ISSN: Print: 2811-1672
Online: 2811-1680

www.njbams.asmeda.org

NJBAMS

Nigerian Journal of Basic and Applied Medical Sciences

An official publication of the National

ASSOCIATION OF SPECIALIST MEDICAL DOCTORS IN ACADEMICS (ASMeDA aka MEDSABAMS)

https://doi.org/10.53994/NJBAMS.202221.3

Neuroprotective Effects of Adansonia digitata L. Fruit Flavonoids on Mercury Chloride-Induced Neuronal Damage in a Rat Model

Aribiyun Y.S.[1], Musa S.A.[1], Gaya B.I.[2], Makena W.[3], Omoniyi A.A.[1], Ishaku B.[3]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria.

ABSTRACT

Background: Plants contain flavonoids, natural substances with varying phenolic structures. Flavonoids have gotten much press in the last ten years because they are now considered essential in a wide range of nutraceutical, pharmaceutical, and medicinal applications. As a result, the study aimed to see if flavonoid fractions of *Adansonia digitata* (L) fruits (FAD) could protect experimental rats from HgCl2-induced changes in brain sialic acid, dopamine, acetylcholinesterase, oxidative stress parameters, as well as brain histology.

Methods: The rats were split into six groups, each with five rats. For 28 days, all the rats were given their assigned treatment once a day. The rats in group I were given only distilled water (2 ml/kg). Group II was given $HgCl_2$ (0.5 mg/kg), while groups III-VI were given FAD (25 mg/kg, 50 mg/kg, and 75 mg/kg, respectively) and then challenged with $HgCl_2$ (0.5 mg/kg), and Group VI was given Ascorbic Acid (200 mg/kg) and $HgCl_2$ (0.5 mg/kg) 30 minutes later.

Results: When compared to the control group (group I), rats given $HgCl_2$ showed a decrease in SOD, CAT, AChE, DA and brain sialic acid, as well as an increase in MDA levels ($p < 0.001$), whereas FAD prevented the effect (decrease of SOD, CAT, and brain sialic acid).

Conclusion: Histological cytoarchitecture degeneration of the cerebral and cerebellar cortices was prevented by FAD and ascorbic acid. Degenerative changes in the cerebral and cerebellar cortex were caused by $HgCl_2$ exposure. FAD is a promising treatment option with neuroprotective properties.

Keywords: Mercury Chloride, Flavonoids, *Adansonia Digitata*, Cerebrum, Cerebellum

Corresponding Author:
Name: Wusa Makena
Address: Department of Human Anatomy,
Faculty of Basic Medical Sciences, College of Medicine,
University of Maiduguri, Maiduguri, Nigeria
Email: wusamakena@gmail.com
Contact: +2348068037900

INTRODUCTION

Mercury is one of the potential environmental xenobiotic toxins linked to long-term neurological and developmental disorders in both animals and humans (1,2). Mercury contamination of any form poses serious health and environmental risks (3). Mercury ions are taken up by and accumulate in many organs after exposure, including the brain, intestine, kidney, liver, and placenta (4). One of the most vulnerable organs to mercury toxicity is the central nervous system (5). Mercury exposure, both acute and chronic, has also been linked to several neurological and psychiatric disorders (5).

Mercury chloride ($HgCl_2$) destroys free radical scavenging systems like superoxide dismutase and catalase and increases reactive species levels, which disrupts the prooxidant-antioxidant balance system and causes oxidative stress (6-8). The brain has a high rate of oxidative metabolism, accounting for 20% of cardiac output. At the same time, in comparison to the lung, liver, and other organs, the brain has relatively low levels of enzymatic and non-enzymatic antioxidants and high levels of unsaturated lipids, making it more vulnerable to oxidative stress (5). Excessive production of free radicals in the brain and an imbalance between oxidative species and antioxidant defences have been linked to the pathogenesis of neurodegenerative diseases (9).

Despite drug development research, treatment for neurological disorders remains abysmal due to modern drugs' failure to regenerate central nervous tissues and their adverse side effects. Because oxidative stress is a common occurrence in metal toxicity, it is critical to comprehend the interaction between antioxidants and neurotoxicants like mercury chloride. The antioxidant activity of medicinal plants is primarily responsible for the benefits associated with antioxidant consumption (10).

Ethno-pharmacological studies revealed that the leaves of A. digitata have a variety of beneficial biological activities, including anti-diabetic, antioxidant, and neuroprotective activities (11,12). The quantitative estimation of A. digitata's phytochemical constituents revealed that the medicinal plant is high in total phenols and total flavonoids. Flavonoids are used to prevent and treat various diseases primarily caused by free radicals (13). It has been reported that compounds containing hydroxyls, such as flavonoids, are responsible for most plants' radical scavenging effects (13). Flavonoids work by scavenging or chelating free radicals (14). As a result, we hypothesized that A.digitata flavonoid fractionates administration could protect against mercury chloride-induced cortical neuronal degeneration by inhibiting oxidative stress. As a result, the current study aims to assess the protective flavonoid fractionate of *A. digitata* against $HgCl_2$-induced oxidative stress and histo-architectural degeneration of the adult Wistar rats' prefrontal cortex and cerebellum.

