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IMPAIRED HIPPOCAMPAL-DEPENDENT LEARNING BEHAVIOUR IN RATS EXPOSED TO CADMIUM-TREATED WATER

[1*]Olorunfemi O. J., [2]Okoseimiema S. C. and [1]Azibalua A. A.

[1]Department of Human Physiology, College of Health Sciences University of Port Harcourt, Port Harcourt, Choba, Rivers State, Nigeria.
[2]Department of Human Anatomy, College of Health Sciences University of Port Harcourt, Port Harcourt, Choba, Rivers State, Nigeria.

***Corresponding Author: Olorunfemi O. J.**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba Nigeria.

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ABSTRACT
Cadmium is a toxic heavy metal that exists naturally as a divalent cation complexed with other elements existing in the earth's crust and has been known for causing neurotoxicity but its specific effects on the hippocampal spatial relationship has not been fully elucidated hence the design of this study. The use of the Inverted Screen, Navigation maze box, Hand grip and Rotarod were used at investigating the effects of cadmium toxicity in the hippocampal region of the brain as it affects the spatial behavior in wistar rats in a standard laboratory. Twenty-five albino wistar rats were weighed and placed into five groups of five animals each. Normal saline water was given to the control. Cadmium contaminated water was prepared and administered orally to the treatment groups 2-4 at relative doses of 0.5ml, 0.7ml and 1ml/100g depending on the body weights of the wistar rat. Vitamin E (as antioxidant) was also administered orally to the rats in group 5. Muscular endurance threshold, spatial task and motor balance and coordination were analyzed using the animal's ability to endure and be stable for a period of time after administration of the doses. Adaptive locomotion was also analyzed using the rat's ability to locate the end of the maze box for a period of 5 minutes after administration. Statistical analysis was performed with ANOVA, while Post Hoc multiple comparison test was used in the comparison of the effects to the negative control group and with the Vitamin E as the positive control group. Results obtained were statistically analyzed and showed that some values were statistically significant when compared to both the positive and negative control. From the results, study indicated that there is a direct relationship between Cadmium exposure effect and significant ($p < 0.05$) decline in cognitive activities, motor coordination, stability of extremities and also on memory and learning on albino wistar rat. At a high dose of 1ml/100g, the rats experienced instability of their extremities and also had a significant effect on memory and learning. Therefore, cadmium toxicity as part of its neurotoxic effect, could significantly interfere with hippocampal spatial relationships with regards to spatial learning steady coordination and episodic memory and these effects were dose-dependent as observed in the study.

KEYWORDS: Cadmium, neurotoxicity, Inverted screen, Handgrip, Rotarod, wistar rat.

INTRODUCTION
Long-term exposure to cadmium through air, water, soil, and food leads to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous, and respiratory systems. Cadmium (Cd) being a heavy metal is a naturally occurring one known for its existence as a divalent cation, complexed with other element existing in the earth's crust at about 0.1 part per million.[1] Cadmium is found and recognized as a form of impurity in Zn or Pb deposits smelting.[2] [3] Referred to cadmium as a silvery-white, lustrous, soft, ductile metal known which chemically resembles zinc and is found in many zinc ores through isomorphous replacement. Cadmium is a soft, bluish-white metallic element that occurs naturally in the earth's crust. It is often found as a mineral combined with other elements such as oxygen, chlorine and sulphur. It is classified as carcinogen group 1 by the international Agency for research on cancer.[4] Cadmium metal does not occur naturally. The only cadmium mineral is greenockite (CdS), which exists as a coating on the zinc sulphide ore. It is generally produced as a byproduct from the smelting of these metals, particularly zinc.[5]

Cadmium is majorly used in electroplating of other metals or alloyed with other metals to form easily fusible compounds which can be used as coatings for other

materials and welding and in soldering process.[6] Cadmium compounds are also used in printing, in textiles, in television phosphors, photography, lasers, in semiconductors, solar cells, in dental amalgams and as pesticides. Cadmium is found in superphosphate fertilizers; the levels Cadmium can only be reduced in food and drinking water by reducing food contacts and the uses of agro-chemicals that contains low cadmium compounds and proper disposal wastes from factories.[5] Cadmium in free condition is available as soft, malleable, ductile and moderately active just like stable metals such as zinc and mercury basically due to the oxidation state of +2 like Zinc and low melting point characteristic like mercury that cadmium possesses.[7]

The hippocampus is described as an essential part of the limbic located in the medial temporal lobe of the brain.[8] The hippocampus plays important role in the formation of recent memories in the brain basically due to is connection via the fornix to the mammillary body, which connects via the mammillo-thalamic tract to the anterior nuclei of the thalamus and thus back to the cingulate cortex.[9] Rat hippocampus development of begins prenatally and continues post natally.[10] reported that rat neurons hippocampal formation (the dentate gyrus and the hippocampus) are born over a protracted period, from gestational day G 15 into adulthood.

