

AMELIORATIVE EFFECTS OF AQUEOUS EXTRACT OF NEEM(AZADIRACHTA INDICA) LEAF AND QUERCETINE IN MERCURY CHLORIDE INDUCED NEUROTOXICITY ON MEDIAL GENICULATE BODY IN ADULT WISTAR RAT**Fakunle Ponle Bamidele¹, Ehiremen Samuel Ehimare^{2*}, Omotosho Dhulqarnain Akanji², Taiwo-Ola Dorcas Olubunmi², Emmanuel Chinomso Jonathan³ and Adeniji Adebisola Ayomiposi²**¹Department of Anatomy, Ladoke Akintola University of Technology, Ogbomosho Oyo State.²Department of Anatomy, Olabisi Onabanjo University Ago-Iwoye, Ogun State.³Department of Histopathology/Morbid Anatomy, Olabisi Onabanjo University Teaching Hospital Sagamu, Ogun State.***Corresponding Author: Ehiremen Samuel Ehimare**

Department of Anatomy, Olabisi Onabanjo University Ago-Iwoye, Ogun State.

Article Received on 28/01/2024

Article Revised on 18/02/2024

Article Accepted on 10/03/2024

ABSTRACT

Introduction: Heavy metals such as mercury accumulate in air, water, and land due to increased industrial activities, mercury chloride (HgCl₂) exposure resulted in neurotoxicity and cognitive impairment. Herbal traditional medicine, medicinal plants have been employed to combat the toxicity of heavy metals and treating of many diseases in traditional medicine. **Aim:** To examine the ameliorative effect combine therapy of aqueous extract of *Azadirachta indica* leaf and quercetin in mercury chloride induced neurotoxicity in medial geniculate body in adult wistar rat. **Methodology:** After a period of 14 days of acclimatization, 25 healthy male wistar rats weighing 150±50g were randomly allocated to five groups, group1(distilled water only), group2 (2mg/kg mercury chloride only PO), group3 (2mg/kg mercury chloride + 200mg/kg extract of *A.indica* PO), group4(2mg/kg mercury chloride+ 200mg/kg extract of *A.indica* PO) and group 5 (2mg/kg + 15mg/kg) The rats were subjected to mercuric chloride, aqueous seed extract of *Azadirachta indica* and Quercetin for fourteen (14)consecutive days. **Result:** The impact of the extract on the median geniculate body weight, full blood count as well as the histology of the media geniculate body were scrutinized. The data revealed that the aqueous seed extract of *Azadirachta indica* and Quercetine in a dose-dependent manner, there was no significance difference in the level of Haematocrit in all the animal groups (P>0.05.) The relative weight of the medial geniculate body exhibited a significant decrease in the treated groups compared to the mercury chloride-only group. The histology of the cerebellum delineated pathological changes arising from the exposure to mercury chloride, while *Azadirachta indica* and Quercetin brought about regenerative changes. **Conclusion:** In summary, the study posits that owing to the presence of phytochemicals in *Azadirachta indica* and Quercetin, it was effective in ameliorating the neurotoxicity induced by mercury chloride in the medial geniculate body of the rats.

KEYWORDS: Neurotoxicity, *Azadirachta indica*, Quercetin, Medial Geniculate Body and Mercury chloride.**INTRODUCTION**

Heavy metals such as mercury, lead, and nickel accumulate in air, water, and land due to increased industries resulting from development.^[1] Mercury (Hg) exposure has increased globally due to industrial activities, gold mining, medicinal uses, and fossil fuel combustion.^[2] Occupational mercury chloride (HgCl₂) exposure resulted in neurotoxicity, including cognitive impairment, motor dysfunction, central auditory system dysfunction, nephrotoxicity, hepatotoxicity, and mortality.^[3] It primarily damage brain tissue, causing focal brain damage that can affect the function of brain parts like the cerebellum, hippocampus, and visual cortex and cause cognitive and behavioral changes.^[4]

Many investigations have shown that mercury causes toxicity by producing reactive oxygen species (ROS) and rapidly depleting beneficial antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSP). ROS promotes mitochondrial malfunction, neuroinflammation, and apoptosis, all of which contribute to neurodegenerative diseases and neuronal cell death.^[5] Mercury accumulation in brain tissue increases the expression and reactivity of N-methyl-D-aspartate (NMDA) receptors. Activation of NMDA type glutamate receptors enhances Ca²⁺ entry into neurons, which motivates neuronal cell death pathways. Furthermore, Ca²⁺ promotes ROS production via the mitochondrial pathway.^[6]

Visual and auditory hallucinations, perceptions that occur in the absence of corresponding sensory stimuli, can arise from a wide range of drug-induced, pollution or medical and psychiatric conditions, as well as in the general population.^[7] It has been proposed that hallucinations appear because of interference with the visual or auditory pathways, followed by defective information processing.^[8] The thalamic lateral geniculate body (LGB) is the main relay center in the visual pathway, and the nearby medial geniculate body (MGB) in the auditory pathway. These geniculate nuclei are therefore candidate sites for damage that could result in over activity of the primary visual and auditory cortices. Attempts to find histopathological changes in the MGB in conditions where hallucinations are common, such as schizophrenia, have so far been unsuccessful.^[9]

