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THE ANTI-OXIDATIVE AND COGNITIVE PROPERTIES OF *ZINGIBER OFFICINALE* RHIZOME ETHANOL EXTRACT AND ITS DICHLOROMETHANE AND N-HEXANE FRACTIONS AGAINST ALUMINIUM CHLORIDE-INDUCED NEUROTOXICITY IN SWISS MICE

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Keywords:

Anti-oxidants, Aluminium chloride, Morris water maze, Neurotoxicity, *Zingiber officinale*

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ABSTRACT: Aluminium neurotoxicity is implicated in Alzheimer's disease, this multifactorial disorder, with neurodegeneration and dementia as hallmarks. Aluminium toxicity is due to imbalances between free radicals and antioxidants formation, leading to oxidative stress. However, protection against aluminium toxicity is yet to be fully realized. Therefore, this study investigated *Zingiber officinale* (ZO) neuroprotective potential against aluminium chloride ($AlCl_3$)-induced neurotoxicity in Swiss mice. Forty-eight animals were divided into eight groups (n=5) with treatment lasting three weeks: Group 1 (control) received 1 ml/kg of distilled water, while groups 2-8 respectively, received $AlCl_3$ (100 mg/kg), donepezil (2.5 mg/kg), ZO ethanol extract (474 mg/kg, 949 mg/kg and 1,423 mg/kg), ZO dichloromethane extract (949 mg/kg), and ZO n-hexane extract (949 mg/kg). Morris water maze test was performed, and the mice were sacrificed. Brain homogenates were assayed for catalase (CAT) and glutathione peroxidase (GPx) levels. $AlCl_3$ treatment led to significantly ($p<0.05$) high escape latency and less latency in the hidden platform quadrant and reduced brain CAT and GPx levels compared with the control. The groups treated with Donepezil and ZO ethanol extract showed no difference ($p>0.05$) from the control, but with significantly ($p<0.05$) less escape latency and high brain CAT and GPx compared with the $AlCl_3$ group. The ZO dichloromethane and n-hexane fractions didn't show consistent actions. Conclusively, ZO ethanol extract improved cognitive activities and had anti-oxidant potentials. The 1,423 mg/kg ethanol extract showed the best results: the dichloromethane and n-hexane fractions didn't show consistent results.

INTRODUCTION: Aluminium is a trivalent cation and toxic metal that is highly ubiquitous as an environmental toxicant and in lots of medical agents, food products including pieces of bread, cakes, and pastries, glace fruits, dairy products [11, 16, 19].

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Aluminium builds up in lots of tissues, including the brain, kidney, liver and ultimately results in certain medical conditions such as dialysis and other neurological problems such as dementia [11]. In the brain, Alumimium exhibit a wide variety of intracellular targets in neurons that affect physiological function [6, 16].

The brain regions most affected by Al neurotoxicity are those involved in learning and memory, which could be attributable to the unique distribution of transferrin receptors and neuroanatomical linkages between brain regions

involved in cognitive functions. It is established that aluminium toxicity is due to imbalances between free radicals and antioxidants formation, leading to oxidative stress. However, protection against aluminium toxicity, which is vital for better health, is yet to be fully realized [11]. Although several studies have been done to unravel the mystery behind the pathogenesis of Alzheimer's disease caused by neurotoxicity yet, there are no available significant treatment options, including the use of synthetic medications [21, 17]. Medicinal plants have been reported to show protection [8, 14] and rich in anti-oxidant properties [2]. It, therefore could be a helpful adjuvant in treating Alzheimer's disease-related problems. Therefore, this study aimed to investigate *Zingiber officinale* ethanol extract and its fractions (dichloromethane and n-hexane) in aluminium chloride-induced neurotoxicity on Swiss mice.

MATERIALS AND METHODS: The research was conducted at the Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. The study was conducted by following the guidelines of the National Institutes of Health's for the Care and Use of Laboratory Animals[15] and the College of Health Sciences Animal Ethics Committee of the University of Uyo with the number 18/PG/BMS/PHS/PHD/004 assigned to it. Forty-eight healthy adult female Swiss mice 12-24 weeks old and weighing 21-27 g were acquired from the animal house of Pharmacology and Toxicology, Department of Pharmacology and Toxicology**,** University of Uyo, Uyo. The mice were kept in standard cages that were adequately ventilated and maintained at typical environmental conditions (12h-12h light-dark cycle). Before the trial, the mice were allowed two weeks of acclimatization and were randomly divided into eight groups.

