Asian Journal of Medicine and Health

18(12): 7-16, 2020; Article no.AJMAH.64195
ISSN: 2456-8414

Neurodegenerative Observations on the Cerebral Cortex of Adult Wistar Rats Following Mercury Chloride Induced Cortical Damage in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PBF and TSO managed the analyses of the study. Author TSO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2020/v18i1230280
Editor(s):
(1) Dr. Janvier Gasana, Kuwait University, Kuwait.
Reviewers:
(1) Mona Ismael Shahin, Ain Shams University, Egypt.
(2) Manuel Arturo Gallardo-Flores, Anglo Americana Clinic, Peru.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64195

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 01 February 2021

ABSTRACT

Background: Mercury is a widespread environmental and industrial pollutant that is used in food preservation, cosmetics, pharmaceutical companies and laboratories. The aim of this present study was to investigate the possible effects of mercury chloride ($HgCl_2$) exposure on the cerebral cortex of adult wista rats; and also to evaluate the effects of mercury on biochemical parameters.
Materials and Methods: Thirty six (36) adult wistar rats of both sexes, weighing between 110 g-300 g were randomly divided into four groups A, B, C and D with nine animals per group. The animals in groups B, C, and D were administered mercury chloride orally at the concentration of 0.2 mg/kg, 0.4mg and 0.5 mg/kg body weights respectively while group (A) served as control and was given distilled water. The administration lasted for a period of 21 days. The brain was carefully removed and weigh immediately with sensitive balance, part of it was homogenized for biochemical analysis (MDA, GSH and NO). The remaining part was then fixed in 10% formol calcium fluid and processed for histopathological studies using H and E stains.

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Results: The results revealed a decrease in animal body weights from all the groups in comparison with the control group (A) which showed an insignificant decrease (P>0.05), group B showed an insignificant decrease (P>0.05) while group C and D showed statistically significant decrease (P<0.05). The brain weights revealed statistically insignificant decrease in the treated groups when compared with the control group. The biochemical evaluation revealed a statistically significant increase (P<0.05) in the level of MDA (Malondialdehyde) in the treated groups when compared with the control group, GSH (Glutathione) revealed statistically significant decrease (P<0.05) in the treated groups and NO (Nitric Oxide) revealed statistically significant increase (P<0.05) in the treated groups as compared to the control group. The histological observation revealed degenerative changes in the cortex of treated groups that were characterized by clustered Pyknotics pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma. Perineural spaces were seen surrounding degenerating neurons. Axons and dendrites are scarcely appreciable around neurons in these groups.
Conclusion: The findings from this study showed that ingestion of mercury chloride has potentially deleterious effects on brain as shown in the histopathology, cellular loss in the brain of wistar rat.

Keywords: Mercury; cerebral cortex; degenerative changes; pyramidal neurons.

1. INTRODUCTION

Mercury has been described to be a toxic metallic substance which occurs in nature and found in in three different forms; elemental, organic and inorganic in the environment. Individuals are exposed to the potential risk of heavy metals via different routes which can lead to many health issues and challenges. Mercury is a widespread environmental and industrial pollutant which is used for food preservation, cosmetics, pharmaceutical companies and laboratories. It is found in the environment in three forms; elemental mercury or metallic mercury [Hgo], inorganic mercury [mercury chloride HgCl] and organic mercury [Mgl-lg]. Elemental mercury is liquid at room temperature and it can be released into the atmosphere as mercury vapor because of its high vapor. Inorganic mercury exists in two oxidative states (mercurous, mercuric), which are generally in solid states as mercurous or mercuric salts and mercuric compound [1]

Mercury chloride is among the heavy chemicals reported to cause devastating health problems, it is a crystalline white solid that is poisonous, soluble and has the tendency to sublime. It is majorly used in insecticide, fungicide, wood preservative and photography. Inorganic mercury has been used for many years in medication; teething powders, skin creams and germicidal solutions thereby exposing humans to different toxicological effect [2]. Exposure to inorganic mercury is known to cause paresthesia, fatigue, progressive weakness and neuropsychiatric disorders [2]