METHODS

Materials

Mercury chloride, (BDH Chemicals Ltd, England) was used as neuronal toxicant. Vitamin C (Ascorbic Acid; 70mg/tablet) produced by Micro Labs Limited with NAFDAC number A4-6634, was obtained from a reputable pharmaceutical store (M.U.B Pharmaceutical Enterprises Ltd.) in Sabon Gari, Zaria, Kaduna state, used as standard drug for Antioxidant. Ketamin (Sigma-Aldrich Co. LLC St. Louis, USA), Phosphate Bufffer Saline (PBS), 70% Ethanol (Sigma-Aldrich Co. LLC St. Louis, USA).

Cite this article as: Aribiyun Y.S., Musa S.A., Gaya B.I., Makena W., Omoniyi A.A., Ishaku B. ENeuroprotective Effects of Adansonia digitata L. Fruit Flavonoids on Mercury Chloride-Induced Neuronal Damage in a Rat Model. Nig. J. Basic & Applied Med. Sci. 2022;1(1);51-56.

Plants collection and Authentication

The fruit of *A.digitata* L. were purchased from Sabo market of Sabo Gari local Government of Kaduna State. Identification and authentication of the fruit were done by using standard botanical monographs. They were further confirmed by the Botany Department, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria. The specimen voucher number of the plant (2512).

Extraction

The fruit pulp of *Adansonia Digitata* L. was extracted with water in a soxhlet apparatus for 10 hours using the procedure recommended by the Association of Official Analytical Chemists (15). The dried fruit pulps were grounded into a powder form and then stored in an airtight container until needed. The crude extract of *A.digitata* was dissolved in n-hexane, then suspending the insoluble residue in distilled water and adding diethyl ether to it. The distilled water fraction was then segmented using n-butanol. The water portion was then thrown away. By treating the n-butanol fraction with 1% KOH and then separating it, the first n-butanol fraction was obtained (saponins). To obtain the second n-butanol fraction, a solution of HCl was added to the remaining 1 percent KOH portion, which was then partitioned with n-butanol until exhaustion (flavonoids) as described by Won et al. (16). The crude flavonoid fraction was used in this study as the final product. A total of 6 kg of *A. digitata* fruit was used in this experiment. A total of 1,020 mg of flavonoids was obtained.

Experimental animals

Thirty (30), apparently healthy Wistar rats of both sexes with weights ranging from 150 g to 220 g (six - seven weeks old) were purchased from Human Anatomy Animal House, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were kept in the Department of Human Anatomy animal house and maintained in standard laboratory condition at room temperature. The animals were fed on standard feed grower mash diet and water *ad libitum*. The animals were allowed to acclimatized for 2 weeks prior to commencement of the administration. The Committee on Animal Use and Care at Ahmadu Bello University in Zaria has given its approval to the current experimental protocols, which have the number ABUCAUC/2018/088. The experimental techniques strictly adhered to the "Guide to the Care and Use of Laboratory Animals in Research and Teaching," as detailed in NIH publications volume 25, No. 28, amended in 1996 (17).

Experimental procedure

The thirty Wistar rats were randomly assigned to six different groups, each with five rats. For 28 days, the control group was given distilled water via oral gavage. For 28 days, the $HgCl_2$ control group received intraperitoneal injections of 5mg/kg $HgCl_2$. The treated experimental groups received 0.5mg/kg $HgCl_2$ of intraperitoneally, thirty minutes (30 min) after receiving 25 mg/kg, 50 mg/kg, and 75 mg/kg FAD via oral gavage for 21 days. The final group received an intraperitoneal injection of $HgCl_2$ (0.5 mg/kg), followed by 200 mg/kg oral gavage for 29 days. The doses of mercury chloride were chosen based on previous research (18).

Morphological Studies

The experimental animals were weighed weekly, before and during the study. On day 29, the experimental animals were humanely sacrificed following ketamin anaesthesia (75mg/kg: ip) (19).

Biochemical Studies

Wistar rat brain tissues were collected and prepared using the methods described by Zatta *et al.* (20) and Habila *et al.* (21). Under anaesthesia, treated and control animals were decapitated, and brain tissue was immediately removed and placed on an inverted Petri dish on ice. Brain tissue was excised, washed in ice-cold saline, and then homogenized in Phosphate Buffer Saline (pH 7.4) using a Teflon homogenizer. Brain homogenate was then centrifuged at 5,000 x g for 15 minutes to remove cellular debris. Supernatant of the resulting brain homogenates were kept at -20^0C until use. Some biochemical parameters like the level of superoxide dismutase activity (SOD), catalase activity (CAT), malondialdehyde (MDA), Acetylcholinesterase, Total Sialic Acid, and, Dopamine was determined using the supernatants. The method described by Akanji et al. (22) was used to measure lipid peroxidation. The method described by Aebi (23) was used to determine CAT activity. Fridovich's (24) method for determining SOD activity. According to the manufacturer's instructions, the activities of brain sialic acid, acetylcholinesterase enzymes and Dopamine were measured using an enzyme-linked immunosorbent assay (ELISA) kit (ACON Labs, USA).

Histological Studies

The brain was dissected out, the prefrontal cortex and cerebellum were carefully excised, and the tissue was post-fixed in Neutral buffer formalin for 24 hours before processing for histological staining with H&E for demonstration of cortical histoarchitectural as well as histochemical staining with cresyl fast violet (CFV) stain for demonstration of nissil substances (25).