Cadmium being a toxic heavy metal in the environment is highly accumulative and can be present in air, water, and soil and has been reported to have caused lots of health problem ranging from brain damage, neurotoxicity and several miscarriages.[11] The exposure of cadmium being a toxic metal can be through consumption of contaminated food and water subsequently leading to a toxic effect on the central nervous system. In addition to this aforementioned effect on the body, cadmium is mutagenic, teratogenic, carcinogenic and embryotoxic.[12] [13]

Cadmium exposure through contaminated water can lead to the production of both acute and chronic tissue injury and can damage various organs including lung, liver, kidney, bone, testis and brain depending on the dosage, route and duration of exposure causing several damages (Jarup,[14][15] Reported that cadmium can result to devastating effects on the nervous system basically due to its ability to cross the blood-brain barrier and exert neurotoxic effects.[15] [16] Neural pathology includes diminished brain function and significant decreases in critical neurotransmitters.[17] Different works have been done to show how toxic metals affect the limbic region of the brain but how cadmium contaminated water affects spatial behavior in wistar rats has been scanty. This work is to further investigate the effects of cadmium contaminated water on the limbic hippocampal spatial behavior in wistar rats.

MATERIALS AND METHODS

Experimental animal

A total of Twenty-five (25) albino rats of both sex weighing 100-135g was obtained from the Animal house unit of the Department of Human Physiology and Toxicology, Faculty of Basic Health Science, University of Port Harcourt, Port Harcourt city, Nigeria. The rats were divided into five groups of five rats per group. They were kept in clean disinfected wooden cages with saw dust as beddings in the animal house, with 12 hours light/dark cycle and 50-60% humidity at a temperature of about 30ºC and was provided with clean feed and water. They were also allowed to acclimatize to the new environment for the period of two weeks before the commencement of the experiment.

Drug and Administration

Cadmium salt was sourced from Pure and Industrial Chemistry laboratory from the University of Port Harcourt. and Vitamin E capsule manufactured by S & S Pharmaceuticals were purchased and dissolved in 200ml distilled water and administered orally to the rats.

EXPERIMENTAL DESIGN

Experimental group	Number of rats	Treatment
Group 1 (control)	5	Saline treated
Group 2	5	0.5ml/100g Cadmium salt solution
Group 3	5	0.7ml/100g Cadmium salt solution
Group 4	5	1ml/100g Cadmium salt solution
Group 5	5	200mg/kg Vitamin E solution

Group 1 (control) was allowed access to food and water ad libitum and subjected to adaptive locomotion test, muscular endurance test, endurance threshold and motor balance and equilibrium test in four daily trials per week using the Hand grip, Inverted screen, Navigation maze and the Rotarod apparatuses.

Group 2 was given daily treatment of 0.5ml/100g cadmium salt solution as stated above with subsequent exposure to adaptive locomotion test, muscular endurance test, endurance threshold and motor balance and equilibrium test in four daily trials per week using the Hand grip, Inverted screen, Navigation maze and the Rotarod apparatuses.

Group 3 was given daily treatment of 0.7ml/100g cadmium salt solution as stated above with subsequent exposure to adaptive locomotion test, muscular endurance test, endurance threshold and motor balance and equilibrium test in four daily trials per week using the Hand grip, Inverted screen, Navigation maze and the Rotarod apparatuses.

Group 4 was given daily treatment of 1ml/100g cadmium salt solution as stated above with subsequent exposure to adaptive locomotion test, muscular endurance test, endurance threshold and motor balance and equilibrium test in four daily trials per week using the Hand grip, Inverted screen, Navigation maze and the Rotarod apparatuses.

