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Studies on the effects of mercury exposure on spatial learning and memory of adult wistar rats

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ABSTRACT: *Mercury is a heavy metal that has been reported to cause devastating health problems worldwide. This work studies the effects of mercury chloride exposure on the histomorphological changes on the hippocampus, spatial learning and memory on adult wistar rats. Twenty four male and female wistar rats were randomly divided into four groups of six animals each. Group I serves as the control group which receives normal saline. While groups II, III and IV were treated orally with 12.45 mg/kg, 28.9 mg/kg and 49.8 mg/kg body weight of mercury chloride respectively for twenty one (21) days. Animals were anasthesized and sacrificed using chloroform. The brain was fixed in Bouin's fluid and tissues were processed histologically using H and E stain. The result shows that there was distortion of the pyramidal cells and congestion, necrosis and sparse distribution of the hippocampal cells were observed. The latency time was decreased in the control group while among the treated groups show that there was an increase in latency time that was statistically significant. It was concluded from this study that mercury chloride exposure has degenerative effects on the hippocampus and also causes memory and learning impairments.*

KEYWORDS: *Hippocampus, memory, mercury chloride, Wistar rats*

I. INTRODUCTION

Man in his environment is exposed to many potential hazards by heavy metals via bioaccumulation and biodegradation which are transferred in man via food chain due to anthropogenic activities [1]. The name mercury was derived from the word Hydragyrus meaning water and silver or silver water [1, 2]. Mercury exists in three forms [3- 6], these forms include: Elemental mercury also called metallic mercury, is element in its pure, un-combined form. Inorganic mercury compounds or mercury salts example mercuric chloride ($HgCl_2$) and organic mercury which is formed when mercury combines with carbon and other elements such as dimethylmercury. Mercury and its compounds can be obtained from Industrial sources, fossils fuels power, mining co-operations, and natural forms such as Mercury chloride that is found in higher densities in rocks and volcanic activities [7- 9] Burning of fossil fuels such as petrol and gas, fumes, battery disposals, broken mercury thermometer and coal combustion are other high sources, of emitting mercury and its compounds [5, 10]. Consumer products such as photographic plates and toners contain high amount of mercury chloride [11]. Some cosmetics also contain mercury examples include creams, perfumes, soaps and mascara.

There are many routes of exposure to mercuric compounds but the evidence of exposure is dependent on the levels of toxicity [12, 13]. These exposure routes include: Oral exposure via consumption of food products and grains preserved with mercuric compounds [12, 14, 15]. Inhalational exposure route can be from fumes, industrial actions of fossil fuel power, odor and sewages in the form of mercury oxide [3, 12, 16, 17]. Dermal exposure can be through the use of mercuric ointments, creams and some soaps which can result in disease conditions [2, 18, 19].

Mercury poisoning or toxicity also known as mercurialism refers to a situation when the metallic mercury is absorbed via the skin and ingested or the vapor is taken via the lungs [20]. Children and women within the reproductive age are more susceptible to mercury poisoning [21]. In children mercury poisoning is known as acrodynia or pink disease. Mercury and its compounds has been shown to have effects on the respiratory, cardiovascular and reproductive systems, blood, hair, skin, and enzymes and many other organs and tissues [4, 21- 26]

Mercuric compounds are used in embalmment and in preservation of anthropological species and specimens, treatment of syphilis, healing injuries and fractures before the advent of antibiotics. Mercury chloride was used in Pharmaceutical companies (thimerosal) in vaccines and in syrup, pills and blue mass. In agricultural sector, mercury is used as fungicides and in preservation of food stuffs. Mercuric compounds are widely used in production of cosmetics such as cream, ointment, perfumes, mascara and others. Other uses include mercury switches, liquid mirrors, batteries, thermometer and many more. The aim of the study was to investigate the effects of mercury chloride exposure on the histomorphological structure of the hippocampus and on spatial learning and memory of adult wistar rats using Morris water maze method.