Statistical Analysis

The data obtained from these studies were analyzed with Statistical Package for Social Science (SPSS) software, version 20 (IBM, USA), using one-way Analysis of Variance (ANOVA) and Turkey's multiple comparison post hoc test, which compared the level of significance between the control and test groups. All data were expressed as mean ± SEM, and $p < 0.05$ was considered significant.

Table 1. Summary of Experimental Design

GROUPS	TREATMENT	DURATION
1	Distilled Water (2 ml/kg)	28 days
2	0.5 mg/kg of $HgCl_2$	28 days
3	25 mg/kg of FAD + 0.5 mg/kg of $HgCl_2$	28 days
4	50 mg/kg of FAD + 0.5 mg/kg of $HgCl_2$	28 days
5	75 mg/kg of FAD + 0.5 mg/kg of $HgCl_2$	28 days
6	200 mg/kg of Ascorbic Acid + 0.5 mg/kg of $HgCl_2$	28 days

RESULTS

Fig. 1 shows the effect of mercury chloride and FAD on the change in body weight and organ weight of the experimental animal. The result shows that there is a significant decrease ($p < 0.05$) in the change of body weight and organ weight of rats treated with only $HgCl_2$ when compared with the control and $HgCl_2$ + FAD (25, 50, and 75 mg/kg), and $HgCl_2$ + 200 mg/kg of AA experimental groups. The rats treated with 75mg/kg of FAD treated rats group showed no significant difference ($p > 0.05$) when compared with the control rats.

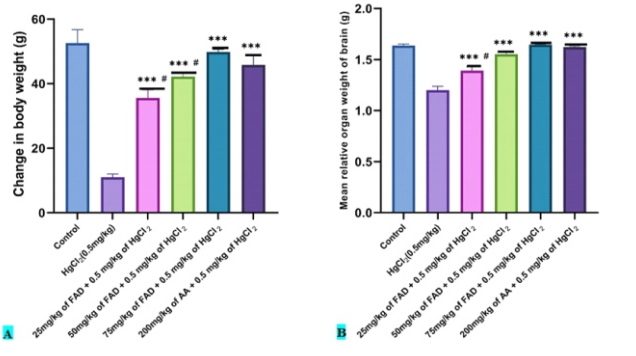


Figure 1: The study's (a) Change in body weight, (b) Organ weight data are shown in bar charts. *, indicates a significant difference when compared to Normal control ($p < 0.05$); #, indicates a significant difference when compared to $HgCl_2$ Control ($p < 0.05$). ($p < 0.05$).

Fig. 2 shows the effect of mercury chloride and FAD on the level of oxidative stress indicators (SOD, CAT, and MDA). The results of the study showed a marked and significant decrease ($p < 0.05$) in SOD and CAT biomarkers in $HgCl_2$ treated rats compared with the control group and rats that were given $HgCl_2$ + FAD (25, 50, and 75 mg/kg). For the MDA oxidative stress biomarkers, a significant increase ($p < 0.05$) was observed in $HgCl_2$-treated rats compared with the other experimental groups.

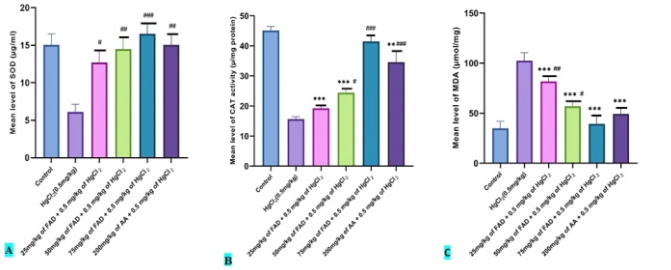


Figure 2: Bar charts of Oxidative stress parameters (a) SOD, (b) CAT, (c) MDA of the experimental study._*, indicates a significant difference when compared to Normal control (*$p < 0.05$); #, indicates a significant difference when compared to $HgCl_2$ Control ($p < 0.05$).

Fig. 3 shows the effect of mercury chloride and FAD on the level of acetylcholinesterase, brain sialic acid, and dopamine. The results of the study showed a marked significant decrease ($p < 0.05$) in acetylcholinesterase and total sialic acid in $HgCl_2$ treated rats compared with the control rats and rats treated with $HgCl_2$ + FAD (25, 50, and 75 mg/kg) respectively. For dopamine, a significant decrease ($p < 0.05$) was observed in $HgCl_2$ treated rats compared with the other experimental groups. There was no significant difference ($p > 0.05$) between $HgCl_2$ + 50 mg/kg, 75 mg/kg FAD, and 20 mg/kg AA-treated rats and control rats.

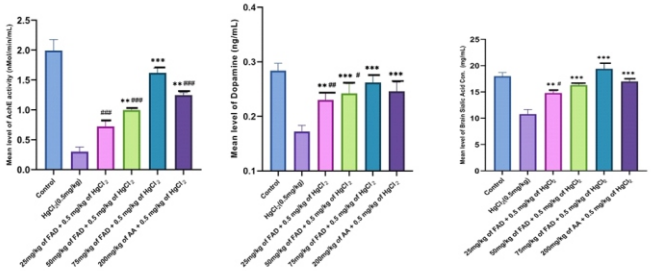


Figure 3: Bar charts of kidney function parameters (a) AchE, (b) Dopamine and (c) Brain Sialic acid of the study. *, indicates a significant difference when compared to Normal control ($p < 0.05$); #, indicates a significant difference when compared to $HgCl_2$ Control ($p < 0.05$). ($p < 0.05$).