Group 5 was given daily treatment of 200mg/kg cadmium salt solution as stated above with subsequent exposure to adaptive locomotion test, muscular endurance test, endurance threshold and motor balance and equilibrium test in four daily trials per week using the Hand grip, Inverted screen, Navigation maze and the Rotarod apparatuses. The rats were sacrificed after four (4) weeks of the treatment.

Assessment of Spatial Behavior

For assessing spatial behavior in rats, the following tests were done; Adaptive locomotion test (Navigation Maze box), Muscular endurance threshold test (Inverted screen apparatus), Endurance threshold test (Hand grip apparatus)**,** Motor balance and equilibrium test (Rotarod apparatus).

Adaptive Locomotion Test

The experiment was carried out on the Wistar rats using the Navigation Maze Box with modified method of Sharma and colleagues (2010).

The animals were given the appropriate doses of the cadmium solution through oral route of administration accordingly for two weeks. They were allowed to rest for a period of 5 minutes. Each rat was then put into the navigation maze box (one at a time) and the stop watch was started immediately. The rat was allowed for a maximum time of 5 minutes to locate the end of the maze box. Immediately the rat reached the endpoint, the result was properly recorded and the rats were withdrawn from the box. If the rat doesn't locate the endpoint and the 5 minutes' elapses, the rat was also withdrawn from the box and the result was taken as incomplete.

Muscular endurance threshold test

The test was carried out using the inverted screen apparatus with a modified method of Kondziela, 1964. Each rat was placed at the center of the screen hanging upside down on the inverted screen. Four limbs of the rat were well gripped, after the firm grip, timing started and immediately the rat lost its grip, recording was stopped. Results were taken in seconds.

Forelimb grip strength test

The test was carried out using the hand grip equipment with a modified method of Connolly et al, 2001. Each rat was placed on the hand grip making sure the paws of the fore limbs are firmly gripped. After the firm grip, recording started. Immediately the rat lost its grip, recording was stopped Results were taken in seconds.

Motor balance and equilibrium

The test was carried out using the rotarod apparatus described by Dunham and Miya (1957). Each rat was placed in the rotarod and allowed to maintain balance**.** After the rat was well balanced on the Rotarod apparatus, the rotating beam was turned on, and recording was started**.** The apparatus was rotated gently in a clockwise motion. Immediately the rat lost its balance and fell, recording was stopped. Results were taken in seconds.

Method of Data Analysis

Quantitative data of the trials were obtained, recorded and tabulated on a broadsheet using the Microsoft Excel (Microsoft office 2013). The quantitative data was then analyzed statistically using Statistical Package for Social Sciences Software (SPSS version 22). Results were represented as Mean ± SD and with the ANOVA analysis techniques, these variables were compared. The results were presented in charts. Statistical significance was set at 95% confidence level ($p < 0.05$*).

RESULTS

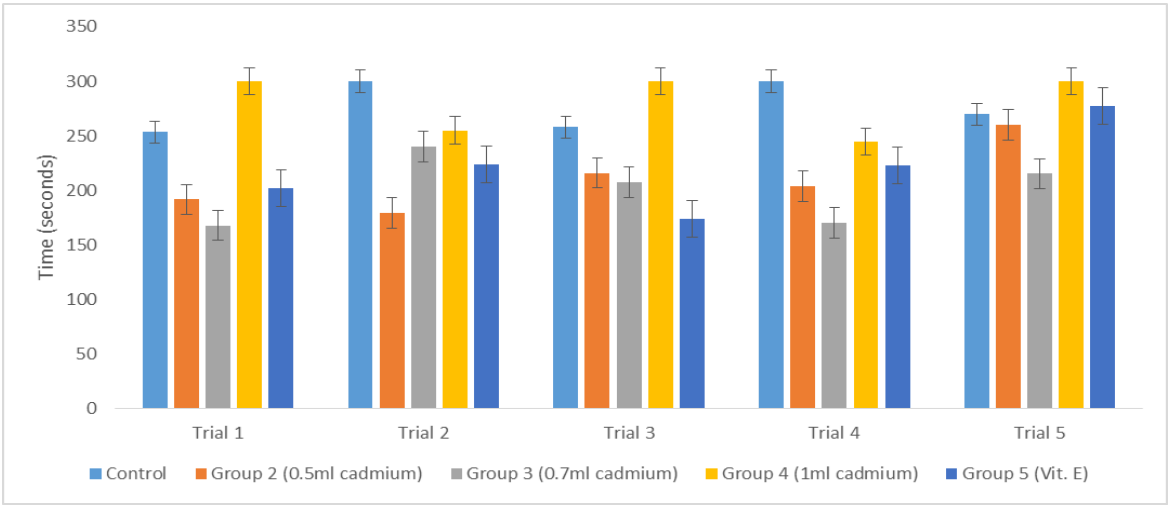

Fig 1: Patterns of adaptive locomotion in the test groups (cadmium treated) and control group in week 2 using Navigation maze test.