II. MATERIALS AND METHODS

2.1 Experimental design

Twenty six (26) adult male and female wistar rats weighing 190 – 220 g were purchased from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University,Zaria. and were housed in polyesther cage with wire gauze and the animals were allowed to acclimatize for two (2) weeks in the animal house covening of the Human anatomy Department Ahmadu Bello University, Zaria. Animals were fed with animals feed (Grower's mesh) purchased from Mae-Syl Agrochemical Company situated at Sokoto road, Samara, Zaria, Kaduna, Nigeria. The animals' feed was prepared in pellets to reduce spillage and were fed twice daily and clean water was provided in plastic drinking bottle and animals were allowed to feed and drink ad-bilitum

2.2 Chemical substances

Mercuric chloride (May and Baker limited, XN202, Dagenham England). The chemical was purchased from Steve Moore chemicals limited Samaru, Zaria. Kaduna. Nigeria. The LD_{50} of Mercury chloride was adopted from Berlin [2] and ATDRS [27] as 166 mg/kg body weight 30%, 15% and 7.5% of the LD_{50} adopted was used was used for various concentrations.

2.3 Experimental Procedure / Protocol

Animals were weighed and randomly divided into four (4) groups with six animals per group; the administration lasted for twenty one (21) consecutive days which was done orally. Animals were grouped as GI, GII, GIII, and GIV. GI was used as control group and were administered normal saline, while groups GII, GIII and GIV served as experimental groups (Table 1)

Table 1: Animals grouping, concentration and frequency of administration of mercury chloride

S/no.	Group	Concentration	Administration
1	I	Normal saline	Once daily
2	II	12.45 mg/kg (7.5%)	Once daily
3	III	24.90 mg/kg (15%)	Once daily
4	IV	49.80 mg/kg (30%)	Once daily

2.4 Neurobehavioral test; spatial learning and memory test using Morris water maze

Morris water maze test was used to develop and test spatial learning and memory in animals according to Morris methods which was further developed by Mark [28], Charles [29], Liu et al, [30]. According to this method, a platform was submerged beneath the surface of the maze pool; the animal task is to find the hidden platform. The animals' starting point was changed from time to time so as to build a cohesive spatial representation of the pool in order to find the platform during training trials and the latency to find the platform location was recorded both during the training and experimental periods. Animals were placed in a circular pool of clear transparent water which was partitioned into four quadrants. The animals starting point was in a random location. The animal will swim from one quadrant to the other searching for an escape route. The latency time to find the platform was recorded.

2.5 Animal sacrifices

At the end of the 21st day of mercury chloride administration the animals were anasthesized using chloroform and brain tissues were removed by opening through the sutures of the skull using the brain opener obtained from the Department of Human Anatomy Ahmadu Bello University, Zaria The brain was then removed and transferred into specimen bottles containing Bouin's fluid for fast fixation.

2.6 Tissue processing technique

The tissues were allowed to stay in the fixing fluid for 48 hrs for proper fixation. The tissues were taken to tissue processing unit Histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria. The tissues were prepared using routine H and E staining technique. The brain tissues were processed routinely and stained using routine H and E technique.

2.7 Statistical analysis

Data obtained was expressed as Mean ± SEM and one way analysis of variance (ANOVA) was used to compare the level of difference between and within the groups at $P < 0.05$. Statistical analysis was performed using EZanalyze v3.0; a post hoc test of Bonferroni was applied.

III. RESULT

3.1 The effect of mercury chloride exposure on Morris water maze test

The results of the effect of mercury chloride exposure on spatial learning and memory using Morris water maze test, showed that there was a decreased in the mean time taken for the animals to complete Morris water maze task in the control group though the decreased was not significant (Table 2). The results showed that animals in group II had an increased time in Morris water maze activity though the increase between week 1 and week 2 was not significant but significant increase was observed between week 1 and week 3. The result showed a significant increased in time in Morris water activity between week 1 and 2, between week 1 and 3 and between week 2 and 3 respectively in both groups III and IV (Table 2).