Damage to MGB neurons has been described in a mercury-exposed dog^[10], and bismuth has been seen to accumulate in the MGB of mice.^[11] In rats exposed to various forms of mercury, mercury was visible in the LGN and MGN, though usually only at higher levels of exposure.^[12]

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 HgCl₂. The antioxidant activity of medicinal plants is primarily responsible for the benefits associated with antioxidant consumption.^[13]

Azadirachta indica plant is a native of India, where it is known as divine tree; "life giving tree". It belongs to maliceae family. Away from India, it is commonly found in Africa and America. It occurs naturally in tropical region and sub-tropical zones. However, it can still be planted or cultivated. *Azadirachta indica* tree is an incredible therapeutic plant^[14] that has been declared the tree of the 21st century by the United Nations.^[15] The plant kingdom represents a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as a lead for the development of novel agents having good efficacy in various pathological disorders in the coming years.

Azadirachta indica plant is considered to be the richest sources of drugs for traditional medicine, modern medicine, nutraceuticals, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs.^[16] Some of the phytochemicals contained in *Azadirachta indica* plant have been isolated, quantified and identified through Intensive studies. These bioactive chemicals have provided leads in the development of several lifesaving drugs, which are in use today.^[17] Extract from *Azadirachta indica*, which is referred to as dogonyaro in some parts of Nigeria are mostly recommended in ancient medical

texts. The leaves can be used as drug for diabetes, eczema and fever.^[17]

Quercetin (Que; 3, 39, 49, 5, 7-pentahydroxyflavone) is one of the most omnipresent flavonoids (Mao *et al.* 2018). Quercetin is highly concentrated in apples, onion, potatoes, peanuts, soybeans, red wine, and other fruits and vegetables. Owing to its chemical structure, Quercetin has strong anti-oxidative and cytoprotective effects on oxidant-induced endothelial cell apoptosis. Additionally, Quercetin prevents oxidative damage and cell death by preventing lipid peroxidation and scavenging oxygen free radicals. Quercetin may cross the blood-brain barrier and exerts neuroprotective effect in many brain injury model. Quercetin has also been shown to protect germ cells from Cd-induced toxicity. It also has anti-inflammatory, antitumor, antiviral, and liver- and cardiovascular-protective effects.^[18]

MATERIALS AND METHODS

Ethical concerns in animal study

All animal experiments and protocols adhered to the guidelines and regulations set forth by the National Research Council in regards to laboratory animal care and utilization (2011). Following the conclusion of experiments, animal carcasses were buried, no less than two feet beneath the natural surface, and covered with lime, disinfectant, and soil.

Ethical Approval: Ethical approval was obtained from the Anatomical Sciences Research Ethics Committee of Olabisi Onabanjo University Sagamu Campus, Ogun State Nigeria.

Animal Housing

In carrying out this study, Olabisi Onabanjo University animal house was used. The animal house is located in a serene and very quiet environment to avoid noise pollution for the animal and to achieve expected and good result. It is free from predators, harsh weather condition, flood and other environmental features that are toxic to the animals. And there is high availability of water (borehole water) for washing and feeding of animal.

Conclusion of Cages

Plastic cages were bought from SABO Market, Sagamu Ogun State. These cages were modified to suit the need of the rats. This was done by perforating them using a hot knife for easy ventilation. A four corner shaped cuttings were made on the cover and on each side of the cage and replaced with iron net and binding wire with the use of plier to allow in fresh air. It took 2 days maximum to get this done because of the much cage to be done.

Feeding and Water Trough Procurement

Plastic plates were used as water and feeding trough. These were bought from market, Sagamu Ogun State. The sizes were not too big in order to make it easy for

the rats to feed and drink and to avoid drowning of the rats and also to avoid easy movement of the rats in the cages.

There feeding were done 2 times in a day for the first 1 month and later reduced to once in a day till sacrifice is done.

Flooring of the Cage

The cages were floored with saw dust which was changed every 2 days with adequate monitoring for good hygiene and wellbeing of the rats. Two different types of saw dust were gotten (smooth and rough). Initially, these two types of saw dusts were used simultaneously. It was later discovered that the rats were more comfortable with the smooth saw dust when compared to the rough one but the rough has one great advantage, it helped the rats for covering as warmth when there probably cold and this smooth saw dust is very good when carrying out the neurobehavioral test (buried test). This discovery came to be when I noticed that the water trough was always filled with the rough saw dust thereby contaminating the water. This led to the use of the smooth saw dust throughout the course of this research.

Rat Procurement

Twenty-five^[25] male wistar rats within the weight range of 150-200g were gotten from a certified institute. The animals were kept in and housed in a well-ventilated animal house and were given food and water ad-libitum.

Feed Procurement

The rats were fed with finisher mash which was gotten from a reliable feed mill (Joyful feeds). This feedmill is located at Isale-Okò in Sagamu, Ogun state, Nigeria. The feedmill is well known for its quality and its cheap price and great quantity and also very close to the research project building (Animal house).