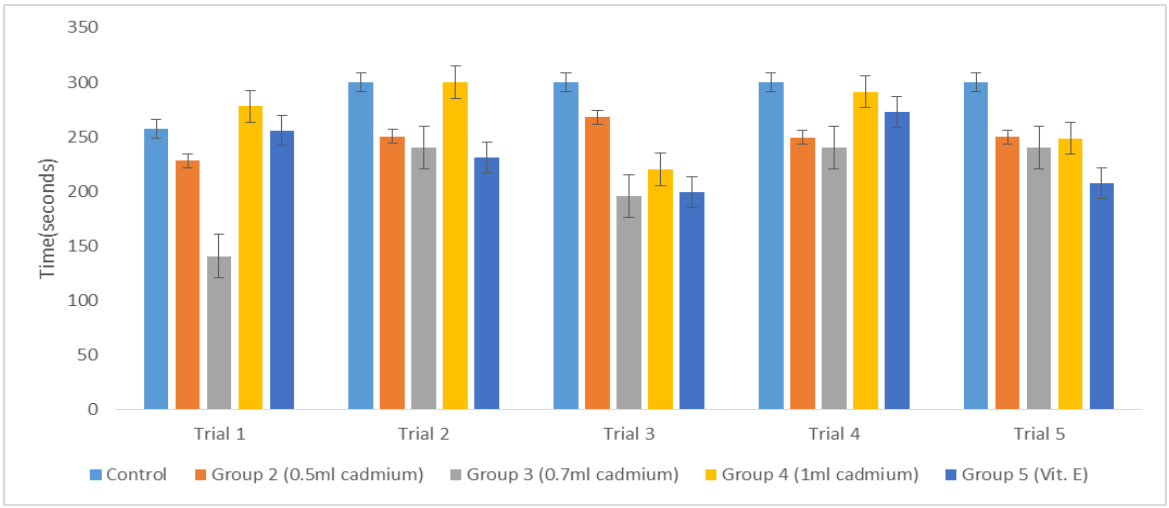

Fig 2: Patterns of adaptive locomotion in the test groups (cadmium treated) and control group in week 4 using Navigation maze test.

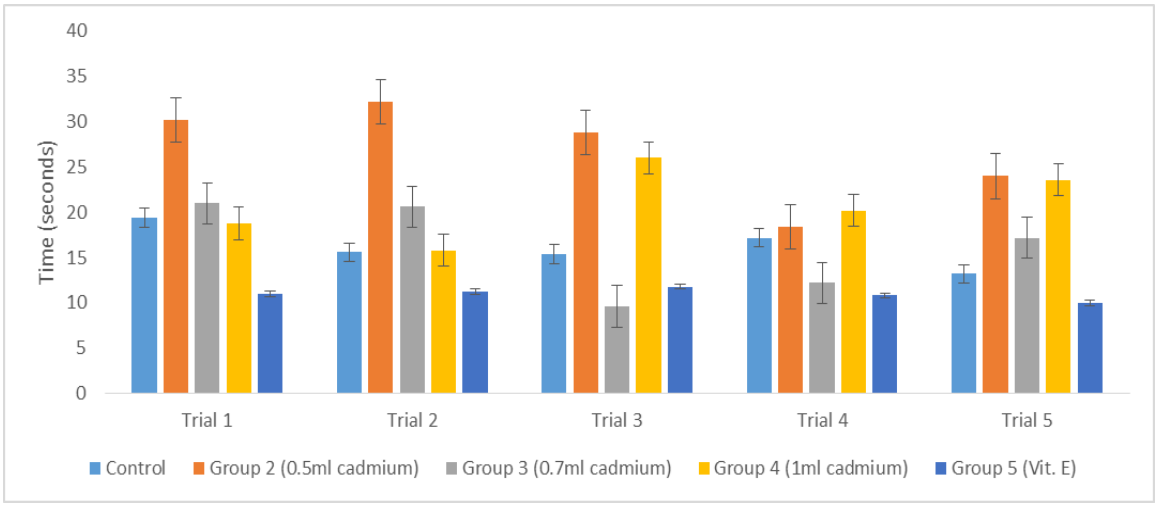

Fig 3: Pattern of muscular endurance threshold using four limbs in the test groups (cadmium treated) and control group in week 2 using inverted screen test.

