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Neuroprotective Properties of the Aqueous and Ethanolic Extracts of *Artocarpus heterophyllus* (Jack Fruit) on the Cerebral Cortex and Hippocampus of Mercury Chloride-Induced Neurotoxicity in Adult Male Wistar Rats

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ABSTRACT

Mercuric chloride intoxication in rodents causes spatial learning and memory impairment. The present study was carried out to investigate the possible protective role of jackfruit against mercuric chloride (HgCl₂)-induced changes in rat brains. Thirty-two adult male rats (160g-200g) were randomized into eight groups of four rats each after acclimatization viz: A: Control, tap water; B: HgCl₂ (0.5 mg/kg body wt.); C and D: Jackfruit ethanolic and aqueous extract (200 mg/kg body wt.) respectively; E and F: HgCl₂ + Jackfruit ethanolic and aqueous extract (200 mg/kg body wt.) respectively; G and H: HgCl₂ + Jackfruit ethanolic and aqueous extract (400 mg/kg body wt.) respectively. All treatments were oral by gastric gavage and lasted 14 days. Morris water behavioural tests were conducted on the 15th day, after which rats were euthanized through cervical dislocation, and histology of rat hippocampus with regard to micro-anatomical parameters were examined in all groups. Mercuric chloride (HgCl₂) induced a significant increase in time taken; among the treated groups, it was shown that there was an increase in latency time, while latency time was decreased in the control group, which was not statistically significant. However, concurrent administration of *Artocarpus Heterophyllus* extract with HgCl₂ caused a reversal of these parameters relative to control and neuronal process seen in the hippocampus. Therefore, aqueous and ethanolic extract of *Artocarpus Heterophyllus* demonstrated protective effects against HgCl₂-induced neurobehavioral and histological changes in spatial learning and memory.

Keywords: Jack fruit, Neuroprotective, mercury chloride, Spatial learning and memory

INTRODUCTION

Neurotoxicity refers to any detrimental effects on the structure or functioning of the central and peripheral nervous system due to exposure to biological, chemical, or physical agents [1, 2, 3]. According to [4], mercury (Hg) is a hazardous element known for its documented adverse health impacts. It is extensively used in various human activities such as agriculture, manufacturing, and medicine, leading to its widespread distribution globally and making it a significant environmental contaminant [5]. Different forms of mercury exist, including elemental mercury (Hg), inorganic, and organic compounds, which can be found in the environment due to human activities and natural processes [5]. Human-related sources of mercury include industrial use of fossil fuels, solid waste incineration, topical medications, cement production, devices measuring mercury, dental amalgam, cosmetics, and small-scale artisanal gold mining. Seafood consumption is a primary route of mercury accumulation in humans [5]. Mercury exposure can occur through ingestion, inhalation, or skin contact [6]. Mercury exposure has been linked to various neurological and mental disorders, both acutely and chronically [5]. The central nervous system (CNS) is particularly vulnerable to the toxic effects of mercury [7]. Mercury chloride (HgCl₂) is a highly toxic mercury and chlorine compound characterized by its water solubility, lack of odour, and colourless crystalline