Collection of *Azadirachta indica*

Phase one: The leaves of the plant (*Azadirachta indica*) were obtained within sagamu Local government, Sagamu, Ogun state, Nigeria. The leaves were identified and authenticated at the Department of Pharmacology, Olabisi Onabanjo University Teaching Hospital. The leaves were thoroughly washed with distilled water and dried in the laboratory under the shed to avoid loss of phytochemicals.

The dried leaves were crushed using laboratory mortar and pestle and then ground to powder using a laboratory grinder. 500g of the powdered sample was dissolved in 2000ml of distilled water and allowed to stay for 24 hours with periodic stirring. The sample was filtered using Whatman number 1 filter paper, the filtrate was then concentrated in a water bath at 400 C for 5 days. The crude slurry was placed in the oven at 400 C, complete drying took two days.

Procurement of Quercetin

Quercetin was bought from a known pharmacy store in sagamu, Ogun state, Nigeria.

Induction of Toxicity

First induction of mercury chloride was given to the mercury groups, after 24hours, the second inducement was given to the mercury groups at a given dose of 2mg/kg.

Treatment with *Azadirachta indica* Quercetin

Twenty-four (24) hours after toxicity inducement, treatment with both *Azadirachta indica* and Quercetin commences and span for 14 days after which the animals were sacrificed.

The grouping and extract administration was administered as follows

Group 1: was administered with water (control)

Group 2: was administered mercury chloride 2mg/kg only

Group 3: was administered mercury chloride 2mg/kg + 200mg/kg of *Azadirachta indica*

Group 4: was administered mercury chloride 2mg/kg + 200mg/kg of *Azadirachta indica* + 15mg/kg Quercetin

Group 5: was administered mercury chloride 2mg/kg + 15mg/kg Quercetin.

Preparation of Tissues for Histological Examination;

The median geniculate body tissues were duly prepared and subjected to histological techniques at the Histological Laboratory of the Department of Anatomy, Olabisi Onabanjo University's Sagamu Campus.

- **Fixation;** The tissues were immersed in a formal saline solution comprising of 0.85 parts of NaCl, 90 ml of water, and 10 ml of formaldehyde for a duration of approximately 24 hours, following which the process of dehydration was initiated.
- **Dehydration;** The tissues were dehydrated in the following solution at different stages; 60% alcohol, 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol, first absolute alcohol and finally second absolute alcohol all at one (1) hour interval each
- **Clearing;** Clearing was done by using xylene (a hydrophilic clearing agent) to remove the alcohol from the cerebellar tissue, which was changed at one hour interval in first xylene initially and finally, second xylene.
- **Infiltration;** The tissues were infiltrated with paraffin wax at a temperature between the ranges of 50-60C for an hour. The tissues were then embedded in a paraffin wax with proper orientation.
- **Embedding;** The tissues were then embedded in paraffin wax with proper orientation. The embedding took place in a LUKAT embedding mild coated with glycerol. The paraffin wax was allowed to solidify forming a visible scum before cooling at a temperature of about 10-15C.
- **Sectioning;** The embedded tissue was affixed onto the microtome, which was adjusted to a thickness of

three(3) micrometers. To mitigate the generation of heat, ice blocks were employed during the sectioning process.

H&E Staining Technique for Paraffin Sections

- Removal of wax
- Hydration with graded alcohol (100% to 50%)
- Staining with hematoxylin
- Differentiation using 1% acid alcohol
- Blueing
- Counterstain with eosin
- Dehydration through graded alcohol (50% to 100%)
- Clearing in Xylene
- Mounting under a coverslip using DPS
- Allow it to dry

Preparation of Normal Saline

Normal saline is a mixture of sodium chloride in water and has a number of uses in medicine. 9g of Sodium Chloride (NaCl) to 1000ml of water was used.

Preparation of Formal Saline

40% formaldehyde: 100ml
Sodium chloride: 9g
Distilled water: 900ml

Graph Pad

Graph pad prism 9 was used to plot the graphs. The TURKEY test and one-way ANOVA were used for this research work.

Photomicrograph

Image acquisition and analysis: A bright light microscope (10 - 40x magnification objective) used. Digital camera - OMAX Toupe view 3.7 attached to P.C - HP used. Java Application Software (image J Software) used.

Statistical analysis

The descriptive statistic of mean, standard deviation and inferential statistics were used for this study, the data was subjected to statistical test and analysis with the aid of Statistical Packages for Social Sciences (SPSS) version 21 and Microsoft excel 2021 for windows using T-test method of data analysis. 0.05 was alpha level of significance ($P < 0.05$) and Graph pad prism 9 was also used to plot the graphs. The TURKEY test and one-way ANOVA were used for this research work.

RESULTS

Mean Changes in Animal Weight, Organ Weight and Relative Organ Weight of Animals

We determine the changes in the body of the animals in all before, during and after the experimental period as shown in table 1.

There was a significant increase ($p < 0.05$) in the weight of animals in week 1, 3, 4 and 5 when compared to second week.

There was no significant difference ($p > 0.05$) in the mean body weight of animals in Mercuric Chloride group when compared with mean of animals in control, group 3, 4 and 5.

Table 1: There was significant increase ($p < 0.05$) in Organ weight of group 2 compared to group 1, 3, 4 and 5.