Human beings exposure to elemental mercury is mainly by inhalation, followed by rapid absorption and distribution in all major organs. It can be from fumes, industrial actions of fossil fuel power, odor and sewages in the form of mercury oxide and some cosmetics in the form of perfumes [3]. Report from previous study as documented by the US Government Agency for Toxic Substances and Disease Registry has raised concern and implicated mercury as the third most toxic element on the planet [4]. The brain is made up of three main parts; the forebrain, midbrain, and hindbrain. The forebrain consists of the cerebrum, thalamus, and hypothalamus (part of the limbic system). The hindbrain consists of the tectum and tegmentum. The hindbrain is made of the cerebellum, pons and medulla. Often the midbrain, pons, and medulla are referred to as the brainstem [5].

It has been shown that mercury compounds are toxic pollutants with some bioaccumulative properties and their anthropogenic natural emissions constitute a great risk to human health thereby showing a great concern [6]

1.1 Exposure by Oral Means

This kind is via consumption of food stuff preserved with mercuric compounds. Fishes and shells for example contain mercury in their muscle, examples of such fishes are Tilapia, King mackerel, Shark and filefishes [7].

1.2 Exposure by Inhalation

This can be from fumes, industrial actions of fossil fuel powder, odor and sewages in the form

of mercury oxide and some cosmetics in the form of perfumes [8]. Metallic mercury in its vapor form is readily absorbed through the lungs and can invariably result in body damage [9] Because of its soluble characteristics, Elemental mercury is highly diffusible in view of its solubility which traverses the cell membranes as well as the blood-brain and placental barriers to reach target tissues and organs [9,10].

1.3 Exposure Via Dermal

The use of mercuric ointments, creams and some soaps can result to disease condition. However, dermal or ocular exposure can occur when coming in contact with mercury and its compound. Inorganic mercury concentration of 0.4 to 2.2 ug/ml present in blood serum or plasma has been reported to cause death in humans [11] Similarly, other investigators have demonstrated that the toxic characteristic properties, toxicokinetics, biological behavior and clinical expressions of mercury compounds are directly associated with their chemical nature and forms [12,13]

Once it is absorbed, mercury chloride is distributed in all tissues and low fractions have been shown to easily cross the brain-blood barrier and the placenta. [14]. The non-occupational exposure of mercury occurs through air, food, drinking water and dental amalgams. Inorganic mercury are absorbed by the gastrointestinal tract after ingestion [13]. Organic mercury are absorbed much more rapidly than inorganic forms, while approximately 80% of mercury vapour is absorbed following inhalational exposure [15] Metallic mercury and mercurous salts are poorly absorbed following oral exposure [16] Absorption of mercury chloride was reported at 48% in rats of the orally administered substance [16] .Human beings exposure to elemental mercury is mainly by inhalation, followed by rapid absorption and distribution in all major organs. It can be from fumes, industrial actions of fossil fuel power, odor and sewages in the form of mercury oxide and some cosmetics in the form of perfumes [3] The primary target organs of elemental mercury are the brain and kidney [2].

It can be through the consumption of food stuff preserved with mercuric compounds. Fishes and shells for example contain mercury in their muscle, example of these fishes are Tilapia, King mackerel, Shark and filefishes [17]. Mercury is a toxic and non-essential metal in the human body. Mercury is ubiquitously distributed in the environment, present in natural products and exists extensively in items encountered in daily life. There are three forms of mercury; elemental mercury, inorgnanic mercury compounds and organic mercury compounds. Inorganic mercury compounds are water soluble, they are also irritants and cause gastrointestinal symptoms. Upon entering the body inorganic mercury compounds are accumulated in the kidney and produce kidney damage. The primary target organs for elemental mercury are the brain and kidney. Mercury vapour readily enters the red blood cells and central nervous system following inhalational exposure [18]. The kidney and central nervous system exhibits greatest concentration of mercury following exposure to inorganic mercury salts [19].