structure. Studies have associated HgCl_2 poisoning with significant damage to rats' hippocampal formation, cortex, and cerebellum [5, 6]. Although the exact mechanism underlying HgCl_2 neurotoxicity remains unclear, recent research suggests several processes, with oxidative stress emerging as a prominent factor in its neurotoxic effects [8, 9, 10]. Research indicates that HgCl_2 exhibits a strong affinity for glutathione enzymes, interacting with their thiol (-SH) group, which leads to intracellular thiol depletion. This depletion reduces the effectiveness of antioxidants like reduced glutathione, glutathione peroxidase, and glutathione reductase in combating free radicals and oxidative damage [11, 12]. Antioxidants are crucial in reducing oxidative stress by scavenging reactive oxygen species and preventing cellular damage [13]. Using natural antioxidants from plant sources is increasingly advocated [14]. Dietary supplementation with antioxidants has shown promise in mitigating cognitive impairment and brain damage. Medicinal plants are recognized for their potent antioxidant properties and are considered reliable sources of supplementary medications [15]. *Artocarpus heterophyllus* Lam. (Family Moraceae), commonly known as jackfruit, is a tropical tree native to India and widely found in Asia, Africa, and parts of South America. Jackfruit is renowned for its nutritional richness, containing minerals, carbohydrates, volatile compounds, proteins, and vitamins. Various parts of the jackfruit tree, including the fruit, bark, leaves, and roots, possess therapeutic properties and are utilized in traditional medical systems for treating various ailments. The fruit and seeds are used in the production of various culinary products. Preclinical studies have highlighted jackfruit's antibacterial, antioxidant, anti-inflammatory, and other beneficial effects [16]. Ripe jackfruits are known for their sweetness, cooling effect, laxative properties, and aphrodisiac qualities and are considered a brain tonic [17]. Jackfruit contains potent polyphenols like resveratrol, which exhibit promising neuroprotective activity [18, 19]. Based on this evidence, this study aimed to explore the potential of aqueous and ethanolic extracts of *A. heterophyllus* fruits against mercury chloride-induced neurotoxicity in rats.

MATERIALS AND METHODS

Preparation of plant materials

The jackfruit was washed and dried at room temperature. The dried samples were ground into powder mechanically using a manual grinder and put away in a fridge before use.

Extraction Procedure

For the aqueous extraction, 500g of the macerated plant powder was soaked in 1000ml of distilled water and kept in a container for 24 hours, while for ethanolic extraction, 500g of the macerated plant powder was soaked in 1000ml of absolute alcohol and kept in a container for 24 hours. The mixtures were then vigorously shaken intermittently for additional 2 hours, to allow complete extraction. The resulting mixture was rapidly filtered through Whatman no 1 filter paper to obtain a homogenous filtrate. Using a rotary evaporator, this filtrate was then concentrated in a vacuum at a low temperature (37-40°C) to about one-tenth the original volume. The concentrates were allowed to open in a water bath (40°C) for complete dryness, yielding 200g of dark brown and light brown gummy substances, respectively. The extract was later reconstituted in distilled water at 1g/ml concentration before administration. The extract was then refrigerated until use.

Experimental Animals and Design

Thirty-two (32) Wistar rats weighing between 120-200g were used for this study. The animals were purchased from the animal house of Abia State University. They were housed in standard cages and left to acclimatize for 14 days under natural conditions in the animal house before the commencement of the experiment. They were fed rat chow and tap water ad libitum. The rats were divided into eight groups: A, B, C, D, E, F, G, and H of 4 rats. Rats in group A received only food and water. All rats in group B were injected with mercury chloride (HgCl_2) at 0.5mg/kg/bw intraperitoneally two times a week for 2 weeks. Rats in group C received aqueous *Artocarpus Heterophyllus* extract, while D received ethanolic, respectively. Groups E to H were injected with mercury chloride (HgCl_2) at 0.5mg/kg/bw intraperitoneally two times a week for 2 weeks according to the method reported in NTP, 1993 and concurrent administration of aqueous and ethanolic extract of *Artocarpus Heterophyllus* respectively. All *Artocarpus Heterophyllus* administration was done orally using a gavage. Groups E and G received 200mg/kg per body weight of aqueous and ethanolic leaf extracts of *Artocarpus Heterophyllus*, respectively, and F and H received 400mg/kg per body weight of aqueous and ethanolic leaf extracts of *Artocarpus Heterophyllus* respectively.

