JOPAT Vol 23(2), 1598- 1606, July – December, 2024 Edition.
ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v23i2.16

Mercury Chloride-Induced Hippocampal Toxicity in Wistar Rats: Antioxidant Activity of Folic Acid

Enogieru, Adaze Bijou [1]*; Ezennia, Martha Chinyere [1]
[1]Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

ABSTRACT

Mercury chloride ($HgCl_2$) is a well-known neurotoxicant with detrimental effects on the central nervous system, particularly the hippocampus, a region crucial for learning and memory processes. Folic acid (FA), also known as the synthetic form of vitamin B9, plays a crucial role in various bodily functions. Accordingly, this study investigated the possible neuroprotective activity of FA against $HgCl_2$-induced hippocampal toxicity in Wistar rats. Forty-eight Wistar rats were assigned into six groups (n=8) with the following treatment regimen for 28 days: A (control); B (4 mg/kg bw $HgCl_2$); C (5 mg/Kg bw FA + 4 mg/kg bw $HgCl_2$); D (10 mg/kg bw FA + 4 mg/kg bw $HgCl_2$); E (5 mg/kg bw FA); F (10 mg/kg bw FA). After treatment, neurobehavioral, antioxidant enzymes, lipid peroxidation, mercury concentration, and histological assessments were done. Results showed significantly ($p < 0.05$) impaired cognition and antioxidants, as well as elevated lipid peroxidation and mercury concentration in the $HgCl_2$-treated rats when compared to the control. In addition, the hippocampus of $HgCl_2$-treated rats exhibited severe atrophy and vacuolated pyramidal cells and astrocytes, signifying hippocampal dysfunction. However, pretreatment of $HgCl_2$-treated rats with FA significantly ($p < 0.05$) mitigated these neurobehavioural, biochemical, and histological alterations. Taken together, the neuroprotective activity of FA against $HgCl_2$-induced hippocampal toxicity is mediated, possibly through its antioxidant and metal-chelation effects.

Keywords: Folic Acid; Neurodegeneration; Hippocampus; Antioxidant; Mercury Chloride

***Corresponding author**: Adaze Bijou Enogieru
Email: adaze.enogieru@uniben.edu +234 7016780198

INTRODUCTION

Neurotoxicity is a significant concern today due to the ubiquity of environmental pollutants and the potential consequences for human health. One neurotoxin of particular concern is mercury, a toxic heavy metal compound found in various industrial and environmental sources [1]. Exposure to mercury compounds has been associated with detrimental effects on the nervous system, including cognitive impairments and neurodegenerative disorders. Mercury chloride ($HgCl_2$) is a well-known neurotoxicant that is reported to accumulate in various brain regions, including the hippocampus, and exert its toxic effects [2]. The hippocampus, an intricate seahorse-shaped structure nestled deep within the brain's temporal lobe, is often hailed as the brain's memory hub. This vital region orchestrates the formation and retrieval of memories, spatial navigation, and various cognitive functions [3, 4]. Yet, beneath its cognitive prowess lies an equally intricate circulatory network responsible for maintaining its metabolic equilibrium. The hippocampus, a neural crucible where experiences, facts, and events are moulded into enduring memories, comprises several regions, including the dentate gyrus, CA1, CA2, and CA3, each contributing uniquely to memory formation and retrieval [5]. Mercury exposure in humans and experimental animals has been linked to oxidative stress, neuroinflammation, and apoptosis [6, 7]. These mechanisms are reported to collectively contribute to hippocampal dysfunction, which often manifests as memory deficits and cognitive impairment.

