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THE EFFECT OF RAUVLFIA VOMITORIA ON MERCURY-INDUCED CHANGES ON THE HISTOLOGY OF THE HIPPOCAMPUS OF ADULT WISTAR RATS

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ABSTRACT

Aim: The present study was aimed at evaluating the effect of *Rauvlfia vomitoria* (RV) on mercury-induced changes on the hippocampus of adult Wistar rats.

Methods: Thirty adult Wistar rats were divided into 6 Groups of 5 animals each. The Control was administered with water, Groups 2 and 4 were administered 49.8mg/kg body weight (bwt) of $HgCl_2$ and 250mg/kg bwt RV, Group 3 was administered with 750mg/kg bwt of RV, Groups 5 and 6 were administered with 49.8mg/kg bwt of $HgCl_2$, 500mg/kg and 750mg/kg bwt of RV respectively. The administration lasted for 35days through oral route daily. Morris water maze (MWM) test for learning and memory was done and the animals were humanely sacrificed while tissue samples were harvested for histological studies.

Results: The result of MWM test showed increase in the mean time taken to locate the platform after mercuric chloride administration but was significantly decreased after the administration of *R. Vomitoria* ($p<0.05$). Observation of the hippocampus showed normal histology in Group 1, while Groups 2, 3, 4, 5 and 6 showed some degenerative and cellular changes.

Conclusion: The administration of *R. vomitoria* has shown to ameliorate the degenerative changes in the hippocampus caused by mercuric chloride toxicity in Wistar rats.

Key words: Mercuric chloride, Hippocampus, *Rauvolfia vomitoria*, Wistar rats

INTRODUCTION

Mercury and its compounds can be obtained from Industrial sources, fossils fuels power, mining Co-operations, and natural forms such as mercury chloride found in higher densities in rocks and volcanic activities (FAO, 1994; Park *et al.,* 2000). 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 (Booth and Zeller, 2005; Burger *et al.,* 2011). Consumer products such as photographic plates and toners contain high amount of mercury chloride (Goyer, 1986). Some cosmetic products contain mercury examples include creams, perfumes, soaps and mascaras (Adepoju-Bello, 2012). There are many routes of exposure to mercuric compounds but the evidence of exposure is dependent on the levels of toxicity (Vimercati and Pesola, 2001; WHO, 2005). These exposure routes include; oral exposure via consumption of food products and grains preserved with mercuric compounds (WHO, 2005; Vupputuri *et al.,* 2005). Children and women within the reproductive age are more susceptible to mercury poisoning (Murphy *et al.,* 1979). 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 (Olivieri *et al.,* 2000; Valera *et al.,* 2008; Rao and Chhunchha, 2009). Mercuric chloride was evaluated in toxicity and carcinogenicity studies because of its extensive use and its occurrence as an environmental pollutant (EHD, 2002).

Neurological symptoms include mental retardation, seizures, vision and hearing loss, delayed development, language disorders and memory loss. In children, a syndrome characterized by red and painful extremities called acrodynia has been reported to result from chronic mercury exposure (WHO, 2007). *Rauvolfia vomitoria* (RV) is a medicinal plant widely distributed all over the world, especially in Asia and West African Countries. The extract from the plant was first extracted by a Swiss Chemist in 1952 and became the first neuroleptic and today the plant is a source of a lot of drugs used in psychiatry (Hansel, 1968). In traditional medicine the root and leaves of *R. vomitoria* are brewed as tea and used in humans for the treatment of hypertention, insanity, snakebites and cholera (Shavarov, 1965). The common name is Swizzle stick (Fapojuwomi and Asinwa, 2013) and the three Nigerian names include asofeyeje in Yoruba, akanta in Igbo and wada in Hausa (Ekutudo, 2003). Research work devoted to phytochemical evaluation of *R. vomitoria* revealed the presence of alkaloids, tannins, saponins, and flavonoids (Akpanabiatu, 2006). The extract has been found to exhibit antioxidant activity, significant hydrogen peroxide scavenging effect relative to ascorbic acid, nitric oxide scavenging effect, and metal chelating activity and ferric reducing power relative to ascorbic acid (Okolie *et al*., 2011). The aim of the present study was to evaluate the effect of *R. vomitoria* on mercuric chloride-induced changes on the Hippocampus of adult Wistar rats.

