo biloba* and ascorbic acid on mercury chloride-induced neurotoxicity on memory and cognitive impairment. Biomarkers of oxidative stress were also examined by assaying for superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) and glutathione (GSH). Finally, the histology of the hippocampus of the rats were observed.

## MATERIALS AND METHODS

### Animals

Animal Use and Care Committee of Bingham University, Karu gave approval for this study after critical evaluation and review. All protocol in this study followed the National Institutes of Health's guidelines for the use and care of laboratory animals and relevant methods were followed to ensure that animals were not subjected to excessive stress and discomfort in the course of this experiment.

### Chemicals and Drugs

The chemical materials used in this study includes: Mercury and Laboratory graded Ascorbic acid manufactured by Central Drug House (P) Ltd, **Batch. No.038P011**, India), were purchased from Covelyn Nigeria Ltd (The Scientific World). *Ginkgo biloba* extract capsule (Bactolac pharmaceutical Inc. **Batch. No. KBPL/GBE/140101**, USA Indore, India) was purchased from God is Able Pharmacy, Masaka, Karu, Nigeria. All other reagents and chemicals used were of analytical grade.

### Experimental design

Adult male Wistar rats (n=42) were procured from the animal care unit (ACU), Ahmadu Bello University, Zaria. The rats were kept and maintained in standard laboratory conditions of room temperature, humidity and under 12-hour dark-light cycle in polyester cage with wire gauze.

Then animals were grouped into seven (7) groups with 6 animals per group. The groupings are as follows:

Group I: Distilled water (1 ml/kg), Group II: mercury chloride (5 mg/kg), Group III: mercury (5 mg/kg) + ascorbic acid (100 mg/kg), Group IV: mercury (5

mg/kg) + ascorbic acid (500 mg/kg), Group V: mercury (5 mg/kg) + *Ginkgo biloba* (100 mg/kg), Group VI: mercury (5 mg/kg) + *Ginkgo biloba* (500 mg/kg), Group VII: mercury (5 mg/kg) + ascorbic acid (100 mg/kg) + *Ginkgo biloba* (100 mg/kg)

**Neurobehavioral observations**

Behavioral studies using Y-maze test was carried out during treatment and after inducement. This Y-maze is used for basically testing for spatial and working memory. Using spontaneous alternation method<sup>14</sup>, Y-maze is a behavioral test for measuring a rodent's willingness to explore new environments, locomotor activity, stereotypic behavior and their memory capacity. The testing occurs in a Y- shaped maze with three arms of the 120° angle from each other. The Y-maze was composed of three arms labelled A.B.C. An alteration was when rat completes entry in the three arms without repeating an entry into any arm. The rodents were introduced at the center of the maze and the animal was allowed to explore the three arms<sup>15</sup>. Each rat was tested for a period of 180 seconds (3 minutes). The results for number of arm entries and time spent in each arm were recorded and apparatus is cleaned and allowed to dry before being used again.

**Animal sacrifice**

The rats were sacrificed by cervical dislocation and cranial decapitation. The cranium of the rat was dissected using forceps and scissors to expose the brain within the cranium, the net weight was taken using the sensitive weighing scale and the brain was excised and immediately fixed in 10% formal saline in labelled specimen bottles to prevent post-mortem changes.

**Biochemical studies**

The brain was harvested and immediately homogenized. The tissue homogenates were

centrifuged at 3000 rpm for 10 min and the supernatant were utilized for different estimations according to the method of Bradford<sup>16</sup>. MDA level, activity of SOD, CAT and GSH concentration were evaluated.

**Histology**

The brain was excised and processed for Haematoxylin and Eosin (H & E) staining techniques. The tissues were processed and embedded in paraffin wax for routine histologic studies. The brain tissues of 5 µ were sectioned with the Letiz rotary microtome. The sections were mounted, stained with H & E method and examined with the light microscope and the photomicrographs were taken.