For several years, researchers have focused on the therapeutic potentials of dietary supplements and antioxidants for the attenuation of metabolic disorders [8, 9], as well as heavy metal toxicity [10, 11]. Folic acid, also known as the synthetic form of vitamin B9, is often regarded as an effective antioxidant comparable to vitamins C and E, which are commonly accepted as effective water and lipid-soluble antioxidants [12]. It is a necessary nutrient involved in several physiological activities, such as DNA synthesis, cell division, and amino acid metabolism [13]. Reports indicate that folic acid may have neuroprotective effects in addition to its well-known function in preventing neural tube abnormalities during pregnancy [14]. It serves as a crucial cofactor in the one-carbon metabolism pathway, facilitating the conversion of homocysteine to methionine and thereby preserving neuronal integrity [14]. Folic acid has been proven to have antioxidant capabilities by promoting glutathione synthesis [15]. It contributes to the production and metabolism of neurotransmitters like serotonin, dopamine, and norepinephrine. Normal brain function depends on a healthy neurotransmitter balance, and abnormalities in these systems have been linked to many neurological disorders [16]. In addition, folic acid plays an important role in maintaining normal neurological functions via the attenuation of neuroinflammation [17]. Despite the multifaceted roles of folic acid in DNA maintenance, oxidative stress inhibition, and anti-inflammatory modulation, there is limited research evidence demonstrating the protective effects of folic acid against $HgCl_2$-induced neurotoxicity. Accordingly, this study investigated such activity in the hippocampus of adult Wistar rats.

MATERIALS AND METHODS

Chemical and Reagents

Mercury Chloride ($HgCl_2$, 99% purity) and Folic acid were manufactured by Molychem, Mumbai, India. Other reagents were all of the analytical grades.

Care and Management of Experimental Animals

Forty-eight Wistar rats weighing 200 g - 230 g were bought and kept in the Department of Anatomy animal holdings. The rats, fed with standard rat chow (Olam Agri Holdings Pte Ltd., Lagos State, Nigeria) and water liberally, acclimatized for two weeks before the beginning of the experiment. The experimental procedures performed on the animals were according to the guidelines of the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria, with approval number CMS/REC/2023/409.

Experimental design

The rats were randomly assigned into six (6) different groups (n=8). The experimental design was as follows:

Group A (control) - received 1 ml of normal saline

Group B ($HgCl_2$) - received 4 mg/kg body weight (BW) of Mercury Chloride ($HgCl_2$) only.

Group C (FA1 + $HgCl_2$) - received 5 mg/kg BW/day of folic acid (FA) and 4 mg/kg BW of $HgCl_2$.

Group D (FA2 + $HgCl_2$) - received 10 mg/kg BW/day of FA and 4 mg/kg BW of $HgCl_2$.

Group E (FA1) - received 5 mg/kg BW/day of FA.

Group F (FA2) - received 10 mg/kg BW/day of FA.

Rats were pretreated with FA one hour before the administration of $HgCl_2$. After 28 days, the rats were evaluated for neurobehaviour.

Neurobehavioural Evaluation

Novel Object Recognition Test: This test was carried out in a wooden open box device (80 × 60 × 40 cm), as previously described [18, 19]. Here, rats explored the device for a 2-minute session of familiarization on the 27th day of the experiment. On the 28th day, a first 5-minute sample trial test (T1) was carried out, with two similar objects (named familiar objects FO1 and FO2) placed at the corners of the box. In the

second 5-minute test (T2), FO2 presented in T1 was substituted with a novel object (NO), and the exploration times for FO1 and NO were recorded. The discrimination between FO1 and NO during T2 was determined by equating the time spent exploring FO1 and NO [18, 19]. For quality control, a discrimination index (DI) was calculated as follows: DI = NO−FO1/NO + FO1.

Y-Maze Test: This test was carried out in a wooden apparatus consisting of three identical arms (33×11×12cm each) which are symmetrically separated at 120° with an equilateral triangular central area, as previously described [20, 21]. The rats were placed at the end of one arm and allowed to move freely through the maze for 5 minutes after which every session was stopped. An arm entry was recorded when the hind paws of the rat were completely within the arm and spontaneous alternation behaviour was defined as three consecutive entries in three different arms [20, 21]. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as the total of alternations /(total arm entries –2×100).