2. MATERIALS AND METHODS

Thirty-six (36) adult, healthy Wister rats weighing between 110-300g of either sex were used for the experimental design. They were housed in standard plastic cages and fed with rat chow daily. All the rats were carefully and routinely screened, inspected and confirmed to be healthy during the period of acclimatization. During acclimatization which lasted for 2 weeks, the animals were fed, monitored and weighed with a weighing scale and the value recorded every week till the end of administration so as to monitor and study the effect of the administration of mercury chloride on the weight of adult wister rat. They were provided with food and distilled water ad libitum. The rats were fed with standard rat pellet purchased from bovajay food mill, Apake, Ogbomoso.

The experimental animals were housed in standard plastic cages in a serene and conducive cross-ventilated room in the Animal Holding of Anatomy department at LadokeAkintola University of Technology, Ogbomoso, Nigeria and maintained in accordance with the 'Guide for the care and use of Laboratory Animals' prepared and compiled by the (National Research Council) [20].

The experimental animals were separated into four (4) groups of nine each. The groups are as follow A, B, C and D.

Group A- this served as the control group having 9 rats, was given normal rat feed and water.

Group B- was given normal rat feed, water and mercuric chloride of 2 mls containing 0.02 g/kg of $HgCl_2$

Group C- was given normal rat feed, water and mercuric chloride of 2 mls containing 0.04 g/kg of $HgCl_2$

Group D- was given normal rat feed, water and mercuric chloride of 2mls containing 0.05 g/kg of $HgCl_2$

Mercury chloride was administered orally, using an oral cannula every morning between the hour of 7am and 8am for twenty one (21) days. The control group feed on distilled water and pellet alone while other experimental groups were given distilled water, pellet and administered mercury chloride. The Mercury chloride stock solution was always kept in a fridge at the end of the administration every day to keep the stock solution at the required temperature.

The experimental animals were sacrificed after the duration of administration of twenty one (21) days ended using cervical dislocation method. The cervical regions of Wistar rats were dislocated to elicit a fracture at the cervical region thereby rendering the animals temporarily unconscious. This process was carried out quickly to prevent the process of autolysis. The brain was carefully removed and weighed immediately with sensitive weighing balance, part of it was homogenized for biochemical analysis (MDA, GSH, and NO). The remaining part was then fixed in 10% formol calcium fluid and processed for histopathological studies using H and E staining technique. The Wistar rats were euthanized on 22nd day of the treatment and the brain of each rat was immediately removed by dissection and fixed in 10% buffered formalin in accordance with previous investigators [21,22] (Zhang et al.; Mai,) for light microscopy using Hand E as previously described [23] Wilson and Gamble.

The normal histological methods of fixation, dehydration, clearing, impregnation, embedding, sectioning and staining (with H and E) was used to produce the sections. The micrographs of the relevant stained sections were subsequently taken with the aid of a binocular light microscope.

2.1 Statistical Analysis

Experimental data that were obtained from this study were expressed as Mean± SEM (standard error of mean) and data were analyzed by analysis of variance (ANOVA). Student's t-test was employed to compare differences between the groups using Graph pad Prism for windows (GraphPad Prism, Inc Chicago). A value of $P < 0.5$ was considered to be significant.

3. RESULTS

The graph above showed insignificant (P>0.05) decrease in brain weights in Group B, C and D as compared with Group A.

Table 1. Effect of mercury chloride on brain weights (g) [Mean ± SEM]

Groups	Weights (g)	Relative brain weight (%)
A	1.79±0.04	0.85%
B	1.70±0.11	0.85%
C	1.79±0.02	1.05%
D	1.71±0.02	1.23%

Values were expressed as Mean ± SEM P < 0.05, (significant)