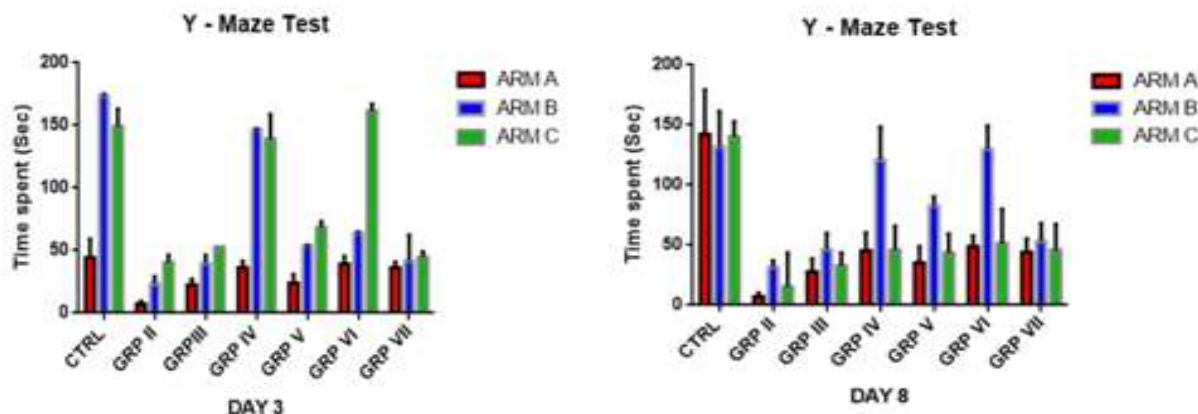
**Data Analysis**

Data were expressed as mean + SEM for quantitative measures. The data comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test. The level of significance was set at p < 0.05, p < 0.01, and p < 0.001. Data tests were conducted using GraphPad Prism 5.00 (GraphPad Prism, San Diego, California, USA).

**RESULTS**

**Neurobehavioral Assessment: Y-Maze**

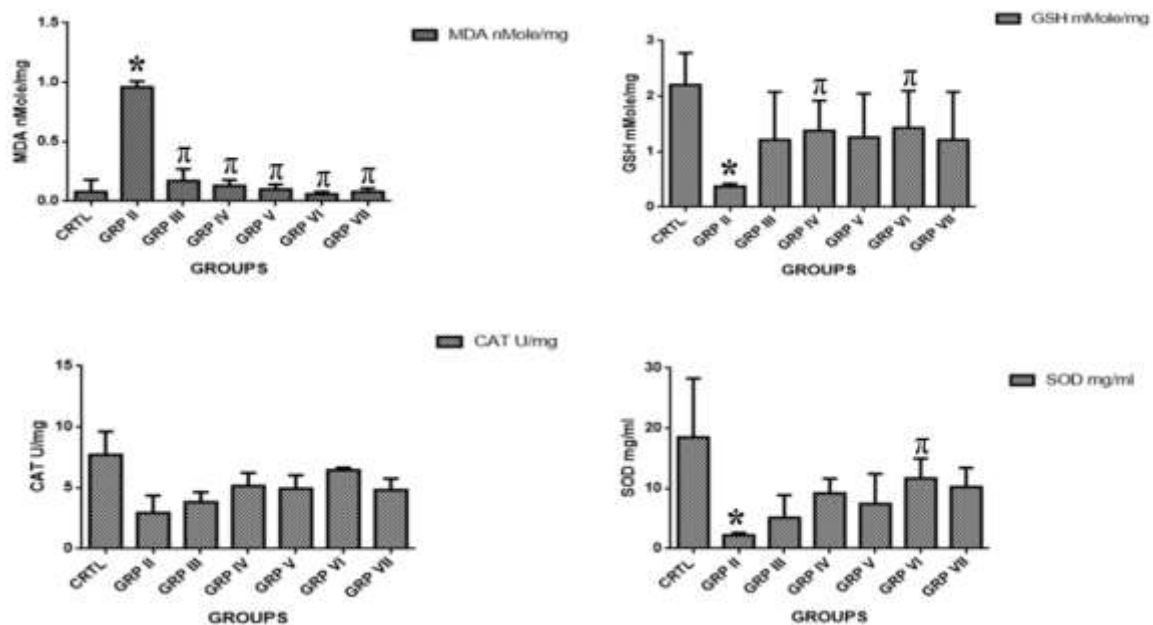
Findings in Figure 2 (Day 3 and 8) respectively, revealed that in all the three arms A, B, C, Group II spent less time in the various arms when compared to the control and other treated groups with Ascorbic acid and *Ginkgo Biloba* that spent more time in the various arms.