Duration(weeks)	Mean animal weight	±SD
Week 1	166.73	32.38
Week 2	133.50	32.35
Week 3	177.14	31.90
Week 4	178.65	30.31
Week 5	179.02	30.24

Timeline for change in weekly body weight of animals

Note: week 1 & 2 = acclimatization week, week 3 = Mercuric Toxicity, week 4 & 5 = treatment or experimental week

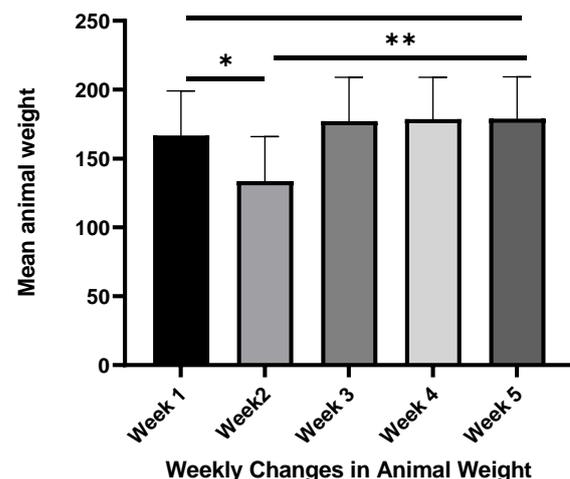


Fig 1 Mean weekly weight of the Animals. Data analyzed by One-Way Anova, followed by Post-hoc brain test.

*= $P < 0.05$ vs week1

Table 2: Mean changes in body weight, Organ (brain) weight and Relative organ weights of the animals.

Parameters	Group 1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)	Group 4 (mean±SD)	Group 5 (mean±SD)
Body weight (g)	183.20±18.06	197.00±20.84	170.20±34.30	191.00±22.66	169.80±36.11
Organ weight (g)	0.13±0.07	1.28±0.73	0.40±0.22	0.42±0.10	0.19±0.10
Relative Organ weights (g)	0.07±0.03	0.70±0.38	0.22±0.09	0.23±0.05	0.13±0.05

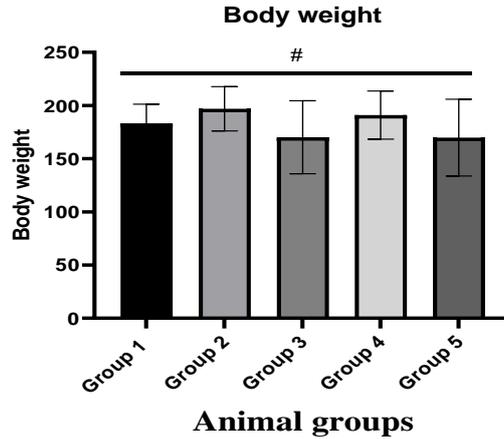


Fig. 2: *=P<0.5 vs group 1. Data analyzed by One-Way anova followed by post-hoc test.

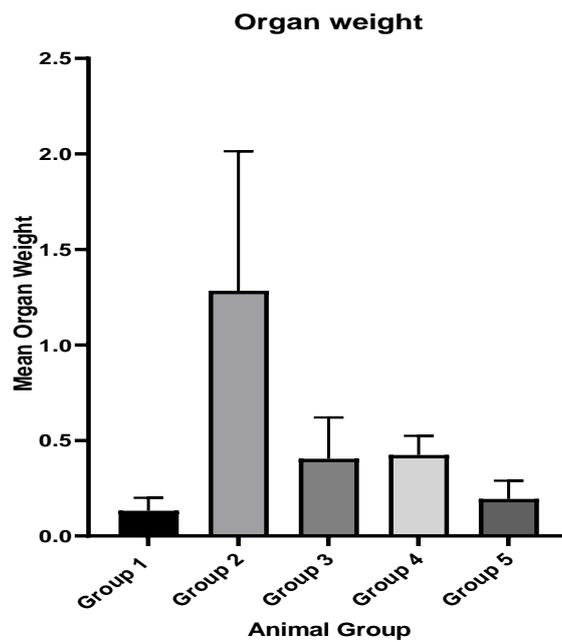


Fig 3: Mean brain weight in all animal group. Data analyzed by one-way anova, followed by post-hoc tukey test. *=P<0.05 vs mercury chloride only, #=P>0.05 vs mercury chloride only.

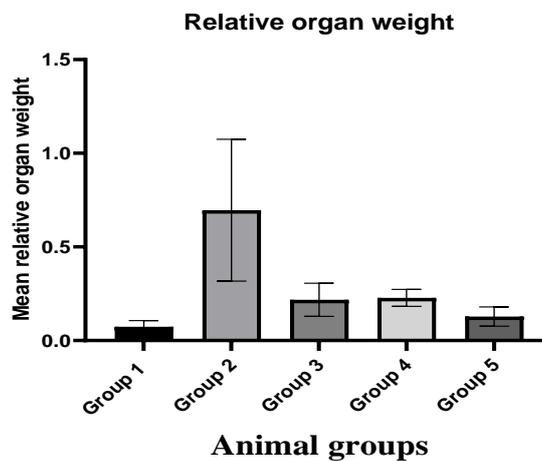


Fig. 4: Mean weight of the animals during induced toxicity in the animal group. Data analyzed by One-way anova, followed by post-hoc tukey test. =P>0.05, no significant difference among the week.