Drugs and Chemicals: Donepezil (Sigma-Aldrich Cooperation, USA) and Aluminium-chloride (Sigma-Aldrich Corporation, USA) were dissolved in normal saline and administered orally using oral tubes. The chemicals used in this experiment were of analytical grade.

Collection and Identification of Plant Extract: *Zingiber officinale* was taken from a local farm in Anua-Offot, in Uyo Local Government Area, Akwa Ibom State. The voucher specimen was deposited in the Department of Botany and Ecological Studies with the herbarium number UUH4099 when it was identified.

Preparation of the Extract of Plant Material: The rhizomes were dried, chopped into little parts, and reduced to powder using an electric grinder. The powdered plant material (1.5 kg) was continuously soaked for 72 hours consecutively in each of the following solvents (2 x 2.5L) to obtain matching fractions of these solvents: ethanol, n-hexane and dichloromethane. The liquid filtrates were concentrated and dried at 40°C using a rotary evaporator before being kept at -4°C until needed [20].

Aluminium Chloride Administration: Aluminum chloride solution was administered orally at a dose of 100 mg/kg body weight daily for three weeks. The dose of Aluminium chloride was selected based on prior literature findings [1].

Treatment Protocol / Experimental Design: Forty-eight animals were divided into eight groups (n = 5) with treatment lasting three weeks: Group 1 (control) received 1 ml/kg of distilled water, while groups 2-8 respectively, received $AlCl_3$ (100 mg/kg), donepezil (2.5 mg/kg), ZO ethanol extract (474 mg/kg, 949 mg/kg and 1,423 mg/kg), ZO dichloromethane extract (949 mg/kg) and ZO n-hexane extract (949 mg/kg)[12].

Morris Water Maze: The Morris water mazetest evaluated the animals' learning and memory [18]. It is a swimming-based model in which the animal learns to go to a hidden platform. It consisted of a large circular pool (120 cm in diameter, 50 cm in height, filled to a depth of 30 cm with water maintained at 26±2°C). With the help of two threads fixed at right angles to each other on the pool's rim, the tank was divided into four equal quadrants. A white-painted submerged platform was placed 1 cm below the water's surface inside the target quadrants of this pool. Throughout the training session, the platform's location was not changed. Each animal was subjected to four consecutive training sessions from day one to day five, with a five-minute interval between trials. The trials were performed on all four consecutive days after 10 minutes of drug administration (Donepezil

2.5 mg/kg, p.o and aluminium-chloride mg/kg, p.o). The mouse was gently deposited in the water between quadrants, facing the pool wall, and given 90 seconds to locate the submerged platform, with the drop location changing for each trial. The fourth day's escape latency time (ELT) to find the hidden platform in the water maze was used as an indicator of learning or acquisition.

The platform was removed on day 5 of the Probe trial, and each mouse was given 60 seconds to explore the pool. The average time spent in each of the four quadrants was recorded. The average time spent in the target quadrant by the animal hunting for the hidden platform was recorded as a retrieval or memory index.

The relative location of the water maze concerning other objects in the laboratory was carefully considered so that prominent visual hints were not disrupted during the investigation. All the tests were done between 10:00 a.m. and 2:00 p.m. for the animals [18].

Sample Analysis: On the same day, the animals were sacrificed by cervical dislocation. The hippocampus and prefrontal regions of the mice's skulls were carefully removed, weighed, and transferred to a glass homogenizer for homogenate preparation.

A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4, stored at 2–8 °C). The homogenate was centrifuged at 3,000 rpm for 10 minutes, and the cloudy supernatant liquid was used for biochemical analysis of catalase (CAT) and glutathione peroxidase activities. Which are biomarkers utilized to detect antioxidation.

