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Cerebellar and Neuro-Behavioural Evaluation of Adult Wistar Rats in Long-Term Exposure to Refuse Dump Site

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ABSTRACT

The present study examined the possible effects of long term exposure of wistar rats to refuse dump site. Young adult wistar rats of approximately 70 days old were housed in the vicinity of a refuse dump site for 5 months, and studied in the Morris water maze (MWM) for learning and memory, in open field for exploratory and locomotor activity and in elevated plus maze for measures of anxiety. The rats were again taken back to the site and kept for a total of 8 months and again studied in the same apparatus. The histology of the experimental animals showed increase vacuolations in the medulla. Also few classic appearance of eosinophilic (degenerating) purkinje neurons with condensed nucleus and bright eosinophilic cytoplasm were seen. There was significant decrease in the % Time in open arms entry after 5 and 8 months (7.45±3.43, 5.744±1.31) of exposures, when compared to control (35.18±13.55). There was no significant difference in % Open arms entry and number of exploratory head dips on the elevated plus maze. In the open field activity, there was significant difference in line crossings only after 8 months (0.71±0.47) of exposure when compared to control (2.83±0.54) and also to 5 months group (3.25±0.30). There was no significant difference in Latency both at 5 months and 8 months of exposure compared to control. Also there was no significant difference in probe trial value of the exposed rats.

Key word: Open arms, Open field, Morris Water and Elevated Plus mazes

INTRODUCTION

Environmental hazard is the state of events which has the potential to threaten the surrounding natural environment and adversely affect people's health. The menace of environmental pollution has been haunting the human world since early times and is still growing due to excessive growth in developing countries¹. Every year around the world, billions of tonnes of waste are generated from domestic and industrial activities², hospitals and clinics³, an inevitable by-product of human activity¹. Open dumping of solid waste remain the prevailing form of waste disposal in developing countries like Nigeria⁴ due to the low budget for waste disposal and non-availability of trained manpower. It poses serious threat to human health, animals and soil productivity⁵.

Rats and mice are the most commonly used animal models in neuro-behavioural research, they are said to be suited model organisms, as they display a variety of behaviours with relevance to human disease⁶. Learning and memory play an important role in the study of neurological disorders with cognitive components, such as schizophrenia and Alzheimer's disease. The most common spatial navigation task is the Morris water maze⁷⁻⁹. The open field and elevated plus maze are

designed to assess anxiety-like behaviour assess exploratory behaviour in different novel environments⁶. This research is aimed at examining the possible effects of long term exposure to refuse dump site on behaviour parameters and histology of wistar rats.

MATERIALS AND METHODS

Area of Study

The study was conducted in Bayelsa State a picturesque tropical rain forest, with an area of about 21,110 square kilometres. More than three quarters of this area is covered by water. It is geographically located within Latitude 04° 15' North, 05° 23' South and longitude 05° 22' West and 06° 45' East.

Animals

Young adult wistar rats of approximately 70 days old were obtained from Imo State University, Nigeria. The animals were allowed to acclimatize prior to the start of the experiment.

Behavioural Studies

Young adult Wistar rats of approximately 70 days old were housed in the vicinity of a refuse dump site for 5 months, and studied in the open field for exploratory and locomotor activity, in elevated plus maze for

measures of anxiety and tested in the MWM for spatial learning and short term memory. The rats were again taken back to the site and kept for a total of 8 months and again tested on the MWM and behavioural apparatus at the end of the 8th month using the methods of Morris⁹.

Histopathology

The rats were sacrificed at the end of the behavioural studies on the 8th month, the cerebellum was dissected and fixed in 10% formal saline, dehydrated in ascending grades of alcohol, impregnated and embedded in paraffin wax. Paraffin sections (5µm thick) were stained with haematoxylin and eosin (H&E).

Statistical Analysis

Values were represented as mean ± SD. Data were analysed by ANOVA and SNK for multiple comparisons. Significant difference was set at p<0.05.

Ethical Approval

This study was approved by the Bayelsa State Ministry of Environment in 2012

RESULTS

The histology of the experimental animals shows increase vacuolations in the medulla. Also few classic appearances of eosinophilic (degenerating) purkinje neurons with condensed nucleus and bright eosinophilic cytoplasm were seen.

There was no significant difference in Latency (amount of time in seconds it took the animals to locate a hidden platform) both at 5 months and 8 months of exposure compared to control. Also there was no significant difference in probe trial value (number of times animals crossed the position of the platform after it had been removed) of the exposed rats and the control. These findings suggest that exposures to refuse dump site have no effect on learning and memory capabilities of Wistar rats (Table 1). There was no significant difference in % Open arms entry and number of exploratory head dips on the elevated plus maze. These indicate an antigenic-like behaviour in exposed groups as evidenced by the less time in open arms (Table 2). In the open field activity, there was significant difference in line crossings only after 8 months (0.71±0.47) of exposure when compared to control (2.83±0.54) and also to 5 months group (3.25±0.30), thus indicating a possible reduction in locomotor and exploratory activities following prolonged exposure (Table 3).