Table 2: Effect of mercury chloride ingestion on spatial learning and memory using Morris water maze test

Weeks	Group I (n=6) Mean ±SEM(s)	Group II (n=6) Mean ±SEM(s)	Group III (n=6) Mean ±SEM(s)	Group IV (n=6) Mean ±SEM(s)
1st	12.43±2.71	17.53±1.77	20.70±2.20	37.30±1.75*
2nd	11.81±1.75	21.74±2.41	24.40±6.20*	41.71±3.60**
3rd	10.53±2.34	26.44±3.41*	36.00±5.32*	52.00±4.24**

*P≤0.05; **P≤0.01; n=number of animals per group; s= time in second

3.2 Histology of the hippocampus

The histological studies showed that animals in the control group I have normal appearance of the hippocampus with normal pyramidal cell layer with pyramidal cells (Fig. 1) and group II animals showed disorientation of the pyramidal cell layer with loss of pyramidal cells (Fig. 2). Figure 3**,** shows the hippocampus of group III, with degeneration of the pyramidal cell layer, loss of some pyramidal cells and clumping of pyramidal cell nuclei. Group IV shows disorientation of the pyramidal cell layer and degeneration of some pyramidal cells with the pyramidal cells appearing to be smaller than normal (Fig. 4).

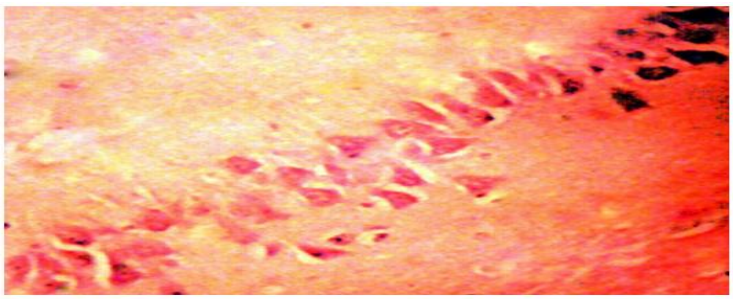

Figure 1: Section of the hippocampus of the control group I) showing normal pyramidal cell layer (PCL) with pyramidal cells (PC) intact (H&E ×250)

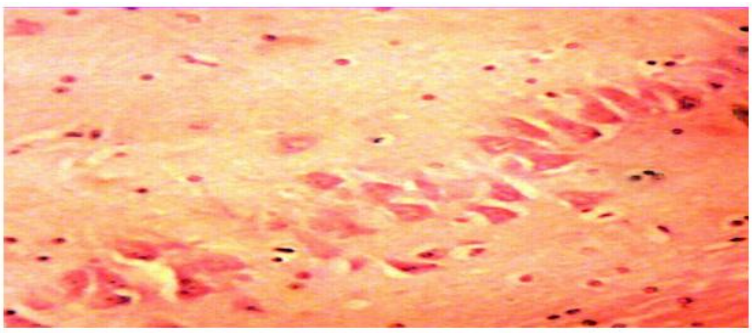

Figure 2: Section of the hippocampus of group II animals showing disorientation of pyramidal cell layer (PCL) with small loss of pyramidal cells (PC) (H&E ×250)