**Figure 2:** Bar Chart showing Mean Time spent in various arms in Y maze test.

### Biochemical analysis

The results revealed increased level of MDA activity in Group II when compared to the control group; it apparently was the highest amongst all the groups as shown in Figure 3. The Results also showed decreased level of GSH, SOD, and CAT (Figure 3) in Group II when compared to the control group.

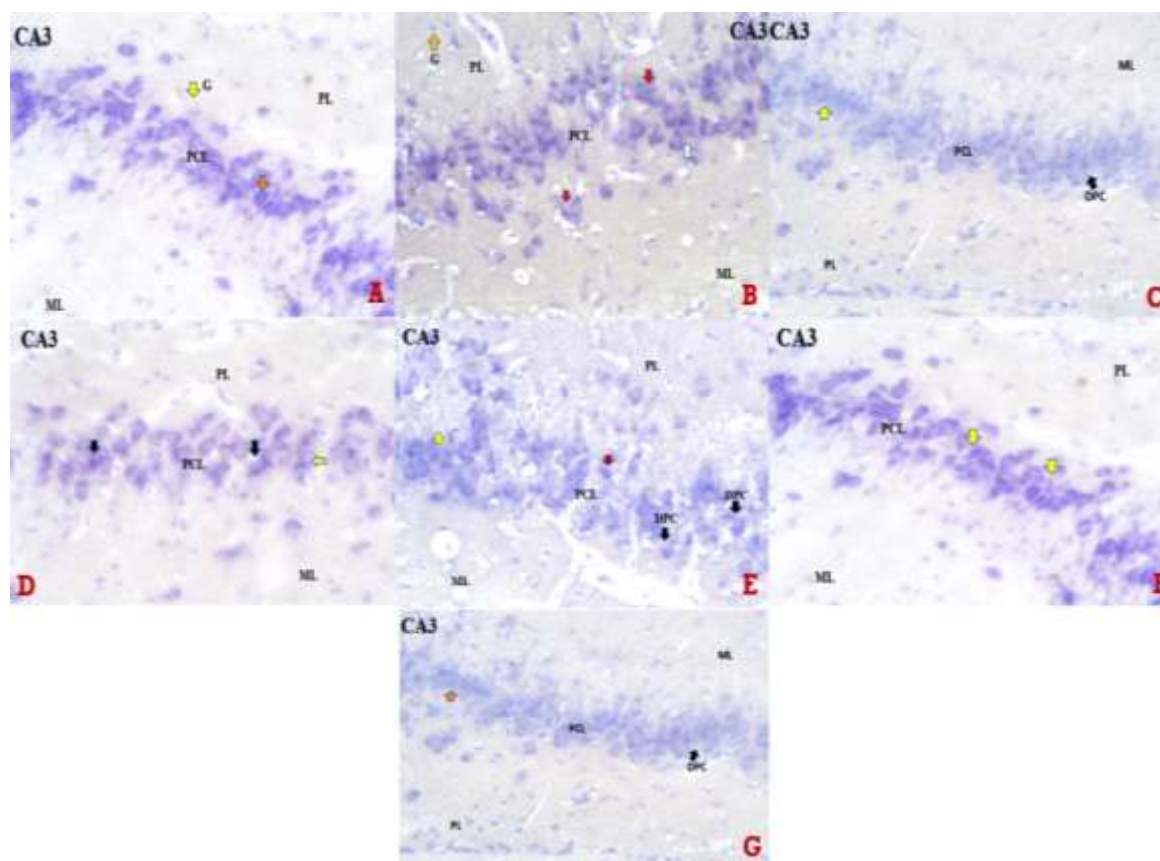


**Figure 3:** Bar Chart showing the effect of *Ginkgo Biloba* Extract and L-Ascorbic Acid on Antioxidant Enzyme Activity. The data are presented as the Mean  $\pm$  SEM (n=6). \*p  $\leq$  .001 versus control, and  $\pi$  p  $\leq$  .001 versus HgCl<sub>2</sub>-treated groups.

### Histological and Histochemical Studies of the Hippocampus

Examination of the histology of the hippocampus showed pyramidal cells, granule cells, molecular layer

and polymorphic cell. CA3 region showed normal architecture with densely packed pyramidal cells, the basic pattern of an ordered sheet of large neurons (pyramidal and granule cells) whose cell bodies are all packed together as shown in Figure 4.



**Figure 4:** Light photomicrographs of CA3 hippocampal sections stained with H&E; (A) showed normal histoarchitecture, with well-defined pyramidal cells, molecular layer (ML), granule cells (G); (B- Group II) showed degenerating pyramidal cells, cytoplasmic vacuolation in the polymorphic layer (PL). Pyramidal cell layer (PCL), degenerating pyramidal cells; (C- Group III) showed degenerating pyramidal cells (DPC); (D- Group IV) showed fewer and less densely packed pyramidal cells, mild shrinking and shriveling cells in PCL; black arrow – degenerating pyramidal cells, yellow arrow – pyramidal cells; (E- Group V) showed irregular arrangement of cells in the PCL, DPC, shrinking cells in the pyramidal layer due to cell alterations; yellow arrow – pyramidal cells; (F- Group VI) showed gradual progressive regeneration of pyramidal cells in the pyramidal layer, more prominent well-defined and packed pyramidal cells; yellow arrows – Pyramidal cells; (G- Group VII) showed degenerating pyramidal cells; orange arrow – pyramidal cells. (Mag x400).