Histological Evaluation

The results from histological observations showed histological changes in the tissues studied. The results of the histological changes showed that rats in the control group have normal histology of the cerebral cortex (Fig 4a), while rats treated with $HgCl_2$ showed extracellular and vacuolated nuclei with some irregularly shaped pyramidal cells lacking axons (Fig 4b). The rats treated with $HgCl_2$ + 25 mg/kg of FAD, respectively, showed mild crowded irregular pyramidal cells with some intracellular vacuoles and deeply stained neuroglial cells with vacuolated neurophils (Fig 4c). Rats treated with $HgCl_2$ + 50 mg/kg and 75 mg/kg of FAD showed near normal pyramidal cells, granule cells, and some vacuolated neutrophils (Fig 4d & e). Rats treated with $HgCl_2$ + 200 mg/kg of AA showed near-normal irregular pyramidal cells, normal granule cells, and some open neuroglia cells (Fig 4f). The cerebellum control rats showed the cytoarchitecture of the molecular, granular, and purkinje cell layers appearing normal (Fig. 6a). Rats treated with just $HgCl_2$ showed a normal molecular layer, with degeneration of the Purkinje cell layer and purkinje cells, while the granular layer showed abnormal crowded granular cells (Fig 6b). Rats treated with FAD show normal molecular and granular layers, with mild degeneration of the purkinje cells within the purkinje cell layer and normal orientation of the molecular layer (Fig 6c, d, e, and f).

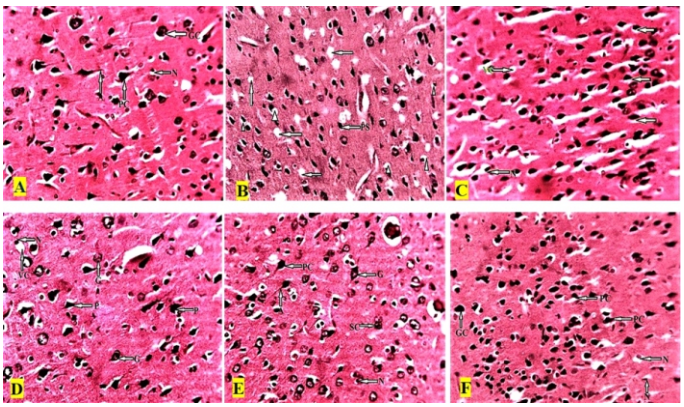


Figure 4: Representative photomicrographs of brain sections of rats (Cerebral cortices) stained by H&E. Control showing normal architecture with pyramidal cell (PC), Stellate Cells (SC), with pale open face nuclei of neuroglia (N) **A**, Extracellular (arrow) and Vacuolated nuclei (arrow head) with some irregular shaped pyramidal cells (PS) in **B,** crowded irregular pyramidal cells with some intracellular vacuoles (arrow) with deeply stained neuroglial cells (N) vacuolated neurophils (VC) in **C,** normal pyramidal cells (P), Granules cell (G), and some vacuolated neuropils (VC) **in D & E,** and showing normal crowded irregular pyramidal cells (PC), normal granules Cells (GC) and some open neuroglia cells (N) in **F**. H&E X250. A= normal control, B= $HgCl_2$ control, C= 5 mg/kg $HgCl_2$ + 25mg/kg FAD, D= 50 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, E=75 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, F=200mg/kg Ascorbic Acid + 0.5 mg/kg of $HgCl_2$.

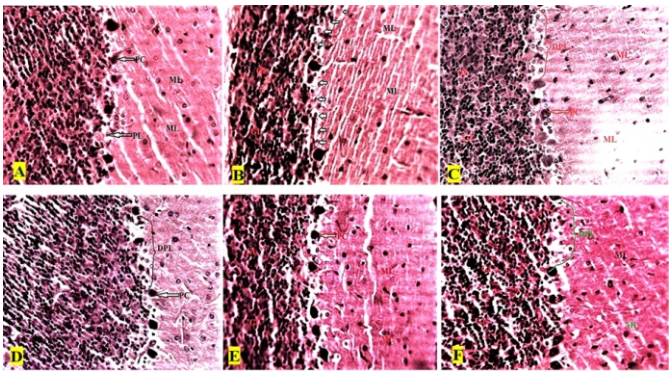


Figure 5: Representative photomicrographs of brain sections of rats (Cerebellar cortices) stained by H&E. Control showing normal orientation of the Molecular layer (ML), Granular layer (GL), and Purkinje layer (PL) with normal Purkinje cells (PC) in **A**, showing densely populated Granular layer (GL), normal Molecular Layer and degeneration of purkinje cells (arrow) in **B,** showing densely packed Granular Layer (GL), slight degenerated purkinje cells (DPL) and normal Molecular layer (ML) in **C,** showing normal orientation of the Molecular layer (ML), Granular layer (GL), and Purkinje layer (PL) with normal Purkinje cells (PC) in **D & E,** and showing densely populated granular layer (GL), normal molecular layer (ML) and slight degeneration of the purkinje layer and cells (DPL) in **F**. H&E X250. A= normal control, B= $HgCl_2$ control, C= 5 mg/kg $HgCl_2$ + 25mg/kg FAD, D= 50 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, E=75 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, F=200mg/kg Ascorbic Acid +0.5 mg/kg of $HgCl_2$.