Evaluation of Biochemical Parameters

Following the end of the novel object recognition test, rats were sacrificed and the hippocampus was dissected out and processed for biochemical and histological assessment. The hippocampus was homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and the homogenate was then centrifuged at 10,000 g for 10 min at 4°C [22, 23]. The supernatant was collected and evaluated for mercury concentration [11], Catalase – CAT [24], Superoxide dismutase – SOD [25], Glutathione peroxidase – GPx [26], Glutathione - GSH [27], and Malondialdehyde – MDA [28].

Histological Evaluation

After suitable fixation of the hippocampus in 10% buffered formal saline for 72 h, processing through the paraffin wax embedding and the Hematoxylin and Eosin staining method was done as previously described [29].

Statistical Analysis

Analysis of data was carried out using the GraphPad Prism Software V9. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test was utilized to determine statistical significance ($p < 0.05$). Values are presented as Mean ± Standard Error of Mean (SEM).

RESULTS

Findings on Neurobehaviour

In the novel object recognition test (Table 1), a significant decrease ($p < 0.05$) was observed in the total exploration time for FO1, NO, and DI of $HgCl_2$-exposed rats when compared to the control. However, a significant increase ($p < 0.05$) was observed in the FA-pretreated rats, for DI, when compared to the $HgCl_2$-exposed rats. No significant difference ($p > 0.05$) was observed between the AA-treated rats and control.

Table 1: Novel Object Recognition Findings across Experimental Groups

Groups	Control	$HgCl_2$	FA1 + $HgCl_2$	FA2 + $HgCl_2$	FA1	FA2
FO1 – 1st Test (s)	30.83 ± 0.95	21.33 ± 1.98 #	27.00 ± 1.58	26.00 ± 1.96	30.29 ± 1.44	31.29 ± 1.54
FO2 – 1st Test (s)	12.33 ± 1.23	12.33 ± 1.43	11.25 ± 1.00	11.00 ± 1.83	12.00 ± 1.23	10.29 ± 1.02
FO1 - 2nd Test (s)	06.83 ± 2.23	12.17 ± 0.95	10.00 ± 1.27	10.17 ± 1.14	08.57 ± 1.73	05.50 ± 1.88
NO	36.33 ± 2.68	17.00 ± 1.57 #	24.33 ± 2.35	27.83 ± 3.32	33.57 ± 1.97	28.17 ± 3.23
DI	0.72 ± 0.09	0.16 ± 0.02 #	0.45 ± 0.03 *	0.47 ± 0.02 *	0.65 ± 0.08	0.73 ± 0.09

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group.

FO1 – Familiar object 1; FO2 – Familiar object 2; NO – Novel object; DI – Discrimination index

In the Y-maze test (Table 2), a significant decrease ($p < 0.05$) was observed in the spontaneous alternation of $HgCl_2$-exposed rats when compared to the control. However, a significant increase ($p < 0.05$) was observed in the FA-pretreated rats when compared to the $HgCl_2$-exposed rats. No significant difference ($p > 0.05$) was observed between the AA-treated rats and control.

Table 2: Y-maze Findings across Experimental Groups

Groups	Control	$HgCl_2$	FA1 + $HgCl_2$	FA2 + $HgCl_2$	FA1	FA2
Total Arm Entries	09.00 ± 1.24	07.50 ± 1.38	09.00 ± 0.63	08.20 ± 2.48	06.83 ± 1.33	07.67 ± 0.56
Total Alternations	06.17 ± 1.20	02.83 ± 0.79	05.20 ± 0.80	04.60 ± 1.89	04.57 ± 0.87	04.83 ± 0.60
Spontaneous Alternation	88.96 ± 5.72	41.85 ± 8.69 #	73.67 ± 7.82 *	72.68 ± 2.61 *	88.09 ± 4.39	84.87 ± 5.53

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group.

Biochemical Findings in the Hippocampus

Table 3 illustrates the activity of antioxidants, and lipid peroxidation as well as Hg concentrations in the hippocampus across experimental groups. A significant decrease ($p < 0.05$) in SOD, CAT, GSH, and GPx, as well as a significant increase in MDA and Hg concentrations, were observed in the hippocampus of $HgCl_2$-exposed rats when compared to the control. However, a significant difference ($p < 0.05$) was observed in these parameters in the FA-pretreated rats following comparison to the $HgCl_2$-exposed rats. No significant difference ($p > 0.05$) was observed between the FA-treated rats and control.