## DISCUSSION

Memory performance investigates the time spent exploring the novel arm, the more time rats spend exploring the novel arm demonstrate better memory performance compared to rats that spend less time. This provides insights into a direct correlation between exploration time and memory function. In this study, rats were administered mercury chloride which is a potent toxicant that can induce neurodegeneration or predispose rats to neurodegenerative conditions, we therefore examined whether mercury impacts the ability of rats to explore the maze arms. The time rats spend in each arm is an indication of their exploratory behavior. Rats with memory deficits or neurodegeneration exhibited altered exploratory

patterns, such as spending less time in novel arms due to impaired memory or cognitive functions. The Results revealed that in all the three arms A, B, C, rats exposed to mercury toxicity spent less time in the various arms when compared to the control and other treated groups with ascorbic acid and *Ginkgo biloba* that spent more time in the various arms. In a study by Farina which investigated the effects of mercury exposure on memory performance using a passive avoidance paradigm in adult Wistar rats<sup>17</sup>, the findings revealed that exposure to mercury chloride led to impaired retention of information, indicating deficits in short-term working memory. This impairment was associated with increased oxidative stress markers and reduced antioxidant enzyme activity in the hippocampus. The Results of the study by Farina and this present study highlight the potential negative impact of mercury



exposure on memory performance in adult Wistar rats<sup>17</sup>. The mechanisms underlying these effects involve oxidative stress, neuroinflammation, and structural alterations in key brain regions involved in memory formation.