Statistical Analysis: The data were analyzed using one-way ANOVA and Turkey's multiple comparison tests and were reported as mean ± SEM. Where $p<0.05$ was regarded as statistically significant. Graph Pad Prism 8.0 software was used to conduct the statistical analysis.

RESULTS:

Neurobehavioral Evaluation:

Acquisition Training using Morris Water Maze for the Assessment of Learning and Spatial Memory: Escape latency for acquisition training was investigated and shown in **Fig. 1.** The result reveals the aluminium chloride treated group had a significant ($p<0.05$) increase in escape latency compared with the control group.

The standard drug, the donepezil group, was observed to have a significant ($p<0.05$) decrease in escape time latency compared with the aluminium chloride treated group and no significant difference compared with the control.

Comparing the various extract with aluminium chloride treated group, there was a significant ($p<0.05$) decrease in escape latency in ethanol low, middle and high dose groups. It was also observed that the *Z. officinale* fractions (n-hexane and Dichloromethane) showed significantly ($p<0.05$) decreased escape latency compared with the aluminium chloride treated group and the control group, respectively.

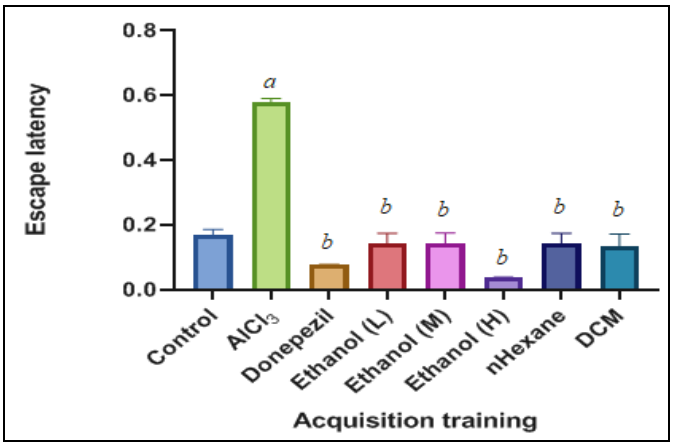

FIG. 1: THE ESCAPE LATENCY FOR ACQUISITION TRAINING. *a* = statistically significant ($P<0.05$) increase compared with the control group. *b*= statistically significant ($P<0.05$) decrease when compared with the Aluminium chloride treated group using one-way Anova followed by Turkey post-test. Each data represents Mean±SEM. (n=5).

Probe Trial During Morris Water Maze Task for the Assessment of Learning and Spatial Memory: The duration of time spent in the quadrant where the hidden platform was initially located (time latency) is reported in **Fig. 2**.

The result showed a significant ($P < 0.05$) decrease in the aluminium chloride-treated group compared with the control group. Comparing the ethanol extract with the aluminium treated group, a significant ($P<0.05$) increase was observed only in high ethanol doses.

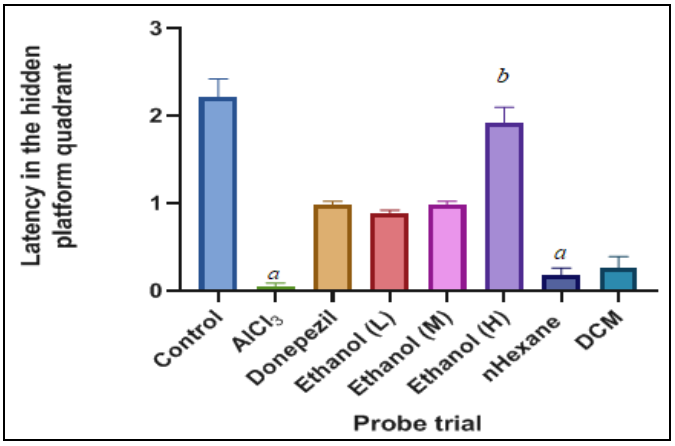

FIG. 2: EFFECT OF *ZINGIBER OFFICINALE* EXTRACT AND FRACTIONS ON TIME LATENCY ON THE QUADRANT THAT EARLIER CONTAINED THE HIDDEN PLATFORM. *a* = statistical significant (P <0.05) decrease in time latency compared with the control group. *b*= statistically significant (P<0.05) increase in time latency compared with the Aluminium chloride treated group using one-way Anova followed by Turkey post-test. Each data represents Mean±SEM. (n=5).