Table 3: Biochemical Findings across Experimental Groups

Groups	Control	$HgCl_2$	FA1 + $HgCl_2$	FA2 + $HgCl_2$	FA1	FA2
CAT (U/mg)	8.91 ± 0.55	4.19 ± 0.54 #	6.99 ± 0.39 *	7.37 ± 0.31 *	9.49 ± 0.75	9.87 ± 0.59
SOD (U/mg)	3.33 ± 0.53	1.59 ± 0.28 #	2.67 ± 0.10	3.09 ± 0.26 *	3.00 ± 0.17	3.14 ± 0.16
GPx (U/mg)	13.91 ± 0.34	05.59 ± 0.37 #	08.59 ± 0.90 *	08.83 ± 0.55 *	12.62 ± 0.40	12.79 ± 0.68
GSH (µM)	22.55 ± 1.40	10.61 ± 1.36 #	16.37 ± 2.27	18.67 ± 0.78 *	24.04 ± 1.90	24.99 ± 1.50
MDA (mol/mg)	03.96 ± 0.21	31.02 ± 0.44 #	18.35 ± 0.55 *	11.72 ± 0.34 *	08.53 ± 0.79	08.14 ± 1.26
Hg (ppm)	0.02 ± 0.00	1.18 ± 0.23 #	0.36 ± 0.10 *	0.28 ± 0.04 *	0.09 ± 0.05	0.06 ± 0.05

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group.

Histological Findings in the Hippocampus

Figure 1 illustrates the histology of the CA1 region of the hippocampus. Figure 1A shows the normal structure of the pyramidal cells and astrocytes of the CA1 region of the hippocampus. Figure 1B shows atrophy and vacuolated pyramidal cells and astrocytes due to the administration of $HgCl_2$. Figure 1C-D demonstrates fewer vacuolations and normal pyramidal cells due to the pretreatment with FA as compared to the $HgCl_2$-exposed rats. Observed in FA-treated rats are normal pyramidal cells and astrocytes having similar morphology when compared to control (Figure 1E-F)

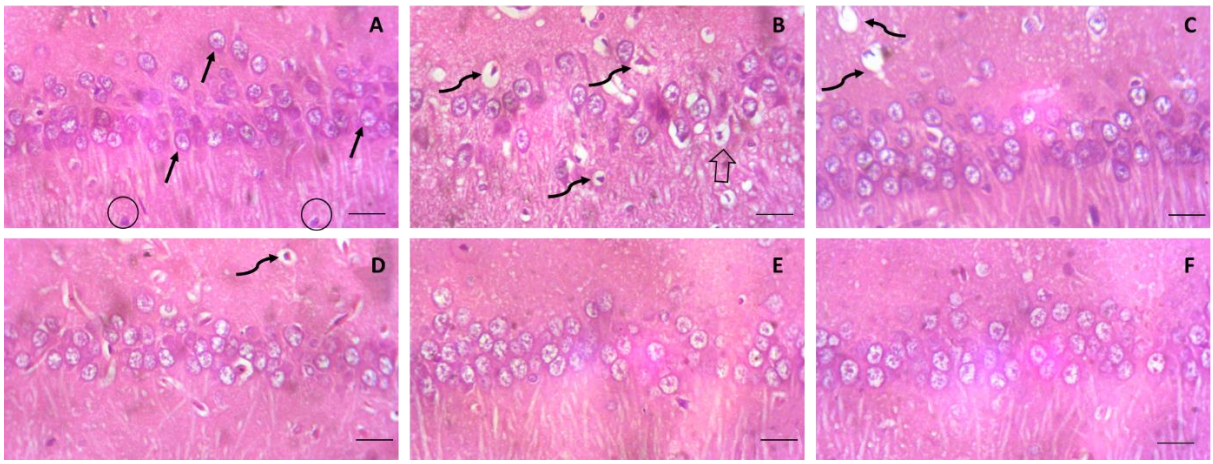

Figure 1: Representative histology of the hippocampus CA1 in control and treatment rats. (A) Control group revealed the normal structure of pyramidal cells (arrows) and astrocytes (circles). (B) $HgCl_2$-treated rats showing atrophy and vacuolated pyramidal cells (arrows) and astrocytes (thick arrow). (C-D) Fewer vacuolations and normal pyramidal cells. (E-F) showing normal pyramidal cells and astrocytes. (H&E 400x; Scale bar: 25µm)