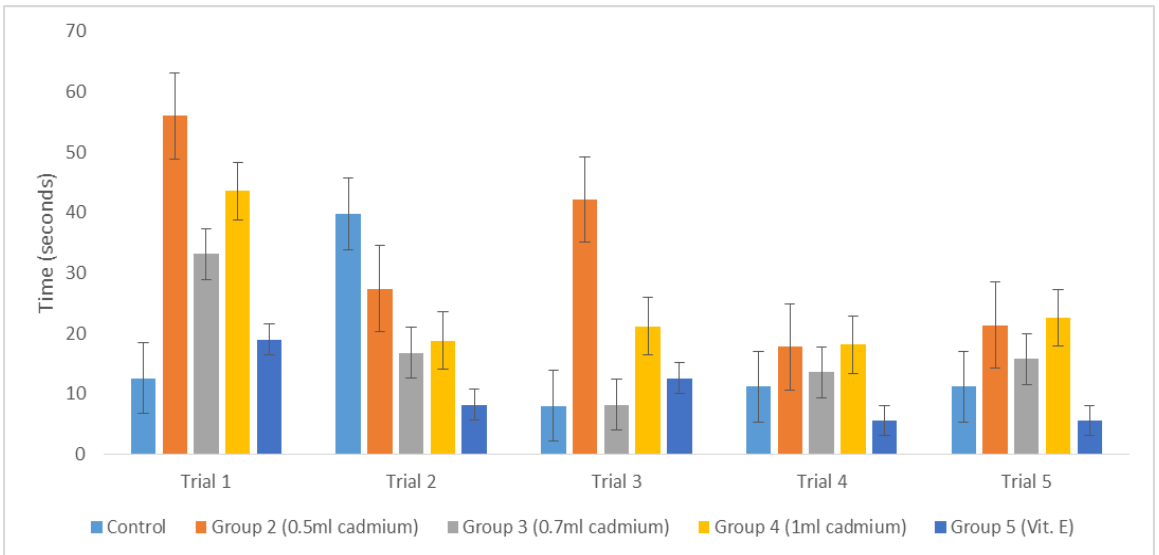

Fig 4: Pattern of muscular endurance threshold using four limbs in the test groups (cadmium treated) and control group in week 4 using inverted screen test.