Blood Cell Counts

The blood count in animal group and the result presented in table.

There was no significance difference in the level of Haematocrit in all the animal groups $\# = P > 0.05$.

The level of Haemoglobin showed no significance difference in group 1 compared to group 2, group 3, group 4 and group 5 $\# = P > 0.05$ but there was significant difference in group 2 when compared to group 4 $\# = P < 0.05$.

The level of white blood cell (WBC) showed no significance difference in group 1 compared to group 2, group 3, group 4 and group 5 $\# = P > 0.05$ but surprisingly, there was significance difference in group 2 compared to group 4 $\# = P < 0.05$.

There was a significant difference $\# = P < 0.05$ in red blood cell in group 2 and group 3 compared to group 1, group 4 and group 5.

There was significant difference in the level of platelets in group 2 and group 4 compared to group 1 $\# = P > 0.05$ but there was no significant difference in group 3 and group 5 compared to group 1, group 2 and group 4 $\# = p < 0.05$.

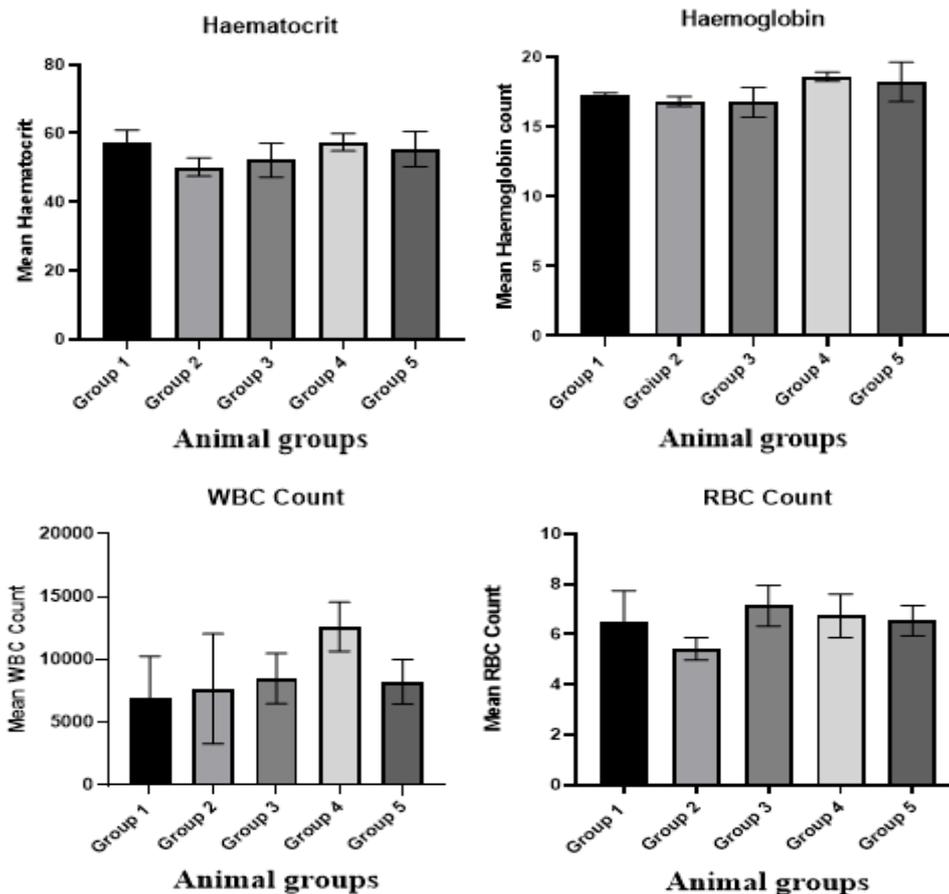
There was no significance difference in the level of Neutrophils in all the animal groups $\# = P > 0.05$.