DISCUSSION

This study investigated the possible neuroprotective activity of folic acid against $HgCl_2$-induced hippocampal toxicity in Wistar rats. The hippocampus is a crucial brain region known for its pivotal role in memory formation, spatial navigation, and emotional regulation [30]. The hippocampus's intricate functions and vulnerabilities are essential for unraveling its role in cognition and behavior and also for developing strategies to protect it from neurotoxic insults [31].

The Novel Object Recognition test is a rapid and efficient method for assessing various aspects of rodents' learning and memory, hippocampal function, and cognitive deficits [32]. In this test, rodents are presented with a familiar and novel object, and assessments are made on their ability to recognize and preferentially explore the novel object, thus indicating recognition memory and cognitive function. Findings from this study indicate that rats exposed to $HgCl_2$ exhibited a significantly diminished discrimination index, thus suggesting that the rats were unable to distinguish between familiar and novel objects when compared to the control group. This is in line with previous studies demonstrating that mercury and heavy metals impair cognition in experimental animals [20, 33]. However, rats pretreated with FA demonstrated a significantly higher discrimination index when compared to the $HgCl_2$-exposed rats, thus highlighting the cognitive-enhancing activity of FA. In the Y-maze test, a noteworthy decrease in spontaneous alternation was observed in the $HgCl_2$-exposed rats when compared to the control group, thus indicating a deficit in cognition. Spontaneous alternation is often deployed as a measure of spatial working memory and can be evaluated by permitting rodents to explore all three arms of the maze [18]. This test is driven by the innate curiosity of rodents to explore previously unvisited areas. Consequently, a rat with intact working memory will remember the arms earlier visited and display an inclination to enter a less recently visited arm. Findings from this study agree with previous studies demonstrating that mercury and other heavy metals impair spontaneous alternation [18, 34]. However, rats pretreated with FA demonstrated a significantly higher Spontaneous alternation when compared to the $HgCl_2$-exposed rats, thus highlighting the memory-enhancing activity of FA against $HgCl_2$.

Oxidative stress plays a pivotal role in mediating $HgCl_2$-induced damage to the hippocampus [2]. For instance, $HgCl_2$ exposure triggers the production of reactive oxygen species, such as superoxide anion radicals (O_2-) and hydroxyl radicals (OH), which are highly reactive molecules that can damage cellular components, including lipids, proteins, and DNA. $HgCl_2$ is also reported to dysregulate the activities of antioxidant enzymes leaving cells vulnerable to oxidative damage. Findings from

this study demonstrate a noteworthy reduction in the activity of SOD, CAT, GPx, and GSH in the hippocampus of rats exposed to $HgCl_2$ when compared to the control group. This is in line with previous studies demonstrating that $HgCl_2$ disrupts the antioxidant defense mechanisms [18, 34]. Inhibition of these enzymes impairs the cell's ability to detoxify superoxide radicals (O_2-) and hydrogen peroxide (H_2O_2). Reports indicate that the inhibition of GSH, a crucial antioxidant, occurs via the binding of mercury to sulfhydryl (-SH) groups on GSH molecules [35], thus forming mercury-glutathione complexes, consequently reducing the availability of GSH for the neutralization of reactive oxygen species. One of the hallmarks of $HgCl_2$-induced oxidative stress is lipid peroxidation. Excessive reactive oxygen species production damages cellular membranes to induce the formation of lipid peroxides and harmful byproducts, which disrupt membrane integrity and function. Elevated levels of lipid peroxides, such as MDA, are indicative of lipid peroxidation [36]. Findings from this study demonstrate a significant increase in hippocampal MDA levels in rats exposed to $HgCl_2$ when compared to the control group, indicating a high level of lipid peroxidation and oxidative stress. This agrees with previous studies showing that $HgCl_2$ induces oxidative stress in the brains of experimental rats [2, 18]. However, rats pretreated with FA demonstrated a significantly higher antioxidant enzymes activity and lower MDA concentration in the hippocampus when compared to the $HgCl_2$-exposed rats, thus highlighting the potent antioxidant activity of FA against $HgCl_2$.