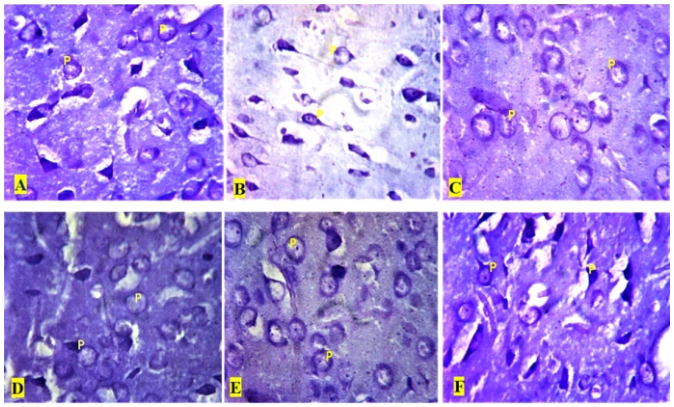


Figure 6: Representative photomicrographs of brain sections of rats (Cerebral cortices) stained by cresyl violet, X250. Control showing normal Nissl substance around the cytoplasm of the neurons in **A,** indicates degenerated neurons without nissl substances in **B,** close to normal neuronal cells with intact nissl substances in **C, D, E and F.** A= normal control, B= $HgCl_2$ control, C= 5 mg/kg $HgCl_2$ + 25mg/kg FAD, D= 50 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, E=75 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, F=200mg/kg Ascorbic Acid +0.5 mg/kg of $HgCl_2$.

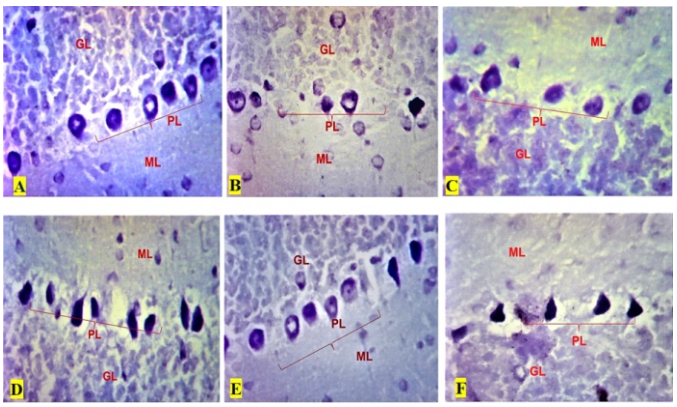


Figure 7: Representative photomicrographs of brain sections of rats (Cerebellar cortices) stained by cresyl violet, X250. Control showing normal Nissl substance around the cytoplasm of the neurons in the three layers of the cerebellum in **A,** indicates degenerated neurons without nissl substances in **B,** close to normal neuronal cells with intact nissl substances in **C, D, E and F.** A= normal control, B= $HgCl_2$ control, C= 5 mg/kg $HgCl_2$ + 25mg/kg FAD, D= 50 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, E=75 mg/kg FAD + 0.5 mg/kg of $HgCl_2$, F=200mg/kg Ascorbic Acid +0.5 mg/kg of $HgCl_2$.

DISCUSSION

The body weight and organ (brain) weight ratio were negatively affected in rats treated with $HgCl_2$, with significant rat body/brain weight loss observed. This work is consistent with the findings of Mariana et al. (26), who found that rats treated with mercury chloride had a lower body weight. The FAD could mitigate the side effect, which could be attributed to the flavonoid's ability to prevent the accumulation of metabolites in tissues that could cause oxidative stress (27). The $HgCl_2$ treated rats showed a significant decrease in the endogenous antioxidant biomarkers SOD and CAT and an increase in MDA in this experiment, indicating the accumulation of high metabolites by the mercury chloride to induce oxidative stress which is consistent with the work done by Agarwal et al. (7). Numerous studies have confirmed that the mechanism of $HgCl_2$-induced neurotoxicity is related to inducing oxidative stress by either overproducing ROS or lowering the antioxidant defence system (28,29). Oxidative damage results in ROS production, leading to peroxidation of cell membrane lipids, disrupting the integrity and function of the cell membrane and causation of neurotoxicity (30). It was discovered that co-administering $HgCl_2$ + flavonoid significantly increased SOD and CAT and decreased MDA in rats. The high dose of flavonoid was more effective in ameliorating the accumulation of metabolites caused by mercury chloride, thereby inhibiting the induction of oxidative stress. This supported the findings of Rice-Evans et al. (31), who discovered that flavonoid compounds' beneficial effects are due to their antioxidant properties.