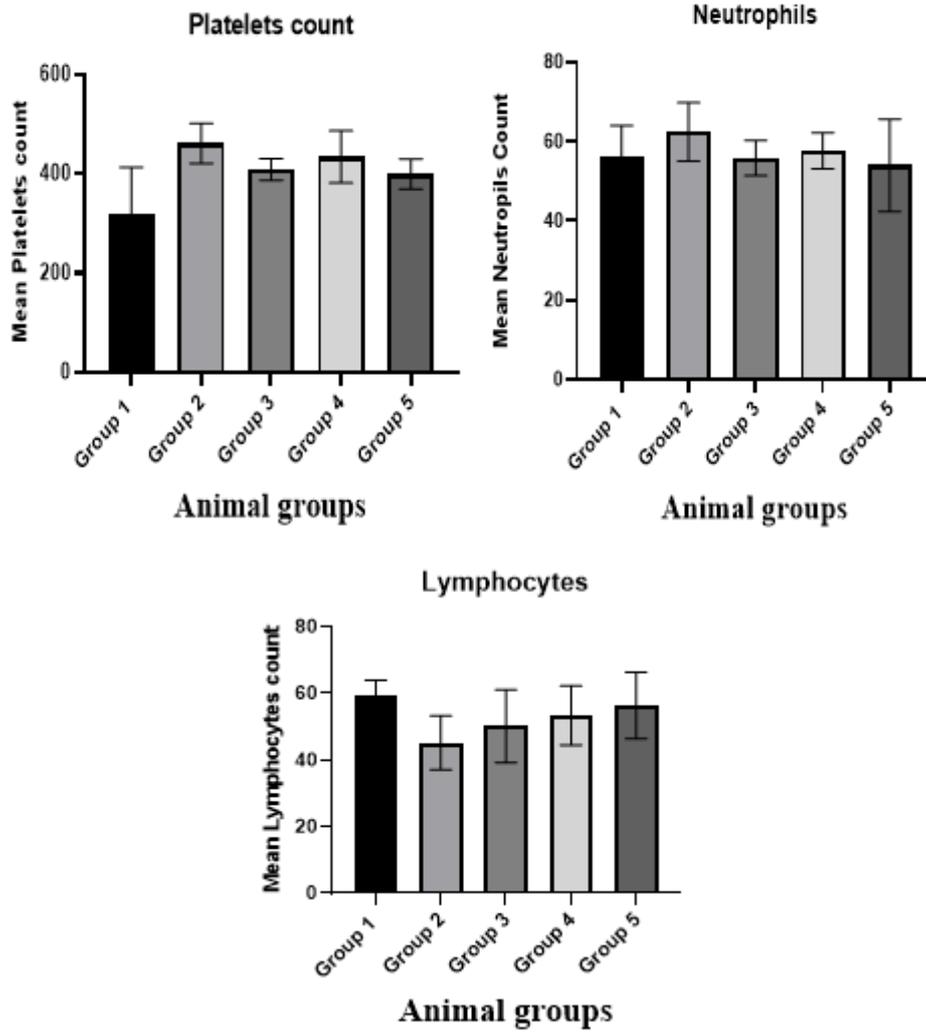
There was no significance difference in the level of Lymphocytes in all the animal groups $\# = P > 0.05$.

MEAN BLOOD CELL COUNT

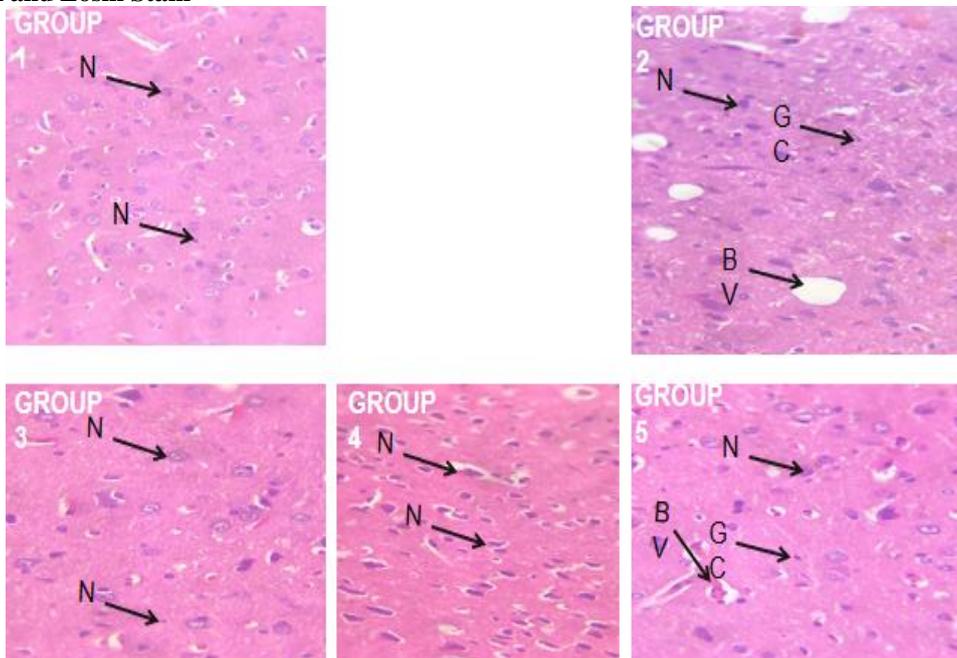
Parameters	Hematocrit (mean ± SD)	Haemoglobin (mean ± SD)	WBC (mean ± SD)	RBC (mean ± SD)	Platelets (mean ± SD)	Neutrophils (mean ± SD)	Lymphocytes (mean ± SD)
Group 1	57.20±3.70	17.24±0.19	6900.00±3364.52	6.50±1.24	318.00±94.71	56.20±7.79	59.40±4.56
Group 2	50.20±2.68	16.82±0.36	7680.00±4383.72	5.42±0.44	461.00±40.06	62.40±7.37	45.20±8.11
Group 3	52.20±4.97	16.76±1.08	8500.00±2012.46	7.14±0.81	408.20±21.38	55.80±4.38	50.20±10.94
Group 4	57.40±2.51	18.60±0.33	12620.00±1954.10	6.74±0.86	433.80±52.70	57.60±4.56	53.40±8.91
Group 5	55.40±5.13	18.24±1.42	8240.00±1786.10	6.54±0.61	398.80±30.30	54.00±11.64	56.40±9.96

Graphical Representation of each Blood Analysis.