Table 1: Performance of exposed rats in the MWM

	Control	5 months	8 months
Latency (s)	7.72±0.64	14.85±2.49	14.93±2.31
Probe Trial	6.171±74	5.91±1.00	4.00±1.23

N – 6 (control), 11 (5 months), 7 (8 months)

Table 2: Performance of exposed rats in the Elevated Plus Maze (EPM)

	Control	5 months	8 months
%OAE	47.78±14.95	21.43±6.96	34.27±6.37
%TIO	35.18±13.55	7.45±3.43*	5.744±1.31*
Hdips	6.83±1.70	4.75±1.12	4.714±1.48

Table 3: Performance of exposed rats in the Open Field Maze (OFM)

	Control	5 months	8 months
LC	2.83±0.54	3.25±0.30	0.71±0.47*?
RATW	10.5±1.29	10.33±1.70	4.57±1.96
CSE	0.67±0.33	0.75±0.41	0.14±0.14
CSD	1.77±0.98	2.303±0.28	2.19±0.56

LC – Line crossing, RATW- Rearing against the wall, CSE- Centre square entry, CSD- centre square duration.

* Significant difference compared to control □ Significant difference between 5 months and 8 months groups P<0.05

DISCUSSION

There was no significant difference in Latency (amount of time in seconds it took the animals to locate a hidden platform) both at 5 months (14.85±2.49) and 8 months (14.93±2.31) of exposure compared to control (7.72±0.64). Also there was no significant difference in probe trial value (number of times animals crossed the position of the platform after it had been removed) of the exposed rats and the control. These findings suggest that exposures to refuse dump site have no effect on learning and memory capabilities of wistar rats. Though the latency increased in the 5th and 8th months but was not statistically different from the control (Table 1). Although some authors have reported impairment of learning and memory capabilities in administered toxic substances¹⁰⁻¹² reported memory deficit in animals following exposure to mercury vapour. These substances can be found and inhaled in refused dump sites at varying concentration, base on our results learning and memory was not altered. There was no significant difference in % Open arms entry and number of exploratory head dips on the elevated plus maze. These indicate an antigenic-like behaviour in exposed groups as evidenced by the less time in open arms (Table 2). In the open field activity, there was significant difference in line crossings only after 8 months (0.71±0.47) of exposure when compared to control (2.83±0.54) and also to 5 months group (3.25±0.30), thus indicating a possible reduction in locomotor and exploratory activities following prolonged exposure.