MATERIALS AND METHOD

Experimental Animals

Thirty adult Wistar rats of average weight of 195g were used for the study and were acclimatized for two weeks and kept in Animal house of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria. The rats were then divided into six groups of five rats per Group for the experiment.

Experimental Chemicals

Twenty five grams of mercuric chloride manufactured by May and Bakers Chemical Laboratory Limited, Dagenham, England was used. *R.* vomitoria root was obtained from Dakaci area of Zaria, Kaduna, Nigeria. Aqueous extraction of *R. vomitoria* root back was prepared according to the method of Handa *et al*. (2008), in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

Animal Experimentation

Thirty adult Wistar rats were divided into 6 Groups of 5 animals each. Group 1 (Control) was administered with distilled water, Group 2 was given 49.8mg/kg body weight (bwt) of mercuric chloride ($HgCl_2$) corresponding to 30% of the LD_{50} of Mercuric chloride (Berlin *et al.,* 2007), Group 3 animals were administered with 750mg/kg bwt of RV, equivalent to 4.29% of LD_{50} of *R. vomitoria* (Amole *et al.,* 2009). Animals in Group 4 were given 49.8mg/kg bwt of $HgCl_2$ and 250mg/kg bwt of RV, while Group 5 animals were administered with 49.8mg/kg bwt of $HgCl_2$ and 500mg/kg bwt of RV and Group 6 rats were administered with 49.8mg/kg bwt of $HgCl_2$ and 750mg/kg bwt of RV. The administration of $HgCl_2$ was for 21 days after which RV was administered for 14 days. The administration was by oral route daily and lasted for 5 weeks while animal feed and drinking water was allowed *ad libitum.*

Neuro-behavioural Assessment

The Morris Water Maze (MWM) which was first established by neuroscientist Richard G. Morris in 1981 was used in order to test hippocampal-dependent learning and memory, including acquisition of spatial memory and long-term spatial memory (Morris, 1981).

Animals Sacrifice

At the end of the administration, the animals were weighed, humanely sacrificed and incision was made through the skin and muscle of the skull. The skull was opened through a mid sagittal incision and the brain was removed and fixed in Bouin's fluid. The tissues were routinely processed and stained using haematoxylin and eosin method.

Statistical Analysis

All data were presented as mean ± SEM and for establishing significant differences, data were analyzed by one-way analysis of variance (ANOVA), followed by Turkey post hoc test. Values were considered significant if p value $\leq$ 0.05.

RESULTS

Physical Observation of the Animals

The result of physical observation of the animals showed that rats in Group 1 were active and friendly while Groups 2, 4, 5 and 6 animals exhibited increased activity and aggression, and drank more water and increased gnawing during $HgCl_2$ administration, Group 3 animals showed little gnawing, calmer and pass watery stools while, Group 4 animals exhibited restlessness and more gnawing during RV administration and Group 5 and 6 animals showed less change in their physical activity.

Morris Water Maze Analyses

At the end of the training period, the animals showed the ability to locate the hidden platform, within a short time seen in the mean training time (T1) for each group. The difference in the mean time taken by the rats to locate the platform after the administration of $HgCl_2$ (T2) was increased significantly in Groups 2, 4 animals and 5 when compared with the time taken by the rats before the administration of the $HgCl_2$ ($p \leq 0.05$). There was a non-significant increase in the mean time taken by the rats to carry out the task at the end of treatment with RV when compared with the time taken by the rats before the administration of $HgCl_2$ while, Group 6 which received the highest dose of the extract showed statistical significant decrease in the time taken to locate the platform as shown in Fig. 1.