Table 1: Experimental Protocol

GROUP	ADMINISTRATION	NO. OF RATS	OF DURATION
A (Normal control)	Food and Water	4	14 days
B (Negative control)	HgCl ₂ at 0.5mg/kg/bw only Intraperitoneally	4	Twice a week for 2wks
C (Ethanolic extract)	HgCl ₂ at 0.5mg/kg/bw + 200mg/kg/bw of Ethanolic extract of <i>A. Heterophyllus</i>	4	14 days
D (Aqueous extract)	HgCl ₂ at 0.5mg/kg/bw + 200mg/kg/bw of Aqueous extract of <i>A. Heterophyllus</i>	4	14 days
E (Low dose group)	HgCl ₂ at 0.5mg/kg/bw + 200mg/kg/bw of Ethanolic extract of <i>A. Heterophyllus</i>	4	14 days
F (Low dose group)	HgCl ₂ at 0.5mg/kg/bw + 200mg/kg/bw of Aqueous extract of <i>A. Heterophyllus</i>	4	14 days
G (High dose group)	HgCl ₂ at 0.5mg/kg/bw + 400mg/kg/bw of Ethanolic extract of <i>A. Heterophyllus</i> .	4	14 days
H (High dose group)	HgCl ₂ at 0.5mg/kg/bw + 400mg/kg/bw of Aqueous extract of <i>A. Heterophyllus</i>	4	14 days

Neurobehavioural tests: The Morris water maze test was carried out on the 15th day of the experiment.

Spatial Learning and Memory Test Using Morris Water Maze (MWM)

This test was conducted to test the effects of aqueous and ethanolic extracts of *A. heterophyllus* fruits in correcting mercury chloride-induced spatial memory deficit and depression. In this test, a platform was submerged beneath the surface of the maze pool; the animal's task was to find the hidden platform. The animals' starting point was changed from time to time to build a cohesive spatial representation of the pool to find the platform during training trials, and the latency to find the platform location was recorded both during the training and experimental periods. Animals were placed in a circular pool of clear transparent water, partitioned into four quadrants. The animal's starting point was in a random location. The animal will swim from one quadrant to another, searching for an escape route. The latency time to find the platform was recorded [20, 21].

Sacrifice and Sample collection

The administration of *Artocarpus Heterophyllus* lasted daily for 14 days. On the 15th day, the rats were subjected to neurobehavioral tests (Morris water maze test), after which they were sacrificed by cervical dislocation. The brains from each group were harvested and fixed in 10% formal saline for histological studies using the H&E procedure.

Statistical Analysis

Data will be presented as the Means \pm Standard Error of Mean and analyzed using one-way analysis of variance (ANOVA) followed by a post-test using a computer-based fitting program (IBM SPSS) version 20. Statistical significance was set at $p < 0.05$. INITIAL and FINAL for each group were compared using students' T-test, and differences between means were regarded as significant at $P < 0.05$.

RESULTS

Behavioural Studies

In this test, the faster the animals swim and get to the escape platform, the more enhanced they are considered to be. The results of the effect of mercury chloride exposure on spatial learning and memory using the Morris water maze test showed that there was a decrease in the mean time taken for the animals to complete the Morris water maze task in the control group, though the decrease was not statistically significant (Table 2). The results showed that animals in the mercury chloride group (HgCl₂ only) had an increased time in Morris water maze activity. Though the increase between exposure and treatment was insignificant, the increase was observed. However, concomitant administration of *Artocarpus heterophyllus* with HgCl₂ showed a decrease in time in Morris water activity in high and low aqueous extract groups, respectively, relative to the control group (Table 2).

Table 2: Effects of *Artocarpus heterophyllus* administration and HgCl₂ treatment on animal Morris water behavioural test following mercury chloride induction