Oxidative stress which is a common pathology which occurs due to imbalance between production and detoxification of reactive oxygen species (ROS) has been implicated in many neurodegenerative diseases<sup>18</sup>. Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various state of the brain. Because of its high oxygen demand, the brain is the most susceptible organ to oxidative damage<sup>19</sup>. Halliwell had reported that increased oxidative stress leads to lipid peroxidation, protein damage and induction of apoptosis<sup>20</sup>. The Results from assessing the levels of the antioxidant enzymes in the current study showed that mercury caused oxidative stress and lipid peroxidation when compared to the Control. However, the groups treated with *Ginkgo biloba* and ascorbic acid had lower levels of MDA and higher levels of GSH, SOD and CAT. These antioxidant parameters are known to be oxidative stress markers and oxidative stress is the instability between the antioxidant defense mechanism and pro-oxidant processes. Malondialdehyde is the final product of lipid peroxidation, therefore, the levels of MDA can be used as a marker of lipid peroxidation. Lipid peroxidation is a free radical related process, which is likely harmful due to the fact that if its uncontrolled, self-enhancing processes can cause disruption of membrane, lipids and other cell components. Aragão *et al.*<sup>21</sup>, demonstrated that chronic exposure to inorganic mercury promoted increased levels of MDA and decreased total antioxidant capacity by promoting oxidative stress in the hippocampus. Their Results of mercury exposure indicated that the neurotoxicity induced by Hg is due to the overproduction of free radicals and products of lipid peroxidation<sup>21</sup>. This is confirmed by the concomitant decrease in total antioxidant capacity. The ability of mercury to inhibit antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase suggest neurotoxicity. However, the rats that were treated with *Ginkgo biloba* and ascorbic acid exhibited notable improvements in the antioxidant profile. These treatments were associated with lower levels of MDA, suggesting a reduction in oxidative stress and lipid peroxidation. Additionally, the increased levels of GSH, SOD, and CAT in the *Ginkgo biloba* and ascorbic acid-treated groups indicate enhanced antioxidant defense mechanisms within brain tissue. *Ginkgo biloba* is known for its potential antioxidant and neuroprotective properties as it contains flavonoids and terpenoids that can scavenge free radicals and reduce oxidative damage<sup>22</sup>. Ascorbic acid, on the other hand, is a well-known water-soluble antioxidant that can directly neutralize reactive oxygen species and regenerate other antioxidants, such as GSH<sup>23</sup>, therefore

the observed decrease in MDA levels and concurrent increase in GSH, SOD, and CAT activities in response to *Ginkgo biloba* and ascorbic acid supplementation suggests that these compounds may play a vital role in reducing oxidative stress-induced damage and promoting antioxidant defense mechanisms in brain tissue.

The CA3 region of the hippocampus of rats in the control revealed normal histoarchitecture with well-defined pyramidal cells and molecular layer, unlike the mercury-treated rats that showed degenerating pyramidal cells, cytoplasmic vacuolation in the polymorphic layer indicating that the administration of mercury chloride had detrimental effects on the histology of the hippocampus. Rats co-treated with mercury and ascorbic acid revealed less densely packed degenerating pyramidal cells (low dose) and mild shrinking and shriveling cells in the pyramidal cell layer (high dose). Rats co-treated with mercury and *Ginkgo biloba* at low dose revealed irregular arrangement of cells in the pyramidal cell layer, degenerating pyramidal cells, shrinking cells in the pyramidal layer, suggesting that low doses of *Ginkgo biloba* may not have a significant protective effect against the toxic effects of mercury. However, at higher dose, co-administration of *Ginkgo biloba* showed gradual progressive regeneration of pyramidal cells in the pyramidal layer with more prominent pyramidal cells, an indication that a higher dose of *Ginkgo Biloba* may have a potential regenerative effect on the histology of the hippocampus affected by mercury chloride toxicity. In rats that received both ascorbic acid and *Ginkgo biloba*, more defined pyramidal cells were present; this suggests a synergistic effect in protecting the histology of the hippocampus against the toxic effects of mercury.

## Conclusion

Mercury has a detrimental effect on the neurobehavioral, biochemical and histological features of the hippocampus of adult Wistar rats. However, both ascorbic acid and *Ginkgo biloba* extract show promising mitigating effect on mercury-induced toxicity.

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## Disclosure Statement

The authors report no conflict of interest.

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