Histological Studies of the Hippocampus

The histological examination of the hippocampus of the animals in Group 1 showed normal histo-morphology of the CA3 region, with neurons closely packed together as shown in Fig. 2. The hippocampus of the animals in Group 2 showed degeneration and necrosis of the neurons of the CA3 region, with non-homogenous cytoplasm, intensely basophilic in appearance and some pyknosis of the hippocampal neurons with some satellitoosis and vacuolation as seen in Fig.3. The hippocampus of the animals in Group 3 showed relatively normal CA3 region of hippoocampus with fewer vacuolation of the hippocampal neurons and more homogeneous cytoplasm compared to the group treated with $HgCl_2$ alone as shown in Fig. 4. The hippocampus of the animals in Group 4 showed relatively normal neurons in the CA3 region with more homogeneous cytoplasm compared to the group treated with $HgCl_2$ as shown in Fig. 5. The hippocampus of the animals in Group 5 showed fewer degeneration and necrosis of the hippocampal neurons with vacuolation of some neurons in the CA3 region of the hippocampus as shown in Fig. 6. The hippocampus of the animals in Group 6 showed slightly more degeneration and necrosis of the hippocampal neurons with vacuolation of the neurons compared to the groups that receive lower dose of RV as shown in Fig. 7.

DISCUSSION

The present study has shown neurodegeneration and necrosis of Pyramidal cells in the CA3 region of the hippocampus of adult Wistar rats induced by mercuric chloride while the hippocampus of the Control group showed normal histo-architecture. The findings from the present study agree with the findings which reported that many heavy metals such as mercury, lead, cadmium and other organic compounds have the capacity to damage the neurons of nervous system (Verina et al., 2007; Sadeeq et al., 2013). Francisco *et al.* (2014) had also reported shrunken, pyknotic, dark stained neurons, spheroid bodies and sparsely distributed neurons in the hippocampus of animals administered mercury. These changes maybe transient but permanent abnormalities may be induced only by sustained exposure of these chemicals in an excessive quantity (Wolf *et al.,* 2003). Gagalli *et al.* (2010) and Melto-Carpes *et al.* (2013), in their separate works had reported decreased neural cell sizes and cell number in mice treated orally with inorganic mercury at high dose for a week. These researches were in agreement with the results of the present work. The present study has shown that RV has ameliorative effects on the pyramidal cells of the CA3 region of the hippocampus. The present study has also shown that there was a direct relationship between the concentration of mercuric chloride, concentrations of RV and the level of neurodegeneration in the hippocampus in the studied specie of rats.

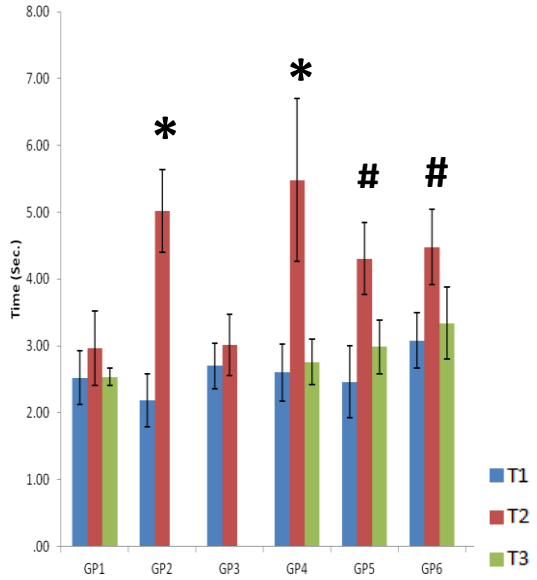

Fig.1: The mean training time (T1) and test time (T2) after $HgCl_2$ administration and after treatment *R. vomitoria* (T3) in Morris Water Maze. Group (GP). *$p \leq 0.05$, #$p \leq 0.01$.