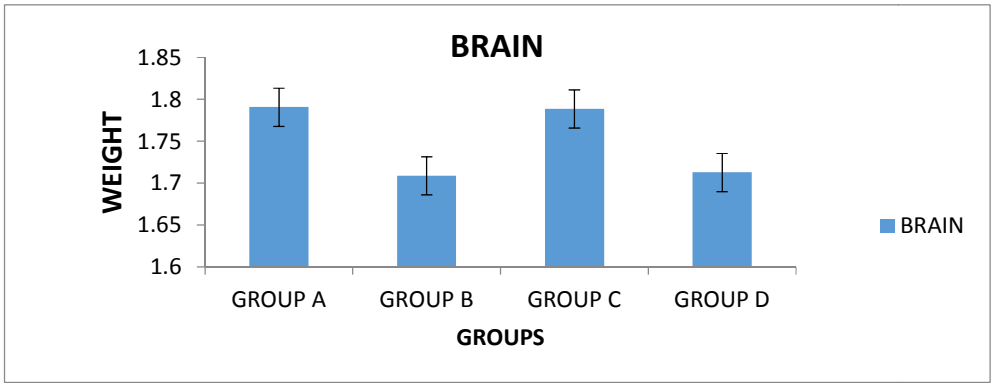


Fig. 1. Showing the mean ± SEM of the brain weight

3.1 Biochemical Evaluation

Table 2 Revealed an increase in the levels of MDA (Malondialdehyde) in the treated groups when compared with the control, it increased significantly (P<0.05) from 0.96 ± 0.17 to 1.84 ± 0.07 in group B, 1.72 ± 0.05 in group C and 1.52 ± 0.03 in group D.

The level of GSH (Glutathione) reduced significantly (P<0.05) in the treated groups when compared with the control, it decreased significantly from 5.11 ± 1.44 to 3.28 ± 0.59 in group B, 2.84 ± 0.22 in group C and 2.76 ± 0.21 in group D.

NO (Nitric Oxide) level increased significantly in the treated groups when compared with the control, it increased significantly (P<0.05) from 10.45 ± 1.43 to 13.83 ± 0.37 in group B, 16.64 ± 0.55 in group C, and 18.48 ± 0.87 in group D.

The graph above revealed statistically significant (P<0.05) increase in the level of MDA in group B, C and D when compared with group A.

Group B, C and D has a statistical reduction (P<0.05) in the level of GSH as compared with group A.

Group B, C and D has a statistical reduction (P<0.05) in the level of NO as compared with Group A.

Table 2. A comparison between different groups on rat brain antioxidant and oxidative status profile in rats

Groups	MDA	GSH	NO
A	0.96 ± 0.17	5.11 ± 1.44	10.45 ± 1.43
B	1.84 ± 0.07**	3.28 ± 0.59	13.83 ± 0.37
C	1.72 ± 0.05**	2.84 ± 0.22	16.64 ± 0.55**
D	1.54 ± 0.03*	2.76 ± 0.21	18.48 ± 0.87**

Values were expressed as Mean ±SEM P < 0.05, (significant)