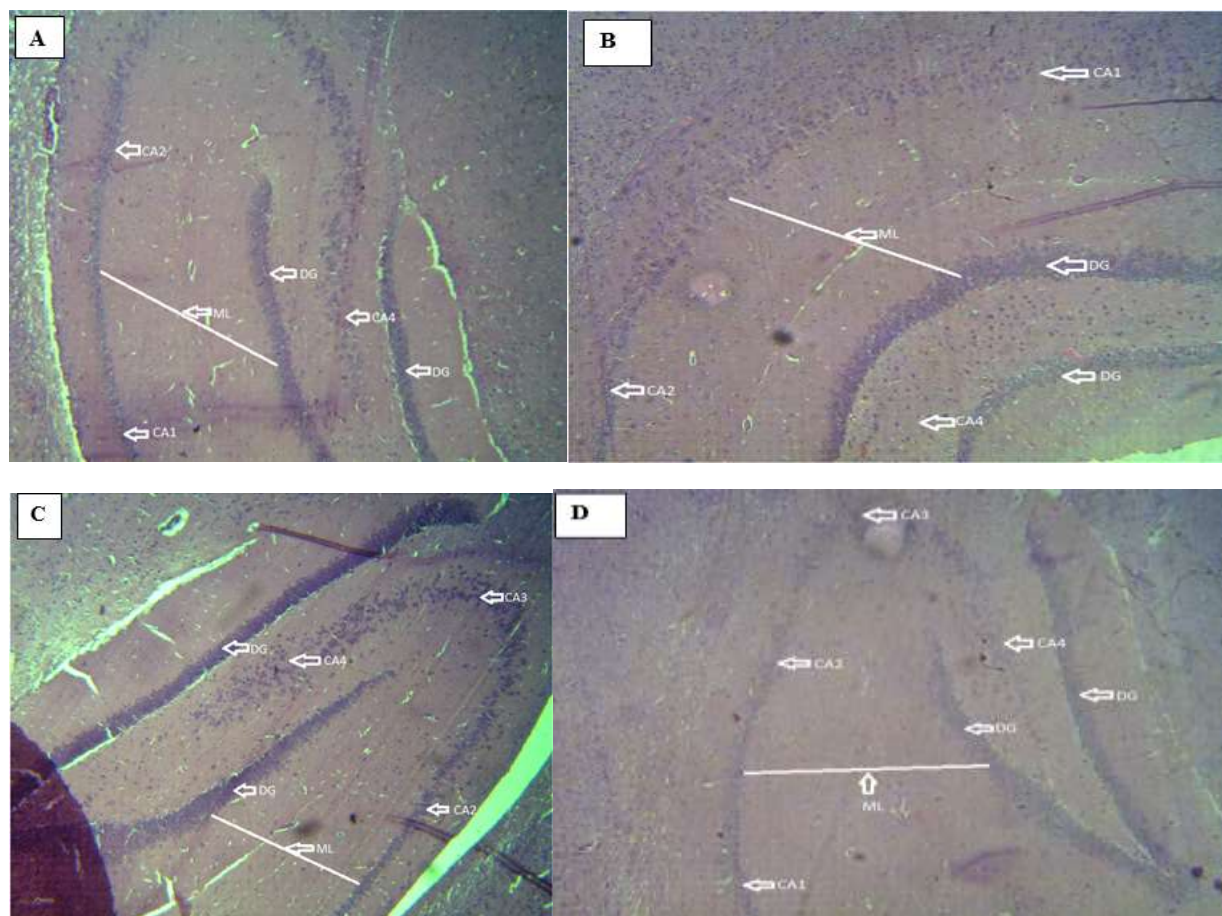
GROUP	Post induction (sec)	Treatment (sec)
Normal	3.80±0.45	2.87±0.47
HgCl ₂	3.76±0.62	6.28±0.93
Ethanollic extract	3.40±0.11	3.20±0.42
Aqueous extract	3.69±0.23	2.53±0.32
Low dose Ethanollic extract	3.22±0.34	6.20±0.08
Low-dose Aqueous extract	3.64±0.72	4.22±0.25
High dose of Ethanollic extract	3.22±1.84	7.10±0.16
High-dose Aqueous extract	3.65±0.82	3.30±0.28

Data is presented as the Mean ± Standard deviation of 5 rats in each group

Histological Studies

The results of histological observation of the hippocampus of animals in the control (water) group showed normal cytoarchitecture of general regions of the hippocampus. DG contains compact granular cells, ML contains neuronal processes, glial cells and few nerve cells, as shown in Figure 1a, while animals in mercuric chloride only (HgCl₂; 0.5 mg/kg) group, showed normal histoarchitecture of the hippocampus, as shown in Figure 1b. The hippocampus of animals in HgCl₂ cotreated with *Artocarpus heterophyllus* group showed normal Pyramidal cells as shown in Figure 1c to H. **CA:** Cornus Ammonis, **DG:** Dentate gyrus **ML:** Molecular layer

The histological studies used the hematoxylin and eosin (H & E) method.