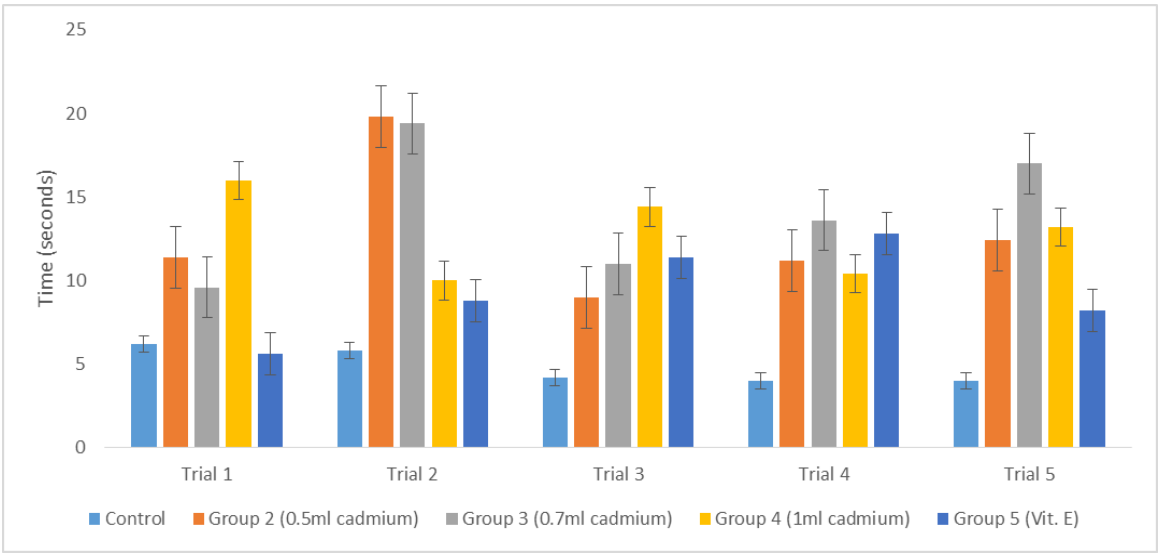

Fig 5: Pattern of endurance threshold in the forelimbs in the test groups (cadmium treated) and control group in week 2 using Hand-Grip test.

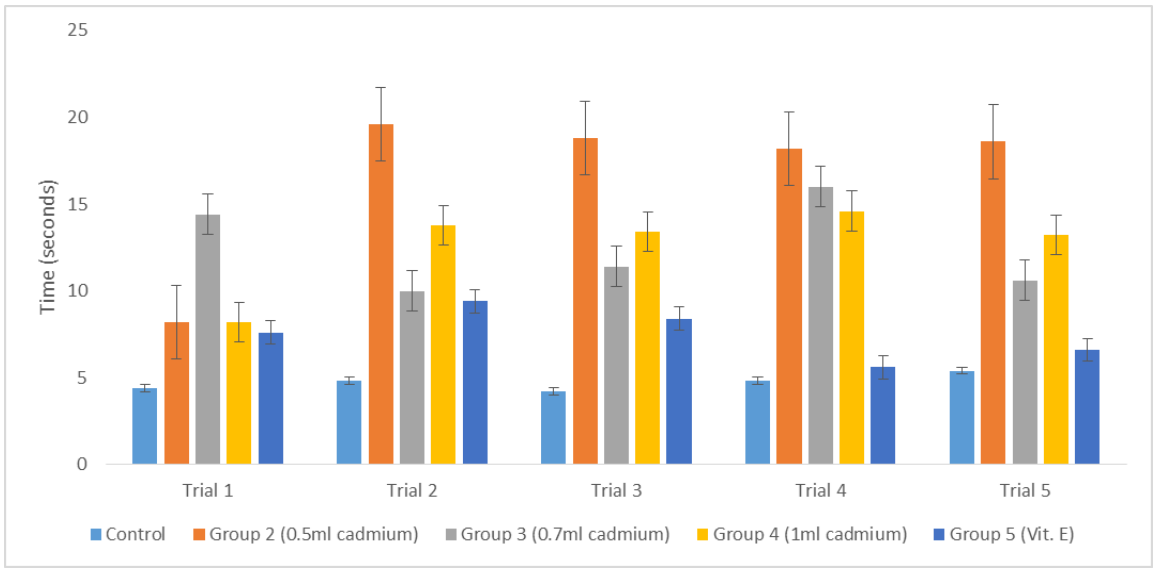

Fig 6: Pattern of endurance threshold in the forelimbs in the test groups (cadmium treated) and control group in week 4 using Hand-Grip test.