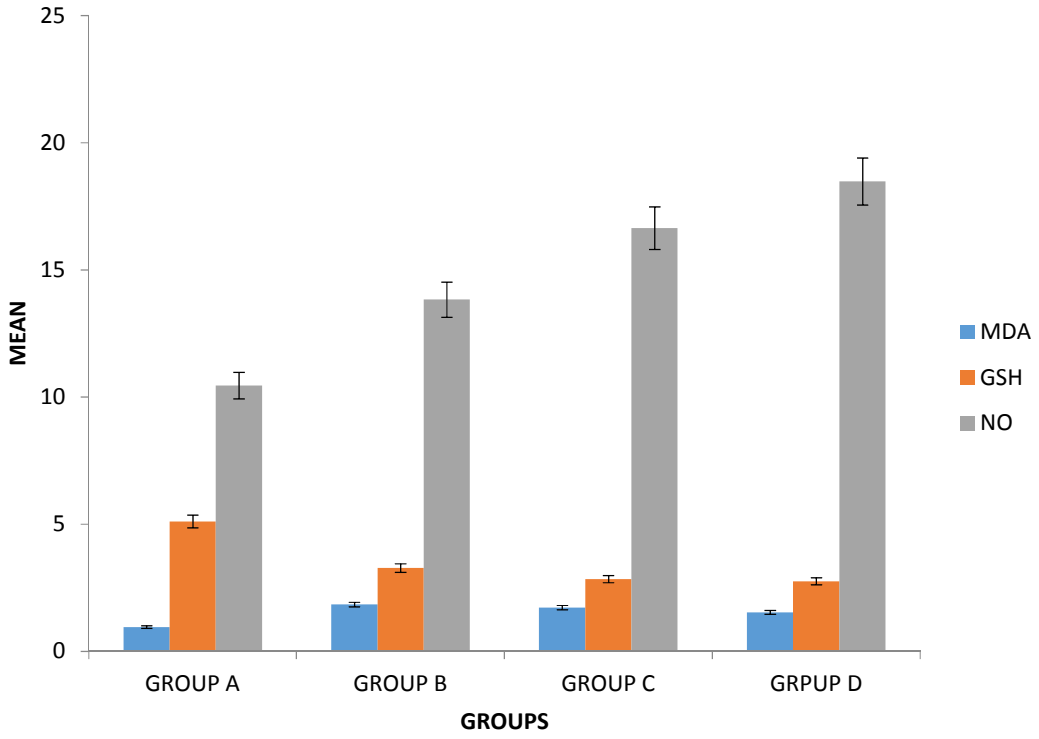


Fig. 2. Histogram showing the mean ± S.E.M of MDA, GSH and NO

3.2 Histological Observation

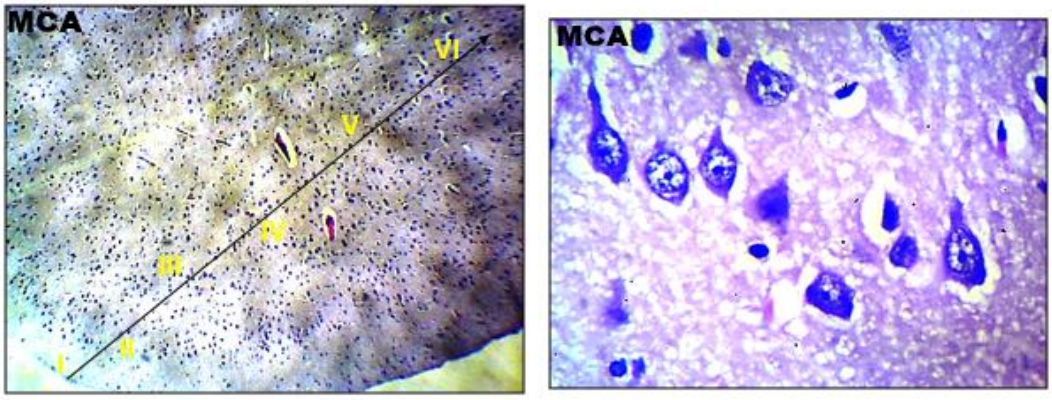
Representative micrographs of H&E staining showing the general cytoarchitecture of the cerebral cortex in wistar rats in group A (control), Group B (Treated with 0.2mg/kg of mercury chloride for 21days), Group C (Treated with 0.4mg/kg of mercury chloride for 21days), Group D (Treated with 0.5mg/kg of mercury chloride for 21days). Magnification: X100, X400 respectively.

Normal histological features of the cerebral cortex in group A demonstrated by large pyramidal neurons with long axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well delineated soma of the pyramidal neurons in this group.

Group B-D treatments caused degenerative changes in the cortex that was characterized by clustered pyknotics pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces can be seen surrounding degenerating neurons (red arrows) Axons and dendrites are scarcely appreciable around neurons in these groups.