Mercury exposure can lead to bioaccumulation in the brain, and elevated mercury levels in the hippocampus have been associated with cognitive impairments and behavioral changes in animal studies [2, 37]. The neurotoxicity of mercury is partly mediated by its effects on neurotransmitter systems and interference with synaptic function [38]. Evaluation of the overall brain and hippocampal mercury levels is integral to unraveling the mechanisms underlying these toxic effects and may offer insights into potential risks to human cognition and memory, particularly in areas with environmental mercury contamination. Findings from this study demonstrate a significant increase in hippocampal mercury levels in rats exposed to $HgCl_2$ when compared to the control group, indicating an accumulation of mercury. This agrees with previous studies demonstrating an elevated concentration of mercury in the hippocampus and other brain regions of experimental animals [2, 37]. However, rats pretreated with FA demonstrated a significantly lower mercury concentration in the hippocampus when compared to the $HgCl_2$-exposed rats, thus highlighting a possible metal-chelating activity of FA. Histological findings of the hippocampus showed significant morphological alterations in the $HgCl_2$-exposed rats, such as atrophy and vacuolated pyramidal cells and astrocytes, thus signifying neuronal damage. These changes are linked to extreme reactive oxygen species generation and oxidative stress, ultimately leading to cell death [32, 39]. These findings are consistent with previous studies demonstrating alterations in the histology of the hippocampus following mercury and heavy metal exposure [2, 40]. However, in the FA-pretreated rats, there were significantly fewer changes in the hippocampus when compared to the $HgCl_2$-exposed rats, suggesting a protective effect of FA against $HgCl_2$.

Altogether, pretreatment with FA has shown promise in mitigating $HgCl_2$-induced hippocampal toxicity in the experimental rats. FA's neuroprotective effects, particularly via its antioxidative and possible metal-chelating activities, contribute to its therapeutic role. Consequently, these findings provide a template for further multidisciplinary studies on the neuroprotective role of FA against mercury toxicity and its associated neurological disorders.

Funding: No funding declared.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgements: The authors appreciate the kind assistance of Mr. Abhelemhen Goodluck Ikpenmwosa and Miss Igbayisemore Matilda Olamerun during this research.

REFERENCES

[1] Wu Y.-S., Osman A.I., Hosny M., Elgarahy A.M., Eltaweil A.S., Rooney D.W., et al. (2024). The toxicity of mercury and its chemical compounds: molecular mechanisms and environmental and human health implications: a comprehensive review. Acs Omega, 9(5): 5100-26.

[2] Aragão W., Teixeira F., Fagundes N., Fernandes R., Fernandes L., Da Silva M., et al. (2018). Hippocampal dysfunction provoked by mercury chloride exposure: evaluation of cognitive impairment, oxidative stress, tissue injury and nature of cell death. Oxidative Medicine and Cellular Longevity, 2018: 7878050.

[3] Kühn S., Gallinat J. (2014). Segregating cognitive functions within hippocampal formation: A quantitative meta-analysis on spatial navigation and episodic memory. Human Brain Mapping, 35(4): 1129-42.

[4] Okhah A.A., Enogieru A.B. (2023). Antioxidant and protective activities of aqueous Theobroma cacao seed extract against aluminium-induced hippocampal toxicity in Wistar rats. Nigerian Journal of Biochemistry and Molecular Biology, 38(4): 197-205.