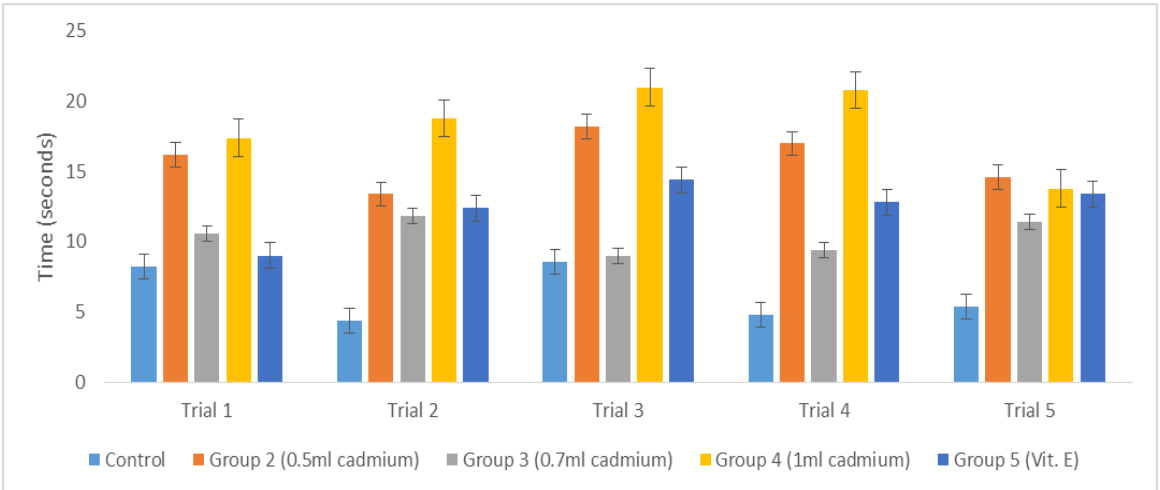

Fig 7: Pattern of motor balance & equilibrium in the test groups (cadmium treated) and control group in week 2 using Rota rod balance test.

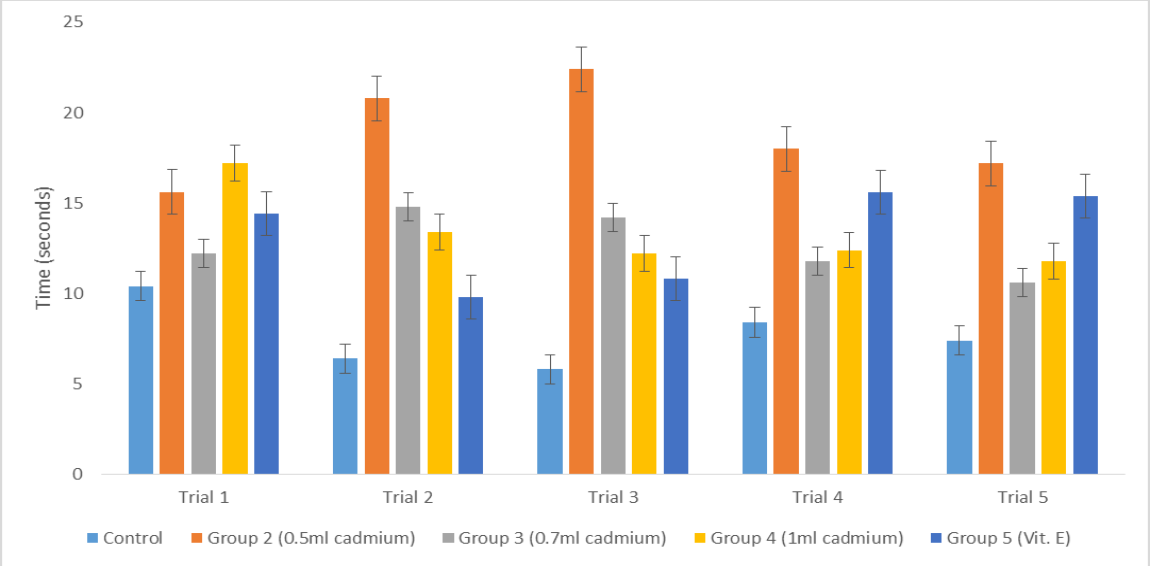

Fig 8: Pattern of motor balance & equilibrium in the test groups (cadmium treated) and control group in week 4 using Rota rod balance test.

DISCUSSION

This present study demonstrated the effect of cadmium contaminated water on the limbic hippocampal spatial behavior in wistar rat. The result showed that chronic Cd exposure caused severe impairment of hippocampal neurons. This study provided direct evidence that relates to the progressive deterioration of the limbic hippocampal as a result of Cd contaminated water.

Navigational maze test is a test for cognitive motor function for rats that rely on cues to navigate from start location to exit end through a labyrinth.[18] Navigational maze is employed in behavioral neuroscience to study spatial behavior and could be a very precise study in learning, memory and spatial working and is also capable of accessing damages to cortical regions of the brain. From the current study, the navigational test involved five trials for the total period of four weeks.