Brain sialic acid was also measured in brain tissue, and it was calculated as a total sialic acid in all groups. Rats treated with only $HgCl_2$ had a significant decrease in total sialic acid. This could impact learning and memory because brain sialic acid has been linked to learning and memory, among other functions in the human body and other animals (32). Mercury chloride acts on cannabinoid (CB1) receptors in the hippocampus and other areas of the brain, causing a decrease in brain sialic acid concentration and, as a result, impairment in learning and memory (33). Flavonoid and ascorbic acid, on the other hand, may alleviate such side effects, as a progressive increase in total sialic acid was observed in flavonoid-treated rats. Several studies have found that the flavonoid has antioxidant properties and also prevents total sialic acid depletion against $HgCl_2$ (34).

Long-term $HgCl_2$ exposure induces neurotoxicity (35), which affects neurotransmitter production and release (36). In this recent work, $HgCl_2$ appears to lower dopamine and AChE activity. Previous research has shown that $HgCl_2$ exposure lowers neurotransmitter activity such as DA and AChE (37, 38). Previous studies in rats and humans have discovered a clear relationship between age-related alterations in AChE, DA, and increased oxidative stress. Free radicals and increased oxidative stress reduce AChE activity (38, 39). Increased lipid peroxidation has also been linked to a decrease in AChE and DA (40). However, FAD administration prevented the toxic and detrimental effect of $HgCl_2$ on AChE and DA activity. **FAD** promotes AChE activity even with the $HgCl_2$ exposure, which could be due to its ability to quench Hg-induced ROS or the reserve of the Hg-AChE interaction. Previous research has found that flavonoids act as inhibitors of acetylcholinesterase (AChE) (41). The current study backs up Jung and Kim's (42) findings that flavonoids regulate various meaningful physiological responses that contribute to neuroprotective effects. These responses resulted in less dopamine neuron loss and dopamine depletion. Flavonoids with their metabolites cross the blood-brain barrier in areas critical for learning and memory, such as the cerebral cortex, cerebellum, hippocampus, and striatum (43).

The findings from the oxidative stress markers were supported

further by histological observations. The findings revealed some degenerative changes in the cerebellum and cerebral cortices in rats treated with only mercury chloride, including some extracellular vacuolation nuclei and irregular pyramidal cell axons in the brain area of the cerebral cortices, a densely populated granular layer, and Purkinje cell degeneration in the cerebellar cortex. The Nissil profiling showed increased chromatolysis changes and a massive reduction in cortical cellular density and the Purkinje layer of the cerebellum. This reflects pathological conditions caused by massive neuronal loss, a mediator of neurodegenerative processes (44). According to the current study, flavonoids protect against the adverse side effects of mercury, which could be due to its high antioxidant activity (45). Flavonoid was able to mediate various actions, including protecting vulnerable neurons, improving neuronal function, and stimulating regeneration, as previously supported by research (46), through interaction with neuronal intracellular signalling pathways involved in neuronal survival and differentiation.

CONCLUSION

FAD at 75 mg/kg was demonstrated to reduce $HgCl_2$-induced brain damage by reducing neuronal cell membrane depolarization, hence maintaining extracellular endogenous antioxidant levels and chelating $HgCl_2$. These enhance neurotransmission, boosting memory and reducing neurodegeneration. As a result, FAD could be used to prevent neurodegenerative disorders.

Abbreviation

AChE: Acetylcholinesterase
AOAC: Association of Official Analytical Chemists
CAT: Catalase
DA: Dopamine
ELISA: enzyme-linked immunosorbent assay
FAD: flavonoid fractions of Adansonia digitata
$HgCl_2$: Mercucy chloride
MDA: Malondialdehyde
NAFDAC: National Agency for Food and Drug Administration and Control
PBS: Phosphate Bufffer Saline
SOD: Superoxide Dismutase

Acknowledgement

Mr Jigo Yaro, Chief Medical Laboratory Scientist, Department of Pathology, Ahmadu Bello University Teaching Hospital, and Mr Ayegbusi Olu, Department of Chemical Pathology, deserve special recognition for taking the time to run the biochemical assay and process the tissues.

Authors Contribution

All authors contributed to the concept and design. Study materials are provided by YSA, SAM, WM, BIG; Data collection: YSA, WM, AAO, & BI. Data interpretation and analysis: All authors; The first draft of the manuscript: WM, YSA, BIG, SAM & BI Manuscript review: AAO, WM, & SAM. The manuscript's final approval: All authors.

Funding

The authors state that they did not receive any funding for this project.

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Consent to participate was obtained from Ahmadu Bello University, Zaria, Committee on Animal Use and Care has given its approval to this animal-related experimental protocols, with the number ABUCAUC/2018/088. The experimental techniques strictly adhered to the "Guide to the Care and Use of Laboratory Animals in Research and Teaching," as detailed in NIH publications volume 25, No. 28, amended in 1996. (National Research Council, 2011).

Consent for publication: Not applicable.

Conflict of Interests: The authors declare that no competing interests exist in the publication.