[5] Adedayo L., Ojo G., Umanah S., Aitokhuehi G., Emmanuel I.-O., Bamidele O. Hippocampus: Its Role in Relational Memory. Hippocampus-More than Just Memory: IntechOpen; 2023.

[6] Teixeira F., De Oliveira A., Leão L., Fagundes N., Fernandes R., Fernandes L., et al. (2018). Exposure to inorganic mercury causes oxidative stress, cell death, and functional deficits in the motor cortex. . Front Mol Neurosci 11: 125.

[7] Durak D., Kalender S., Uzun F.G., Kalender Y. (2010). Mercury chloride-induced oxidative stress in human erythrocytes and the effect of vitamins C and E in vitro. African Journal of Biotechnology, 9(4): 488-95.

[8] Enogieru A., Momodu O., Omoruyi S., Om'iniabohs F. (2015). Changes in Biochemical Markers of Kidney Function and Antioxidant Status of Diabetic Rats treated with Aqueous Leaf Extracts of Ficus exasperata (Vahl). African Journal of Biomedical Research, 18(1): 61-7.

[9] Momodu O., Enogieru A., Omoruyi S., OmIniabohs F. (2014). Extracts of Hunteria umbellata reverses the effect of streptozotocin-induced pancreatic islet-cell destruction. J Exp Clin Anat, 13(12): 66-73.

[10] Enogieru A.B., Iyoha E.N. (2023). Role of Nitric Oxide, TNF–α and Caspase-3 in Lead Acetate-Exposed Rats Pretreated with Aqueous Rosmarinus officinalis Leaf Extract. Biological Trace Element Research: 1-11.

[11] Enogieru A.B., Idemudia O.U. (2024). Comparative protective activity of aqueous Zingiber officinale root and Theobroma cacao seed extracts on lead acetate-induced cerebellar toxicity in rats. Journal of Trace Elements Minerals, 10: 100190.

[12] Gliszczyńska-Świgło A. (2007). Folates as antioxidants. Food Chemistry, 101(4): 1480-3.

[13] Tola F.S. (2024). The concept of folic acid supplementation and its role in prevention of neural tube defect among pregnant women: PRISMA. Medicine, 103(19): e38154.

[14] Xu X., Yang J., Lu Z., Ding J., Lin S. (2024). Folic acid ameliorated the scopolamine-induced memory impairment in mice and the neuroprotective mechanisms. Food Bioscience, 60: 104351.

[15] Asbaghi O., Ghanavati M., Ashtary-Larky D., Bagheri R., Rezaei Kelishadi M., Nazarian B., et al. (2021). Effects of folic acid supplementation on oxidative stress markers: a systematic review and meta-analysis of randomized controlled trials. Antioxidants, 10(6): 871.

[16] Surén P., Roth C., Bresnahan M., Haugen M., Hornig M., Hirtz D., et al. (2013). Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama, 309(6): 570-7.

[17] Darbandi Z.K., Amirahmadi S., Goudarzi I., Hosseini M., Rajabian A. (2024). Folic acid improved memory

and learning function in a rat model of neuroinflammation induced by lipopolysaccharide. Inflammopharmacology, 32(2): 1401-11.

[18] Enogieru A., Omoruyi S. (2022). Exploration of Aqueous Phyllanthus amarus Leaf Extract as a Protective Agent in Mercury Chloride-Exposed Wistar Rats: A Neurobehavioural Study. Journal of Applied Sciences and Environmental Management, 26(4): 629-37.

[19] Enogieru A., Egbon F. (2022). Neurobehavioural and Histological Alterations in Lead Acetate-Exposed Rats Pretreated with Aqueous Leaf Extract of Vernonia amygdalina. African Journal of Biomedical Research, 25(2): 205-13.

[20] Enogieru A.B., Williams B.T. (2024). Cognitive-and memory-enhancing activity of Cinnamon (Cinnamomum zeylanicum) aqueous extract in lead acetate-exposed rats. Journal of Trace Elements and Minerals, 9: 100189.

[21] Enogieru A.B., Inegbedion G.O. (2022). Attenuation of Oxidative Stress and Cognitive Impairment in Cadmium Chloride-Exposed Wistar Rats Pre-treated with Ethanolic Turmeric Root Extract. The Journal of Phytopharmacology, 11(2): 118-24.