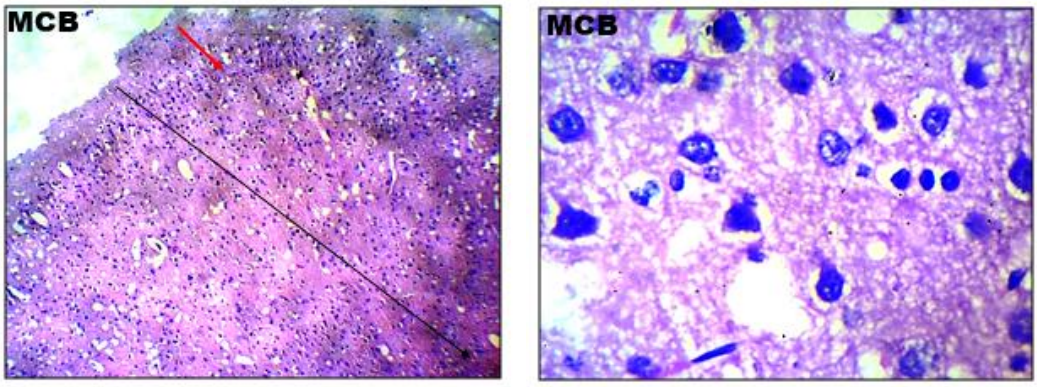
Plate A: photomicrographs showing a normal histological feature of the cerebral cortex, characterized by large pyramidal cell, with long axons that extends well from the delineated soma of the pyramidal neurons, normal molecular layers and external granular layer also appear normal. (H & E X100, X400).

Plate B: photomicrographs (red arrow) shows a slightly mild generative changes in the pyramidal cell, which appear slightly distorted with loss of their process, mild degenerative changes occur in the cytoplasm with condensed nuclei, molecular layer is similar to that of group A (H & E X100, X400).



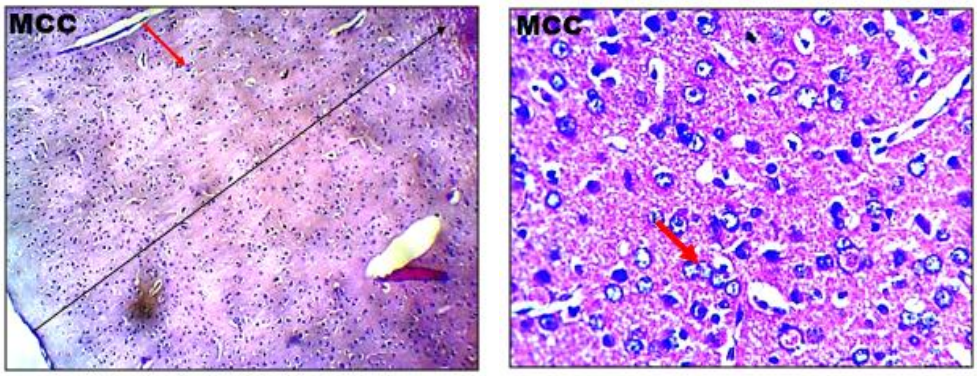
X100 **X400**

Plate A: Control group A



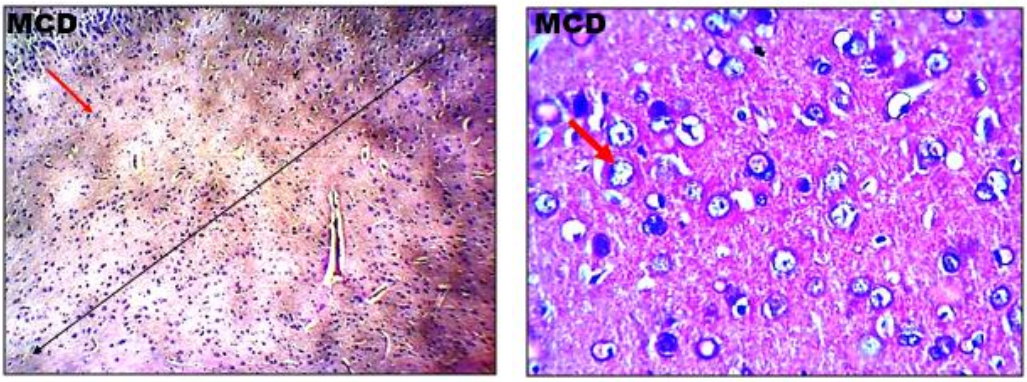
X100 **X400**

Plate B: **Group Treated with 0.2 mg/kg of mercury chloride for 21 Days**



X100 X400

Plate C: Group Treated with 0.4 mg/kg of mercury chloride for 21 days



X100 X400

Plate D: Group Treated with 0.5 mg/kg of mercury chloride for 21 days

Plate C: Photomicrographs (red arrow) shows loss of pyramidal cells due to degeneration, leading to plenty of perineural spaces, molecular layers appear unorganized with lots of spaces, and cell distortion was very obvious. (H & E X100, X400).