Figure 1 and figure 2 revealed the result of patterns of adaptive locomotion in the test groups and control group in week 2. For week 2 and week 4, there appeared to be a gradual distortion in the time spent in navigating the entire stretch of the box in the test groups, though there was no significant difference in the groups including the control except at trial 5 in week 2 where the declining effect of cadmium was been noticed. In week 4, cadmium administration became more debilitating as it slowed down the spatial task performance time and deranged the quality of task performance and the effects appeared to be dose and time-dependent. The general result of the adaptive locomotion test indicates that cadmium affected the general performance of the rats thereby decreasing their ability to locate the exit point of the maze box. The results suggested that a 1-month exposure of Cd affected the memory and learning of the rats. This is in line with the result of Pulido[19] [20] where it was obtained that Cd ingested rat took more than a month for any effect to occur on the spatial behavior of wistar rats.

The inverted screen test (figures 3 & 4) is a test of muscle strength, coordination and balance using all four limbs. It was performed on the trials at different level of treatment to test for the animal's strength, coordination and balance for four weeks. Results from the second week showed that the ability to hang on depreciated significantly form from group 2 to group 4 treated with cadmium. The falling off the screen showed huge unsteadiness and motor weakening as firm gripping became deteriorated by the administration of cadmium. In week 4 of study, the effect of cadmium significantly impaired the agility and endurance of the animal and when compared to the Vit E treated group and the negative control group, the difference was significant ($p< 0.05$). The general result for inverted screen test revealed changes in the rats used for the trials in terms of coordination which can be associated with level of exposure of the animal brain to Cd producing a long-term impairment and changes in the development of the motor coordination. The result gotten from this test also showed the impairment of the extremities of the rats. The general result obtained for inverted screen test (muscle strength and coordination balance) compare favorably with what.[21][22] Obtained but however contradict.[19] Result where it was obtained that rats fed with Cadmium substances had an impairment in motor coordination which restricted their coordination balance.

The handgrip test as shown in figures 5 & 6 is regarded as a neuromuscular assay of muscle strength. It was performed on the trials at different level of treatment to test for the animal's strength for four weeks. For week 2, the hand grip test results for both control and cadmium treated group showed that animals in negative control group spent a significantly shorter time on the tasks compared to groups 2, 3, & 4. The result obtained

showed that at higher dosage rate of 0.7ml Cd in the subsequent week, cadmium treated group developed anxieties making them unstable compared to the lower dosage of treatment. The general result obtained from the handgrip test showed that at higher dosage rate of cadmium, Cadmium affected stability of extremities in rats basically due to the fact that cadmium treated animals spend a statistically significantly longer time on the task compared to control animals. This indicates that cadmium treated animals developed neuromuscular toxicity compared to the control group. However, the study of.[23] At a lower dose showed a lower stability level on cadmium injected rat which quite disagreed with this finding. However.[24] Result compared favorably with this finding where it was reported that rats fed with 25mg/kg Cd diet showed a higher degree of instability and anxiety.

Rotarod test is solely meant for the evaluation of motor coordination, balance and motor learning, it was performed on control and cadmium treated group for four weeks. From the result (figures 7 & 8), it was seen that the cadmium tested group interestingly did better than the control group. The result obtained at 0.5ml cadmium showed that cadmium affected the performance of the rat for the second week of the treatment phase. Subsequent week trials at minimal treatment were not significantly affected. At 0.7ml cadmium, changes on the rat commenced drastically where it was observed that there were changes at different level of the treatment on the trials which affected the cognitive activities of the albino rat used for this study as this rate in Cd ingestion on the rat altered cognitomotor stability, and anxiety level of the rats. This basically may be due to the fact that the effect of Cd on rat distorted the excitability of the rat neurons blocking the calcium channels at the level of the membrane in the presynaptic terminals subsequently causing a form of dysfunction in the motor balance and coordination. This present study is in alignment with the study of Damasio.[24] [21] where shock sensitivity was detected at different dosage rate of Cd on treated rat.

CONCLUSION

In view of the above findings and discussion, it is imperative to say that cadmium accumulation in the brain can damage brain hippocampal spatial relationships. The result from this study indicated that there is a direct relationship between Cd exposure effect and decline in cognitive activities, motor coordination, stability of extremities and also on memory and learning on albino wistar rat. At a high dose of 1ml, the rats experienced instability of their extremities and also had a significant declining effect on memory and learning. Therefore, cadmium toxicity as part of its neurotoxic effect, could significantly interfere with hippocampal spatial relationships with regards to spatial learning steady coordination and episodic memory and these effects were dose-dependent as observed in the study.

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