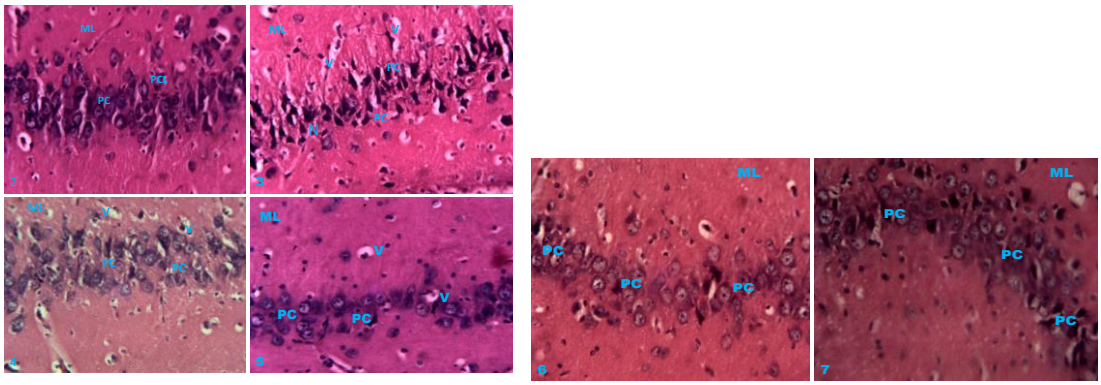

Fig. 2: A Section of CA3 Region of the Hippocampus of the Control Group, showing Pyramidal cell layer (PCL) with normal Pyramidal cells (PC), Molecular layer (ML). Fig. 3: A section of CA3 Region of the Hippocampus of Group 2 showing Pyramidal Cells (P) in region, Area of Vacuolation (V), Necrotic Cells (N) and Molecular layer (ML). Fig. 4: A Section of CA3 Region of the Hippocampus of Group 3 showing Area of Vacuolation (V), Molecular layer (ML), Pyramidal cells (P). Fig. 5: A Section of CA3 Region of the Hippocampus of Group 4, showing Molecular layer (ML), Pyramidal Cells (PC). Fig. 6: A Section of CA3 Region of the Hippocampus of Group 5, showing Molecular layer (ML), Necrosis (N), Normal Pyramidal Cell (P), and Vaculation (V). Fig. 7: A Section of CA3 Region of the Hippocampus of Group 6, showing the Molecular layer (ML), Necrosis (N), Normal Pyramidal cell (P). H&E X250.

The hidden platform version of the Morris water maze test for visio-spatial learning and memory is hippocampus dependant (Eichenbaum *et al*., 1990; McDonald and White, 1994). The overall performance in the Morris water maze experiment after administration of mercuric chloride (T2) showed increase in the time taken to locate the hidden platform when compared to before the administration of mercuric chloride (T1), an indication of the effect of mercuric chloride administration on spatial learning and memory. After administration f RV (T3), there was decrease in the time taken to locate the hidden platform when compared with the time taken after the administration of mercuric chloride; which was an improvement in learning and memory. The present result was consistent with other epidemiological and experimental

animal studies in which mercury exposure was reported to be associated with decreased intelligence, learning and memory ability (Melto-Carpes *et al*., 2013; Sadeeq *et al*., 2013; Francisco *et al*., 2014). The amelioration afforded by RV could be said to be due to its antioxidant ability (Akpanabiotu *et al*., 2009; Bisong *et al*., 2011).

CONCLUSION

Mercury exposure has been shown to induce neurodegeneration in the CA3 region of the hipocampus of the adult Wistar rats and *R. vomitoria* has shown to significantly ameliorate the neurotoxicity induced by mercuric chloride administration. As such, supplement of *R. vomitoria* should be produced for people exposed to mercury poison to consume along with other antioxidants.

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