Plate D: photomicrographs (red arrow) shows severe degeneration in fragmented cytoplasm, condensed nuclei within soma, very large and numerous perineural space surrounds the degenerating neuron, spaces within these pyramidal cells and granular layer is observed. (H & E X100, X400).

4. DISCUSSION

The toxicity of mercury has been shown to depend on the form and s such the various mercury compounds have different toxicities depending on their physical and chemical properties which affect absorption, distribution, tissue affinities and stability within the bio-system [24]. For instance, elemental mercury in the liquid state has unique toxic effects that differ from those of mercury vapor; likewise, organic mercury molecules are toxicologically different from different from inorganic forms [25] $HgCl_2$ is an inorganic compound used in various fields and its numerous effects were evaluated in many toxicity and carcinogenic studies because of its extensive use and wide occurrence as an environmental pollutant [26,25] Once absorbed, $HgCl_2$ is distributed in all tissues and low fractions have been shown to easily cross the brain-blood barrier and the placenta [27]

This project studied the histomorphological effects of mercury chloride revealed on the cerebral cortex, result of weight analysis showed a decrease in the final total body weight of rats in Group B which were treated with 0.2 mg/kg of mercury chloride and it was statistically insignificant ($P>0.05$) when compared with group A.

The body weights result of rats in Group C which were treated with 0.4 mg/kg and Group D which were treated with 0.5 mg/kg showed a statistical significant ($P<0.05$) decrease when compared with Group A. The decrease in body weights was caused by the reduced food and water intake, as a result of treatment with mercury chloride, this result is in accordance with previous findings of [28] which also observed decreased in body weights of experimental animals.

There was no significant effect on the brain weights of the rats treated with mercury chloride when compared with the control group, although there was decrease in the brain weights but it was not statically significant ($P>0.5$)

The results of biochemical parameters investigated revealed increased level of Malondialdehyde (MDA) which is an oxidative stress marker, it increased and it was statically significant ($P<0.5$) in all the treated groups when compared with the control group, this findings correspond with the result previously reported by [29] which evidenced the increase in MDA in the brain and plasma. The level of Glutathione (GSH) which is an antioxidant in animals, capable of preventing damage to important cellular components was decreased significantly ($P<0.5$) in all the treated groups as compared with the control group, this report correspond with previous findings that reported that Hg reduces the GSH levels in the body [30] . The level of Nitric Oxide (NO) which is a vasodilator was assessed; it revealed significant increase in all the treated groups as compared to the control group. Similarly, it has been reported that mercury chloride intoxication increases the production of nitric oxide as observed in this study [31].

General degenerative changes in the cerebral cortex was observed as degenerated cortical areas, necrotic cells and distortion of cellular layers of the cerebral cortex. The pyramidal cells in the cerebral manifested some changes ranging from degeneration and reduction in number of pyramidal cells to the loss of neuronal cell fiber due to the reduced number of cells when compared to the control group which could be as a result of the exposure of mercury chloride. This indicates that the activity of the hippocampus in memory formation and learning will be impaired and the role of the cerebral cortex that is involved in storage and retrieval of information will also be lost. The findings in this study agree with the report of previous studies. [32], who reported that rats exposed to high concentration of mercury vapor, showed neurodegenerative changes in the cerebral which was responsible for memory deficit in such animals. While it had similarly been shown that cell sizes and cell numbers were observed to be decreased in mice treated orally with inorganic mercury at high doses for a week [33]. Additionally, the results of the present study show that cell sizes and cell numbers were observed to be decreased as previously reported.

5. CONCLUTION

The study concluded that exposure of wistar rats to mercuric chloride induced distortion and neuronal loss in the cortical section of the cerebral cortex which may lead to neurodegenerative disorders in the brain of the wistar rats investigated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64195