[22] Enogieru A.B., Momodu O. (2022). Neuroprotective effects of Zingiber officinale against lead-induced toxicity in Wistar rats. Nutrire, 48(1): 2.

[23] Montilla P., Barcos M., Munoz M.C., Bujalance I., Munoz-Castaneda J.R., Tunez I. (2005). Red wine prevents brain oxidative stress and nephropathy in streptozotocin-induced diabetic rats. Journal of biochemistry and molecular biology, 38(5): 539.

[24] Cohen G., Dembiec D., Marcus J. (1970). Measurement of catalase activity in tissue extracts. Analytical biochemistry, 34(1): 30-8.

[25] Misra H.P., Fridovich I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247(10): 3170-5.

[26] Nyman M. (1959). Serum hatoglobin; methodological and clinical studies. Scandinavian journal of clinical and laboratory investigation, 11: 1-169.

[27] Ellman G.L. (1959). Tissue sulfhydryl groups. Archives of biochemistry and biophysics, 82(1): 70-7.

[28] Buege J.A., Aust S.D. [30] Microsomal lipid peroxidation. Methods in enzymology. 52: Elsevier; 1978. p. 302-10.

[29] Drury R., Wallington E. (1980). Carleton's histological technique 5th ed. New York: Churchill Livingstone.

[30] Eichenbaum H. (2017). The role of the hippocampus in navigation is memory. Journal of neurophysiology, 117(4): 1785-96.

[31] Davidson T.L., Stevenson R.J. (2024). Vulnerability of the Hippocampus to Insults: Links to Blood–Brain Barrier Dysfunction. International Journal of Molecular Sciences, 25(4): 1991.

[32] Amhanyunose P., Enogieru A. (2023). Improvement in Cognition, Memory and Hippocampal CA1 microstructure in Aluminium Chloride-exposed Rats Following Pretreatment with Aqueous Psidium guajava Leaf Extract. Journal of Experimental Clinical Anatomy, 20(1): 1-9.

[33] Kim W., Yoo D.Y., Jung H.Y., Kim J.W., Hahn K.R., Kwon H.J., et al. (2019). Leaf extracts from Dendropanax morbifera Léveille mitigate mercury-induced reduction of spatial memory, as well as cell proliferation, and neuroblast differentiation in rat dentate gyrus. BMC Complementary and alternative medicine, 19: 1-11.

[34] Malqui H., Anarghou H., Ouardi F.Z., Ouasmi N., Najimi M., Chigr F. (2018). Continuous exposure to inorganic mercury affects neurobehavioral and physiological parameters in mice. Journal of Molecular Neuroscience, 66(2): 291-305.

[35] Ajsuvakova O.P., Tinkov A.A., Aschner M., Rocha J.B., Michalke B., Skalnaya M.G., et al. (2020). Sulfhydryl groups as targets of mercury toxicity. Coordination chemistry reviews, 417: 213343.

[36] Gaweł S., Wardas M., Niedworok E., Wardas P. (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960), 57(9-10): 453-5.

[37] Cheng J.-P., Wang W.-H., Jia J.-P., Zheng M., Shi W., Lin X.-Y. (2006). Expression of c-fos in rat brain as a prelude marker of central nervous system injury in response to methylmercury-stimulation. Biomedical and Environmental Sciences, 19(1): 67-72.

[38] Branco V., Aschner M., Carvalho C. (2021). Neurotoxicity of mercury: an old issue with contemporary significance. . Adv Neurotoxicol, 5: 239–62.

[39] Huang T.-T., Leu D., Zou Y. (2015). Oxidative stress and redox regulation on hippocampal-dependent cognitive functions. Archives of biochemistry and biophysics, 576: 2-7.

[40] Wu J., Cheng G., Lu Z., Wang M., Tian J., Bi Y. (2016). Effects of methyl mercury chloride on rat hippocampus structure. Biological trace element research, 171: 124-30.