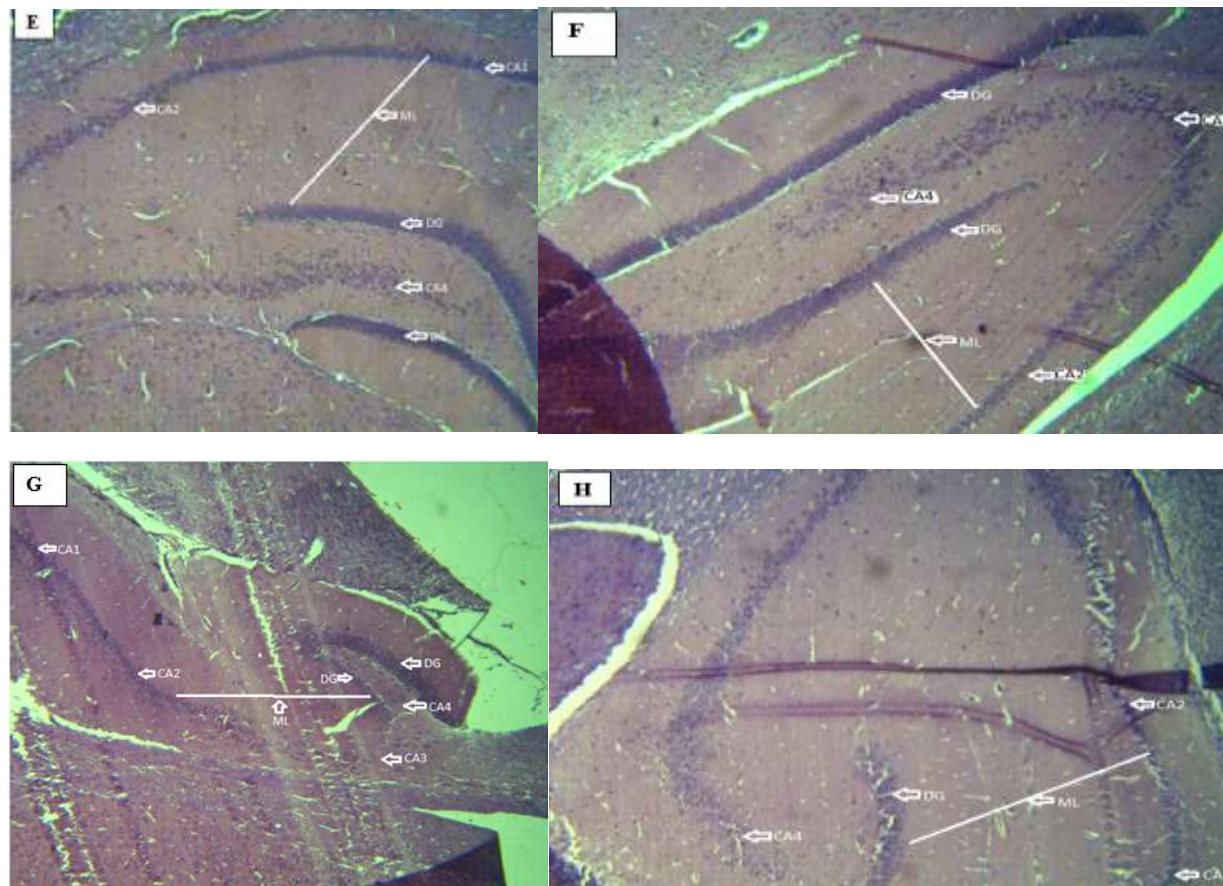


Fig. 1: Effects of HgCl_2 and cotreatment with *Artocarpus heterophyllus* on hippocampus across all groups. (A) normal control. HgCl_2 group (B), ethanolic extract of *Artocarpus heterophyllus* (C) and aqueous extract of *Artocarpus heterophyllus* (D). HgCl_2 and low dose ethanolic extract of *Artocarpus heterophyllus* group (E), HgCl_2 and low dose aqueous extract of *Artocarpus heterophyllus* group (F), HgCl_2 and high dose ethanolic extract of *Artocarpus heterophyllus* group (G), and HgCl_2 and high dose aqueous extract of *Artocarpus heterophyllus* group (H). H & E stain, X100. CA: Cornus Ammonis, DG: Dentate gyrus ML: Molecular layer.

DISCUSSION

Mercury is one of the most toxic heavy metals in the environment in three forms, namely, elemental, inorganic, and organic, causing human health problems [22]. The principal sources of exposure to mercury compounds in the general population are ingestion and inhalation of mercury from dental amalgams and ingestion of fish (fresh water and marine) and seafood [23]. The protective effect of ethanolic and aqueous extract of *Artocarpus heterophyllus* on HgCl_2 -induced neurotoxicity on adult Wistar rats' hippocampus was studied. In this current work, we showed that the *A. heterophyllus* intake prevented deficits in memory and motor promoted by Hg at low concentrations. It has been reported that jackfruit has antioxidant, neuroprotective, antiepileptic, and antidepressant and also has various therapeutic phytoconstituents [24, 25]. These effects and their phytoconstituents might be responsible for therapeutic activities [26]. There was a significant increase in the time the experimental rats spent finding the hidden platform in the Morris water maze test for memory and learning. Conversely, the pyramidal cell layer of the hippocampus appears to be damaged with dead cells, vacuolated spaces, and distortion in the general morphology of the pyramidal cells, which appear smaller than normal. These alterations can consequently result in memory impairments, which could result from neuronal degeneration; the destruction of the pyramidal cells implies that