REFERENCES

1. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Critical Reviews in Toxicology 2006;36: 609–62.
2. Liu W, Xu Z, Deng Y, Xu B, Wei Y, Yang T. Protective effects of memantine against methylmercury-induced glutamate dyshomeostasis and oxidative stress in rat cerebral cortex. Neurotoxicity Research 2013; 24: 320–37.
3. Kim KH, Kabir E, Jahan SA. "A review on the distribution of Hg in the environment and its human health impacts," Journal of hazardous materials 2016; 306, pp. 376– 385.
4. Bridges CC, Zalups RK. "Mechanisms involved in the transport of mercuric ions in target tissues," Archives of Toxicology 2017**;** 91, no. 1, pp. 63–81.
5. Abdel-Moneim, AE. The neuroprotective effect of berberine in mercury-induced neurotoxicity in rats. Metabolic Brain Disease 2015; 015-9652-6.
6. Mahboob M, Shireen KF, Atkinson A, Khan AT. "Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury," Journal of Environmental Science and Health A 2001**;** 36, no. 5, pp. 687–697.
7. Agarwal R, Goel SK, Chandra R, Behari JR. "Role of vitamin E in preventing acute mercury toxicity in rat," Environmental Toxicology and Pharmacology 2010; 29, no. 1, pp. 70–78.
8. Nabil A, Elshemy MM, Asem M, Gomaa HF. Protective Effect of DPPD on Mercury Chloride-Induced Hepatorenal Toxicity in Rats. **Journal** of **Toxicology 2020;** Article ID 4127284, 7 pages. http://dx.doi.org/10.1155/2020/4127284
9. Abdel Moneim AE. Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress and histopathological alterations in brain of female rats. Biological trace element research 2012; 150:328– 336. doi:10.1007/s12011-012-9498-2S.
10. Ayyathan DM, Chandrasekaran R, Thiagarajan K. Neuroprotective effect of Tagara, an Ayurvedic drug against methyl mercury induced oxidative stress using rat brain mitochondrial fractions. BMC Complementary and Alternative Medicine 2015; 15:268. DOI 10.1186/s12906-015-0793-2.
11. Ebaid H, Bashandy SAE, Alhazza IM, Hassan I, Al-Tamimi J. Efficacy of a methanolic extract of Adansonia digitata leaf in alleviating hyperglycemia, hyperlipidemia, and oxidative stress of diabetic rats. BioMed Research International 2019; 2835152.
12. Atuadu VO, Anibeze CIP, Finbarrs-Bello E, Esom EA, Okoh D. Protective role of Adansonia digitata on lead-induced neurotoxicity in the hippocampus of adult Wistar rats. (conference preceedings) IBRO Report. The 14th Society of Neuroscientists in Africa Conference 2019;7:S19.
13. Langenheim JH. Higher plant terpenoids: A phytocentric overview of theirecological roles. Journal of Chemical Ecology 1994; 20(6): 1223-1280.
14. Kamatou GPP, Vermaak I, Viljoen AM. An updated review of Adansonia digitata: A commercially important African tree. South African Journal of Botany 2011**;** 77; 908–919.
15. Association of Official Analytical Chemists (AOAC) Official methods of analysis XI Edition: Association of official analytical chemists. *11th* ed. Washington D.C: The Association; 1970.
16. Won WS, Shin KH, Kang SS. Chemistry and Pharmacology of Flavone –C-Glycoside from *zizyphus* seeds. Korean Journal of Pharmacognosy 1980;11(3–4):141–148.
17. National Research Council. National Institutes of Health Guide for the Care and Use of Laboratory Animals 2011. https://doi.org/10.17226/12910
18. Salman MM, Kotb AM, Haridy MA, Hammad S. Hepato- and nephroprotective effects of bradykinin potentiating factor from scorpion