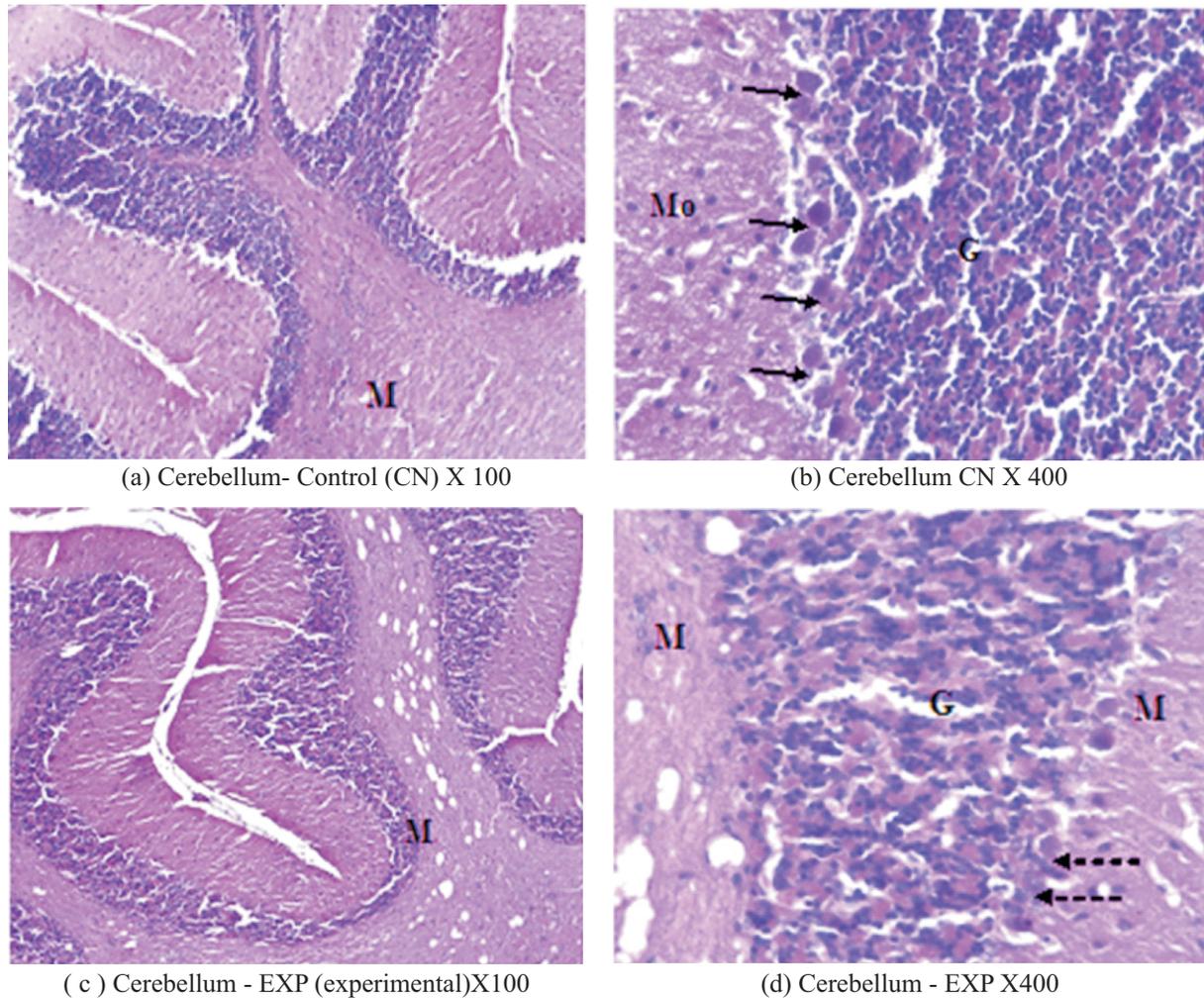


Figure 1 (a-d)

CN- Normal Cerebellum with branching central Medulla (**M**). Three layers of the cortex; Molecular (**Mo**), Granular (**G**) and Purkinje cells (**arrows**). **EXP-** Increased vacuolations in medulla (**M**). Few classic appearance of eosinophilic purkinje neurons with condensed nucleus and bright eosinophilic cytoplasm are seen (**dash arrows**)

Our histological findings which show increase vacuolations in the medulla, few classic appearances of eosinophilic (degenerating) purkinje neurons with condensed nucleus and bright eosinophilic cytoplasm are similar to the findings of Wolf et al.¹² where rats exposed to high concentration of mercury vapour, showed neurodegenerative changes in the hippocampus. It is also similar to the findings of Sadeeq et al.¹³ where the pyramidal cell layer of the hippocampus appears to be damaged with dead cells, and vacuolated spaces.

CONCLUSION

Although our study revealed that exposures to refuse dump site have no effect on learning and memory capabilities of wistar rats, there was presence of degenerating purkinje neurons seen in the cerebellum.

REFERENCES

1. Karishnamurti GSR, Naidu R. Solid-solution equilibria of cadmium in soils. *Geoderma*, 2003; 113:17-30
2. Hester RE, RM. Harrison, "Environmental and Health Impact of Solid Waste Management Activities", *Island Press, Washington*. 2002; 194
3. Pattnaik S, Reddy MV. Assessment of municipal solid waste management in Puducherry (Pondicherry), *India Resour., Conservat. Recycl.*, 2009; 54:512-520
4. Akpoveta OV, Okoh BE, Osakwe SA. Quality Assessment of Borehole Water used in the Vicinities of Benin, Edo State and Agbor, Delta State of Nigeria. *Current Research in Chemistry*, 2011; 3: 62-69.
5. Smith CJ, Hopmans PFJ. Cook Accumulation of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia. *Environ. Pollut.*, 1996; 94 (3): 317-323

6. Cryan J, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov.* 2005; 4:775-90
7. D'hooge R, De Deyn P. Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev.* 2001;36:60-90
8. Brandeis R, Brandys Y, Yehuda S. The use of the Morris Water Maze in the study of memory and learning. *Int J Neurosci.* 1989; 48:29-69
9. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods.* 1984;11:47-60
10. Buraimoh AA, Ojo SA, Hambolu JO and Adebisi SS. Behavioural Endpoints of Adult Wistar Rats, Following Aluminium Chloride Exposure. *British Journal of Pharmacology and Toxicology* 2011; 2(5): 273-276.
11. Jiang S, Su J, Yao S, Zhang Y, Cao F, Wang F, Wang H, Li J, Xi S. Fluoride and Arsenic Exposure Impairs Learning and Memory and Decreases mGluR5 Expression in the Hippocampus and Cortex in Rats. 2014; 23(4):9
12. Wolf U., M.J. Rapoport, T.A. Schweizer. Evaluating the affective component of the cerebellar cognitive affective syndrome, *Journal of Neuropsychiatry and Clinical Neuroscience,* 2003; 21:245-53.
13. Sadeeq AA, Ibegbu AO, Taura MG, Timbuak JA, Adamu LH, Kwanashie HO. Studies on the effects of mercury exposure on spatial learning and memory of adult wistar rats. *International Journal of Pharmaceutical Science Invention* 2013; 2(12):12-16