HISTOLOGICAL SLIDES
Haematoxylin and Eosin Stain



Legend: N=Neurone, GC=Glial cells. BV(Blood Vessels) H&E X200

Photomicrograph of the medial geniculate Body, across group. **Group 1:** shows well differentiated and organized medial geniculate body neurons(N), **Group 2:** induced with 2mg/kg of mercuric chloride shows degenerated neurons(N), glial cells(GC) and dilated blood vessels(BV), **Group 3:** induced with 2mg/kg mercuric chloride and treated with 200mg/kg extract shows regenerated neurons(N) and glia cells without any distortion, **Group 4:** induced with 2mg/kg mercuric chloride and treated with 15mg/kg quercetin shows regenerated neurons(N) and hyperchromatic glia cells, **Group 5:** induced with mercuric chloride and treated with 200mg/kg *Azadirachta indica* and 15mg/kg quercetin shows regenerated neurons(N) dilated blood vessels(BV) and glia cells(GC). H/EX400

DISCUSSION

Heavy metals such as mercury, lead, and nickel accumulate in air, water, and land due to increased industries resulting from development (1). Mercury (Hg) exposure has increased globally due to industrial activities, gold mining, medicinal uses, and fossil fuel combustion (2). Occupational mercury chloride (HgCl₂) exposure resulted in neurotoxicity, including cognitive impairment, motor dysfunction, central auditory system dysfunction, nephrotoxicity, hepatotoxicity, and mortality (3). It primarily damage brain tissue, causing focal brain damage that can affect the function of brain parts like the cerebellum, hippocampus, auditory and visual cortex and cause cognitive and behavioral changes (4).

In this study, the effects of neem leaf on body weight, relative organ weight, and animal organ weight are evaluated. Additionally, the toxicity of mercuric chloride on an oxidative stress marker is examined, and the aqueous extract of *Azadirachta indica* leaf is evaluated for its potential to prevent mercury chloride-induced damage to the MGB architectural structure.

Quantitative analysis of the changes in the body of the rats before, during and after the experimental period revealed there was a significant increase in the weight of animals in week 1, 3, 4 and 5 when compared to second week, however, there was no significant difference in the mean body weight of animals in Mercuric Chloride group when compared with mean of animals in all other groups. There was significant increase in weight of Medial Geniculate Body of mercury toxicity group compared to all other groups. This is consistent with a study by^[20] who deduce that there is no significant change in body weight of animal after inducement with mercury chloride. This is supported by a study by^[6], who conclude that The toxicity of mercury chloride (HgCl₂) causes a decrease in the number of erythrocytes from 1,826,000 cells/mm³ to 1,678,000 cells/mm³ in treatment D (0.15 pmm), an increase in leukocytes from 14,050 cells/mm³ (control) to 16,360 cell/mm³ in treatment D (0.15 pmm), a decrease in hemoglobin level from 7.6 g/dl to up to 6.7 g/dl of treatment D (0.15 pmm)

and a decrease in hematocrit from 13.24% to 10.24% D (0.15 pmm). How-ever this deteriorating effect was ameliorated by the administration of *Azadirachta indica* and quercetin.

In the full blood parameters, there was no significance difference in the level of Haematocrit, Neutrophils and Lymphocyte in all the animal groups Likewise, the level of Haemoglobin showed no significance difference within all the groups however, there was significant decrease in mercury chloride induced group when compared to the group treated with quercetin. The level of white blood cell (WBC) showed no significant difference across the groups Likewise, the level of WBC showed no significance difference within all the groups however, there was significant decrease in mercury chloride induced group when compared to group treated with quercetin. There was a significant reduction in red blood cell in mercury chloride induced group compared to all other groups. There was also a significant increase in the level of platelets in mercury chloride induced group.

Mercury kinetics and metabolism within human cells is complex and only partially understood. In the brain, methylmercury is slowly changed into inorganic mercury, the proximate toxic species. The effect of mercury within cells is likely to be a balance between the ingress of mercury from environmental exposure, the protective mechanisms within cells against mercury toxicity (such as binding to metallothioneins and selenium, and sequestration in lysosomes).

It was observed in the sections stained in H & E and demonstrated in the photomicrographs that the neurons and the glial cells were equally and evenly distributed within the control group and likewise group 3, 4 and 5. In the mercury induced group the blood vessels are dilated with non-uniform neuronal types were seen as different from the distinct neuron types observed in the rats with few and scanty glial cells compared to the control group.

These study is supported by^[19] who concluded that mercury was found in neurons and oligodendrocytes in medial geniculate body that are affected by the disease, and often co-localized with aggregated α -synuclein. Mercury in the motor cortex, thalamus and striatum could result in bradykinesia and rigidity, and mercury in the cerebellum could cause tremor.