(Buthus occitanus) venom on mercuric chloride-treated rats. EXCLI journal 2016; 14;15:807-816. doi: 10.17179/excli2016-777. PMID: 28337111; PMCID: PMC5318677.
19. Wellington D, Mikaelian I, Singer L. Comparison of ketamine-xylazine and ketamine-dexmedetomidine anesthesia and intraperitoneal tolerance in rats. J Am Assoc Lab Anim Sci. 2013 Jul;52(4):481-7. PMID: 23849447; PMCID: PMC3725934.
20. Zatta PM, Ibn-Lkhayat-Idrissi P, Zambenedetti MK, Kiss T. In vivo and invitro effects of aluminum on the activity of mouse brain acetylcholinesterase. Brain Research Bulletin 2002; (59) 1: 41–45.
21. Habila N, Inuwa HM, Aimola IA, Lasisi OI, Muhammad A, Okafor AI, Williams IS. Acetylcholinesterase Activity in the Brain and Blood of Mice Infected with Najanigricolis Venom. Biological Segment 2012; 3(1) BS/1565.
22. Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F. Psidium guajava extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI Journal 2009; 8: 148–154.
23. Aebi H. Catalase in vitro. Methods of Enzymology 1984; 105: 121–126. doi:10.1016/S0076-6879(84)05016-3. PMID:6727660.
24. Fridovich I. Superoxide dismutases. An adaptation to a paramagnetic gas. Journal of Biological Chemistry 1989; 264: 7761–7764. doi:10.1016/S0021-9258(18)83102-7. PMID:2542241.
25. Romero-Sarmiento Y, Soto-Rodríguez I, Arzaba-Villalba A, García HS, Alexander-Aguilera A. Effects of conjugated linoleic acid on oxidative stress in rats with sucrose-induced non-alcoholic fatty liver disease. Journal of Functional Foods 2012; 4:450–8.
26. Mariana M, Taíse F, Pedros C, Oliveira, Vitor AO, Rafael F, Santos, Cezar AB. Safety data for Mercuric Chloride Physical and Chemical Properties. Janssen Pharmaceutical 2016; 3a: 2440 Geel, Belgium.
27. Rendeiro C, Rhodes JS, Spencer, JP. The mechanisms of action of flavonoids in the brain: direct versus indirect effects, Neurochemistry International 2015; doi: 10.1016/ j.neuint.2015.08.002.
28. Franco JL, Posser T, Missau F, Pizzolatti MG, AR. Dos Santos, Souza DO, Aschner M, Rocha JB, Dafre AL, Farina M. Structure-activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. Environmental Toxicology and Pharmacology 2010; 1;30(3):272-278. doi: 10.1016/j.etap.2010.07.003. PMID: 21127717; PMCID: PMC2992974.
29. Othman MS, Aboulkhair SG, Abdel M, Moneim AE. The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. Food and Chemical Toxicology 2014; 69C:175–181. doi:10.1016/j.fct.2014.04.012.
30. Yousef AO, Fahad A, Abdel Moneim AE, Metwally DM, El-Khadragy MF, Kassab RB. The Neuroprotective Role of Coenzyme Q10 Against Lead Acetate-Induced Neurotoxicity Is Mediated by Antioxidant, Anti-Inflammatory and Anti-Apoptotic Activities. International Journal of Environmental Research and Public Health 2019; 13;16(16):2895. doi: 10.3390/ijerph16162895. PMID: 31412628; PMCID: PMC6720293.
31. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 1996; 20: 933–56.
32. Markram K, Gerardy-schahn and Sandi. Selective Learning and Memory Impairments in mice Deficient for Polysialylated Ncam in Adulthood. Neuroscience 2007; 144: 788–796.
33. Dityatev A, Dityateva G, Sytnyk V, Delling M, Toni N, Nikonenko I, Schachner M. Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. Journal of Neuroscience Research 2004; (42)24: 9372-9382.
34. Yadava J, Vermaa AK, Gargb RK, Shiulia KA, Mahdic AA, Srivastava S. Sialic acid associated with oxidative stress and total antioxidant capacity (TAC) expression level as a predictive indicator in moderate to severe Alzheimer's disease. Experimental gerontology 2020;141:111092.
35. Aragão WAB, Teixeira FB, Fagundes NCF, Fernandes RM, Fernandes LMP, da Silva MCF. 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; doi: 10.1155/2018/7878050.
36. Weis J, Smith G, Zhou T, Bass C, Weis P. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. Bioscience 2009; 51, 209-217. http://dx.doi.org/10.1641/0006-
37. Franciscato C, Goulart FR, Lovatto NM, Duarte FA, Flores EM, Dressler VL, $ZnCl_2$ exposure protects against behavioral and acetylcholinesterase changes induced by $HgCl_2$. International Journal of Developmental Neuroscience 2009; 27(5), 459-468.http://dx.doi.org/10.1016/j.ijdevneu.2009.05.002
38. Abu-Taweel GM. Neurobehavioral protective properties of curcumin against the mercury chloride treated mice offspring. Saudi journal of biological sciences 2019; 26(4), 736-743.
39. Molochkina EM, Zorina OM, Fatkullina LD, Goloschapov AN, Burlakova EB. H_2O_2 modifies membrane structure and activity of acetylcholine esterase. Chemico-Biological Interactions 2005; 157:401–404.
40. Jha R, Rizvi SI. Age-dependent decline in erythrocyte acetylcholine esterase activity: correlation with oxidative stress. Biomed Pap Med Fac Univ Palacky Olomouc Czech Rpub 2009; 153:195–198.
41. Uriarte-Pueyo, Calvo MI. Flavonoids as Acetylcholinesterase Inhibitors. Current Medicinal Chemistry 2011;18, 5289-5302.
42. Jung UJ, Kim SR. Beneficial Effects of Flavonoids Against Parkinson's Disease. Journal of Medicinal Food 2018; 00 (0) 2018, 1–12.
43. Passamonti, S, Vrhovsek U, Vanzo A, Mattivi F. Fast access of some grape pigments to the brain. J Agric Food Chem 2005; 53: 7029–34. DOI: 10.1089/jmf.2017.4078
44. Feng C, Liu S, Zhou F, Gao Y, Li Y, Du G, Chen Y, Jiao H, Feng J, Zhang Y, Bo D, Li Z, Fan G. Oxidative stress in the neurodegenerative brain following lifetime exposure to lead in rats: Changes in lifespan profiles. Toxicology 2019; 1;411:101-109. doi: 10.1016/j.tox.2018.11.003.
45. Tembo DT, Holmes MJ, Marshall LJ. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. Journal of Food Composition and Analysis 2017; 58:40–51.http://dx.doi.org/10.1016/j.jfca.2017.01.002
46. Sabogal-Guáqueta AM, Muñoz-Manco JI, Ramírez-Pineda JR, LampreaRodriguez M, Osorio E, Cardona-Gómez GP. The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer´s disease model mice, Neuropharmacology 2015; doi: 10.1016/j.neuropharm.2015.01.027.