Effect of *Zingiber officinale* Ethanol Extract and its Fractions on Catalase Activity: The effect of *Zingiber officinale* ethanol extract and its fractions on catalase activity was reported in **Fig. 3**.

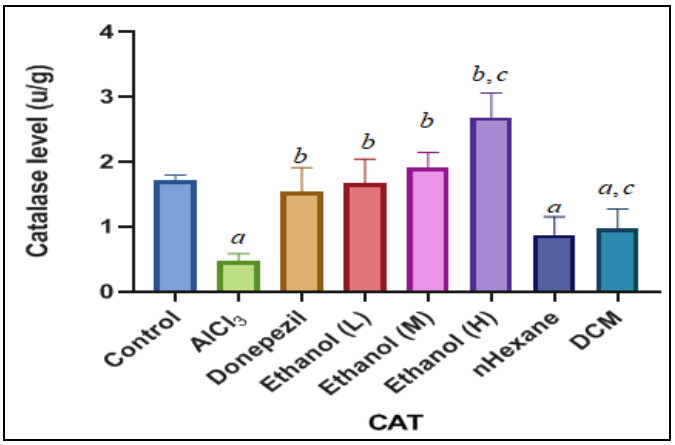

FIG. 3: EFFECT OF *ZINGIBER OFFICINALE* ETHANOL EXTRACT AND ITS FRACTIONS ON CAT (U/G) ACTIVITY A = STATISTICALLY SIGNIFICANT (P <0.05) DECREASE IN THE LEVEL OF CAT COMPARED WITH THE CONTROL GROUP. *b*= statistical significant (P<0.05) increase in the level of CAT compared with the Aluminium chloride treated group *c*= statistical significant (P<0.05) increase in the level of CAT in high ethanol dose when compared with Donepezil group and statistically significant (P<0.05) decrease in the level of CAT in DCM fraction when compared with Donepezil group using one-way Anova followed by Turkey post-test. Each data represents Mean±SEM. (n=5).

The result shows that the aluminium chloride treated group had a significant (P<0.05) decrease in the level of CAT compared with the control group. Comparing the extract and the aluminium chloride-treated group, the low, middle, and high doses showed a significant (P<0.05) increase. Moreover, the high ethanol dose showed a significant (P<0.05) increase compared to the standard drug and the donepezil group. The result also shows that the extract fractions n-hexane and DCM fractions had a significant (P<0.05) decrease compared with the control group. Moreover, there was a significant (P<0.05) increase in the DCM and n-hexane fractions compared with the aluminium chloride-treated group. Also, comparing the DCM fraction with Donepezil treated group, there was a significant (P<0.05) decrease.

Effect of *Zingiber officinale* Ethanol Extract and its Fractions on Glutathione Peroxidase Activity: The effect of *Zingiber officinale* ethanol extract and its fractions on glutathione peroxidase activity was reported in **Fig. 4**.

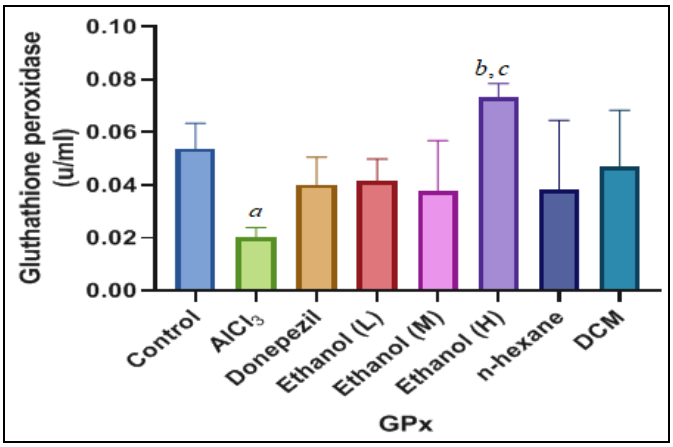

FIG. 4: EFFECT OF *ZINGIBER OFFICINALE* ETHANOL EXTRACT AND ITS FRACTIONS ON GPX ACTIVITY *A* = STATISTICALLY SIGNIFICANT (P-VALUE <0.05) DECREASE IN THE LEVEL OF GPX WHEN COMPARED WITH THE CONTROL GROUP. *b*= statistical significant (P-value <0.05) increase in the level of GPx when compared with the Aluminium chloride treated group *c*= statistical significant (P < 0.05) increase in the level of GPX (ug/ml) when compared with the Donepezil group using one-way Anova followed by Turkey post-test. Each data represents Mean±SEM. (n=5).