activity from the brain region that projects into the pyramidal layer of the hippocampus will also be lost, such as memory and learning ability [27]. [28, 29], reported that exposure to high mercury induced slight cognitive deficits. The neurotoxicity of HgCl_2 in the rat hippocampus was well demonstrated by the neuronal degeneration noted by the pyknotic neurons in the granule layer of the dentate gyrus (DG) of the rats with few nerve cells. The resultant effect of this is reduced acquisition and recall of episodic and spatial memories in such affected animals [3]. To test the curative therapeutic potential of *A. heterophyllus* extract, mice were administered 200 and 400 mg/kg ethanolic and aqueous extract orally after being exposed to

HgCl₂ concurrently. When compared to the control group, the histoarchitecture of the hippocampus revealed normal histoarchitecture. Plants containing alkaloids have potential therapeutic effects against a variety of neurodegenerative diseases [6]; the presence of phytochemicals found in *A. heterophyllum*, such as alkaloid, carbohydrate, flavonoid, polyphenol, and steroid, could explain the observed therapeutic potential of *A. heterophyllum*.

CONCLUSION

The study concluded that HgCl₂ intoxication has effects on the hippocampus of mice as it causes reduced nerve cells and learning and memory impairment. The results also showed an increased time taken to find the hidden platform, which was an indication of memory loss. Learning and memory impairments were attributed to be dose and time-dependent, and mercury intoxication resulted in some level of damage to the hippocampus, which is very crucial in memory and learning. However, the administration of *A. heterophyllum* extract was of therapeutic value against HgCl₂-induced neurotoxicity in adult male mice. Moreover, the finding of ameliorative changes observed behaviorally and histologically across the groups concomitantly treated with *A. heterophyllum* and HgCl₂ suggests the potency of the antioxidant potential of *A. heterophyllum* to reduce most of the above implications in the rats affected.

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