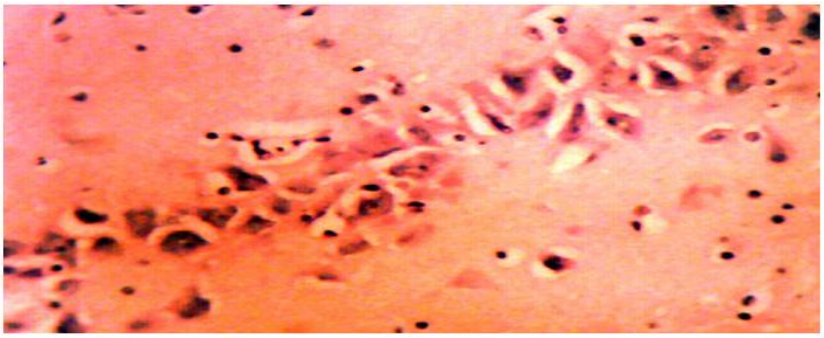

Figure 3: Section of the hippocampus of group III animals, showing, some degeneration of pyramidal cell layer (DPCL) with loss of degeneration of some pyramidal cells (PC) clumping of pyramidal cells nuclei (CN), (H&E ×250)

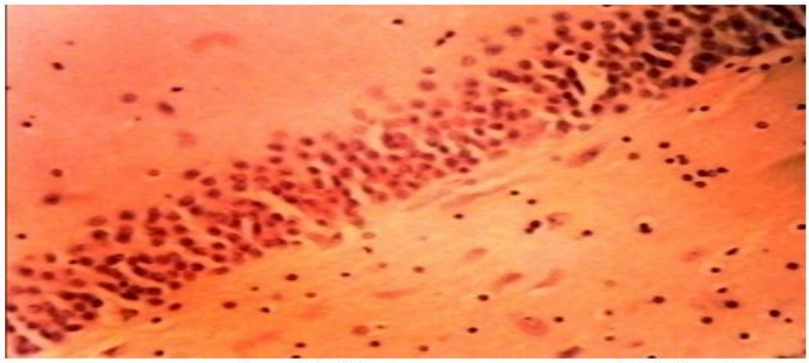

Figure 4: Section of the hippocampus of group IV animals showing disorientation of the pyramidal cell layer ({PCL) with degeneration of some pyramidal cells (DPC).The pyramidal cells appear to be smaller than normal (H&E ×250)

IV. DISCUSSION

The pyramidal cells of the pyramidal cell layer in the hippocampus manifested some changes ranging fron 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 implies that the activity of the hippocampus in memory formation and learning will be impaired and the role of the hippocampus that involved storage and retrieval of information will also be lost. The findings in this study agree with the studies of Wolf et al., [31], who reported that rats exposed to high concentration of mercury vapor, showed neurodegenerative changes in the hippocampus which was responsible for memory deficit in such animals. Similarly, Gagelli, [32] had shown that cell sizes and cell numbers were observed to be decreased in mice treated orally with inorganic mercury at high dosages for a week.

The result from the present study show that, there was a significant increase in the time taken by the experimental rats to find the hidden platform in Morris water maze test, for memory and learning. Conversely, the pyramidal cell layer of the hippocampus appears to be damaged with dead cells, and vacuolated spaces and distortion in the general morphology of the pyramidal cells that appear smaller than normal. These alterations can consequently result to memory impairments which could be as a result of neuronal degeneration; the destruction of the pyramidal cells implies that activity from the brain region that projects into the pyramidal layer of the hippocampus will also be lost such as memory and learning ability [33]. Mutter [34] had reported that short term occupational exposure to high levels of mercury induced slight cognitive deficits. A memory deficit among animals exposed to methyl mercury was not significant in latency time or swim length between the different groups of animals according to Olson [35]. A research conducted on cognitive test using Y-maze for memory and showed that mercury has no effect on memory which disagrees with the results of the present work [34].

V. CONCLUSION

It can be concluded from the present study that mercury intoxication has effects on spatial learning and memory using Morris water maze test which was induced as a result of administration of mercury chloride orally. The results also showed an increased time taken to find the hidden platform which was an indication of memory lost. Spatial learning and memory impairments were attributed to be dose and time dependant and mercury intoxication resulted to some level of damage to the hippocampus which is very crucial in memory and learning.

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Competing Interests: The authors have declared that no competing interest exists in the study

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