The result shows that the aluminium chloride treated group had a significant (P<0.05) decrease in the level of GPx compared with the control group. Comparing the extract and the aluminium chloride treated group, the high ethanol dose showed a significant (P<0.05) increase. Moreover, the high ethanol dose showed a significant (P<0.05)

increase compared with the standard drug and the donepezil group. The result also shows that the extract fractions n-hexane and DCM fractions decreased compared with the control group.

DISCUSSION: In this study, biochemical and behavioral alterations caused by $AlCl_3$ exposure were investigated, and the possible effect of treatment with the *Zingiber officinale* Roscoe ethanol extract and its fraction (Dichloromethane and n-hexane) that has been used traditionally to improve memory and reduce age-related cognitive decline.

According to the present results, spatial learning and memory were affected by $AlCl_3$ exposure for three weeks by increasing the escape time latency during acquisition training (0.6 s) and a reduction in the time spent in the quadrant where the hidden platform was previously placed (0.05 s); it is evident that $AlCl_3$ causes a cognitive impairment characterized by a deficiency of spatial memory. This is consistent with various memory studies in which Aluminium causes cognitive dysfunction and negatively affects the spatial learning and memory capacity of rats, it was reported that chronic exposure to Aluminium reduced spontaneous alternation in male and female rats[21]. Furthermore, as determined by the Morris water maze test, spatial memory is linked to the NMDA receptor/ Ca2+ influx signaling pathway and is dependent on the learning function and memory of the hippocampus [7, 5]. Aluminium may inhibit this receptor. Studies have shown that these receptors have a role in spatial learning, working memory, and reference memory [10].

The study showed that oxidative stress was markedly visible in the $AlCl_3$ group, which was indicated by an increase in the level of MDA (0.06 umol/ml) and a significant decrease in catalase (0.48 umol/ml) and GPx (0.02 u/ml) activities. These findings are consistent with previous research; they could be explained by an increase in lipid peroxidation of biological membranes, as well as changes in membrane integrity, capacity, permeability, and fluidity; affecting membrane-bound enzymes, resulting in cell leakage; and affecting membrane-bound enzymes, resulting in cell leakage [13]. On the other hand, a significant decrease in catalase activity in the brain of Wistar rats exposed to aluminium was reported [3]. Manipulation of aluminum-induced mice with *Zingiber officinale* Roscoe ethanol extract and its fraction (Dichloromethane and n-hexane) revealed a reduction in MDA level and elevation in catalase activity compared to the $AlCl_3$ group. These results confirmed that *Zingiber officinale* Roscoe ethanol extract and its fraction (Dichloromethane and n-hexane) could significantly modulate oxidative stress parameters caused by $AlCl_3$ through its antioxidant composition. These findings support previous studies that ginger's complex phytochemistry contains components that scavenge free radicals generated in food chains or biological systems. In vitro studies have shown that the active components in ginger have antioxidant potential [4]. In rat models, ginger reduced induced lipid peroxidation and increased the levels of antioxidant enzymes and serum glutathione [9].

CONCLUSION: This study revealed that the treatment with the *Zingiber officinalis* ethanol extract and its fraction (Dichloromethane and n-hexane) in $AlCl_3$-induced female Swiss mice improved memory, reduced the oxidative stress status and improved biochemical parameters; this effect has been achieved through its potent anticholinesterase activity and antioxidant capacity. The results demonstrated that *Zingiber officinalis* ethanol extract and its fraction (Dichloromethane and n-hexane) prevented aluminium chloride-induced neurotoxicity in mice brains and could provide an excellent therapeutic approach for intervention against neurological diseases.

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