CONCLUSION

Key findings of this study are that a proportion of people from a wide background of clinic-pathological conditions had mercury in cells of their Median Geniculate Neurones. The distribution of mercury-containing cell types varied between individuals. These results raise the possibility that exposure to mercury, with preferential uptake in the geniculate nuclei, followed by inhibition of geniculate neuronal output and

subsequent over-activity of the primary visual and auditory cortices, could be one factor underlying visual and auditory hallucinations.

ACKNOWLEDGEMENT

The authors will like to Acknowledge Olabisi Onabanjo University for providing Resources and a Conducive Environment for conducting this Research.

Competing Interest: All Authors Declare that there's no competing interest.

Author's Contributions

All authors have equal contributions in completing the present research work. All authors read and approved the final manuscript

REFERENCE

- Ijomone OM, Okori SO, Ijomone OK, Ebokaiwe AP. Sub-acute nickel exposure impairs behavior, alters neuronal microarchitecture, and induces oxidative stress in rats' brain. *Drug and chemical toxicology*, 2018; 41(4): 377-384.
- Siblerud R, Mutter J, Moore E, Naumann J, Walach H. A hypothesis and evidence that mercury may be an etiological factor in Alzheimer's disease. *International Journal of Environmental Research and Public Health*, 2019; 16(24): 5152.
- Huang CF, Hsu CJ, Liu SH, Lin-Shiau SY. Neurotoxicological mechanism of methylmercury induced by low-dose and long-term exposure in mice: oxidative stress and down-regulated Na⁺/K⁺-ATPase involved. *Toxicology letters*, 2008; 176(3): 188-197.
- Owoeye O, Salami OA., Monosodium glutamate toxicity: sida acuta leaf extract ameliorated brain histological alterations, biochemical and haematological changes in wistar rat *African Journal of biomedical research*, 2017; 20(2): 173-182.
- Jakaria M, Park SY, Haque ME, Karthivashan G, Kim IS, Ganesan P, Neurotoxic agent-induced injury in neurodegenerative disease model: Focus on involvement of glutamate receptors. *Frontiers in molecular neuroscience*, 2018; 11: 307.
- Jha A, Saidullah B, Bubber P. A study on prooxidative and neurotoxic effects of mercury chloride in rats. *EC Pharmacology and Toxicology*, 2019; 7: 112-124.
- Waters F, Fernyhough C. Hallucinations: A Systematic Review of Points of Similarity and Difference Across Diagnostic Classes. *Schizophr Bull.*, 2017; 43: 32-43. 10.1093/schbul/sbw132
- Boksa P. On the neurobiology of hallucinations. *J Psychiatry Neurosci*, 2019; 34: 260-262.
- Dorph-Petersen KA, Caric D, Saghafi R, Zhang W, Sampson AR, Lewis DA. Volume and neuron number of the lateral geniculate nucleus in schizophrenia and mood disorders. *Acta Neuropathol*, 2019; 117: 369-384.
- Farrar WP, Edwards JF, Willard MD. Pathology in a dog associated with elevated tissue mercury concentrations. *J Vet Diagn Invest.*, 2014; 6: 511-514.
- Ross JF, Switzer RC, Poston MR, Lawhorn GT. Distribution of bismuth in the brain after intraperitoneal dosing of bismuth subnitrate in mice: implications for routes of entry of xenobiotic metals into the brain. *Brain Res.*, 2016; 725: 137-154.
- Moller-Madsen B. Localization of mercury in CNS of the rat. V. Inhalation exposure to metallic mercury. *Arch Toxicol.*, 2012; 66: 79-89.
- 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(1): 1-8.
- Kumar S, Vandana UK, Agrwal D, Hansa J. Analgesic, Anti-inflammatory and Anti-pyretic Effects of *Azadirachta indica* (Azadirachta indica) Leaf Extract in albino rats. *International Journal Scientific Research*, 2015; 4: 713-721.
- Dhama K, Mani S, Chukrubarty S, Tiwari R, Kumar A, Selvaraj P, Rai RB. Herbal Remedies to combat Cancers in Humans and Animals. A Review *International Journal of Current Research*, 2013; 5: 1908-1919.
- Mahima AK, Verma A, Kumar A, Rahal R, Deb SK. Immunomodulatory Therapeutic Potential of Herbal, Traditional and Ethnoveterinary Medicine. *Pakistan Journal of Biological Science*, 2013; 15: 754-774.
- Padal, M.M., Rastogi, S., Rawat, A.K.S., Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-based Complement. *Altern. Med.*, 2013; 376327.
- Zou H., H. Ye, R. Kamaraj, T. Zhang, J. Zhang, and P. Pavek, "A review on pharmacological activities and synergistic effect of quercetin with small molecule agents," *Phytomedicine*, 2021; 92: Article ID 153736.
- Pamphlett R, Png FY. Shrinkage of motor axons following systemic exposure to inorganic mercury. *J Neuropathol Exp Neurol*, 2018; 57: 360-366.
- Chen, J.G., Jiang, Q.C., Wen, B., Wang, R.Y., Wu, Y.G., Li, X., 2018. Effects of deoxygedunin on Alzheimer-like pathologic dysfunction induced by D-galactose combined with AlCl₃.