Interference of Cadmium and Repetitive Concussion on Cognitive Functions in Adult Wistar Rats.

Frank, Peace Udochi, Olorunfemi O.J.
Department of Human Physiology, College of Health Sciences University of Port Harcourt,
Port Harcourt, Choba, Rivers State, Nigeria.
olorunfemi.oluwadare@uniport.edu.ng

Abstract - Concussion is a brain injury with complex pathophysiological process induced by traumatic biomechanical forces and results to affectations to the neurocognitive domains. Exposure to Cadmium, a major global pollutant, which has found extensive use in industries also exerts several toxic effects on the brain by several mechanisms. Hence the need to examine the effects of administration of Cadmium and repetitive concussion on cognitive functions (learning, memory and perception) in adult Wistar Rats. This study was carried out on 25 rats which were divided into five groups as negative control group, 5 mg/kg b.w. Cadmium-only Group, Repetitive concussion only group, 5mg/kg b.w. Cadmium+ repetitive concussion group and standard drug group which received Epinephrine. The animals were allowed to perform cognitive/spatial tasks (i.e. navigational maze, elevated plus maze, Barnes maze, passive avoidance and inverted screen tests), in a four-day period with five trials each day. The findings showed that repetitive concussion can be implicated in the cases of short term impairment in spatial working memory, spatial reference memory, cognitive flexibility, as well as impairment in acquisition, consolidation and recall, as well as a state of anxiety, while the administration of cadmium could be said to demonstrate its adverse neurocognitive impact according to this study in the area of short term impairment in spatial working memory, spatial reference memory and cognitive flexibility as well as impairment in the acquisition, consolidation and recall. A combination of the two factors showed a progressive loss of motor skills and anxiety.

Keyword; Concussion, Cadmium, Navigation maze, Barnes maze, Cognition.

Introduction

Cognition is a psychological term that includes the processes of learning, memory and attention, as well as perception, language,intelligence, and reasoning. Cognitive phenomena are essentially internal psychological processes which, from the experimentalpoint of view, must be inferred from overt changes in an organism's behaviour (1). Furthermore, executive function refers to cognitive processing that can be described as top-down, active manipulation of information or control of behavioural states (2), which are mediated by overlapping but unique prefrontal cortical circuits and include attention, initiation, set-shifting or mental flexibility,organization, abstraction, planning and problem-solving (3,4).

Concussion defined as a clinical syndrome involving a disturbance in brain function that is generally time-limited and results from biomechanical forces, such as a bump, blow, or jolt to the head or body (5,6). In addition, an earlier definition by McCrory *et al*. (7), referred concussion to a sudden and transient alteration in consciousness induced by traumatic biomechanical forces transmitted directly or indirectly to the brain. Ropper and Gorson (8) also reported that there may or may not be loss of consciousness, although a concussion typically does involve some period of transient amnesia. Cadmium (Cd) is a silvery-white, soft, ductile chemical, heavy metal and a naturally occurring (sparsely distributed) element which is found in the Earth's crust with a concentration of 0.15ppm, as well as relatively poor abundance (64^{th} amongst elements) in the earth's crust (9,10). Its interference with brain function is the focus of this study.

Materials & Method

Animals

A total of twenty-five (25) adult male wistar rats which weighed about 140 – 190 g were used for this study. The animals were purchased from the animal house of Department of Human Physiology, University of Port Harcourt, and housed in clean disinfected wooden cages with saw dust as beddings, with 12hours light/dark cycle and 50-60% humidity at a temperature of about $30^{o}C$ and were allowed to acclimatize to the new environment for two weeks, with free access to clean water and animal feed (standard finisher feeds, Top feed, Nigeria) before the experimental processes began.

Experimental Induction of Concussion

The animals for the study were exposed to mild skull knock with hammer for 5 times per minutes daily. This was done to set up an agitation within the skull, though not hard enough to knock off consciousness in the rats. Appropriate observable characteristics were scored and recorded according to the modified method of Smith *et* aI. (11)

Experimental Intoxication of Cadmium

Cadmium chloride ($CdCl_2.H_2O$, 99% pure) was purchased from Sigma Chemicals (St Louis, MO, USA) was used for this study. The animals in the group which was administered with cadmium receive 5 mg/kg BW Cd^{2+} intraperitonially. The Cd intoxication protocol was chosen based on published research (12).

Animal grouping & Treatment

The entire animals for the study were weighed and randomly divided into five groups of five animals each. The animals in group I which served as the control group were given intraperitoneal injection of 0.5 mL of physiological saline, clean water and feed only, without any of treatment. Those in Groups II were administered with 5 mg/kg BW Cd^{2+} i.p., as well as clean water and feed. Animals in group III were exposed to concussion only using the Skull Hammer and as well as given food and clean water. Group IV animals received 5mg/kg BW of Cadmium plus induced with concussion while Group V animals served as the standard group which received epinephrine.

Determination of Cognitive function

The following experimental protocols were followed in the investigation of the effects of administration of Cadmium and concussion on the cognitive activities (learning, memory & perception) of the animals. This was carried out in a quiet laboratory by using the Barnes-maze, passive –avoidance test, Navigational task and the elevated plus maze.

Navigational Task

This widely used test is essential in behavioural neuroscience to study spatial learning and memory, as it measures both cognitive and motor functions in rats. In this study, each rat was placed in an opaque maze of length 153.1 cm, monitored and the time it took to navigate through the box to the other end was noted using a stop watch. The principle governing the test is based on the ability of the rats to use either spatial or cue information to solve the task. Hence it is used to basically test mnemonic function in rats as the animals are allowed to find their way through the environment without getting lost. This requires memory for locations and routes.

Barnes – Maze Test

This task was designed over four decades ago (1979) and is still found useful and appreciated till date as a means of assessing spatial working memory, spatial reference memory (short and/or long term) and cognitive flexibility (13). This test consists of an elevated circular surface with equally spaced holes (8) of uniform diameter around the edge and one of them with a darkened escape hole that rats can escape the maze.

Inverted Screen Test

Inverted screen test is a Sensorimotor test which evaluates the muscle (grip) strength of the animal. It uses the inverted screen apparatus to evaluate the behavioural motor function (motor skills and muscle strength) of the rat in task performance, with time. The principle is based on the grip strength of the animals in an inverted position by calculating the hanging duration, or the ability to hang on to objects of varying weight. It follows the following procedureas described previously (13, 14).

Passive – Avoidance Test

This stands as a useful test which uses an equal 2-part Plexiglas box that has bright and dark parts for evaluating the effects of novel chemical entities on learning and memory as well as studying the mechanism involved in cognition. In accordance with the guidelines of the American psychological association, a minimal amount shock intensity needed to motivate the animal was used in this task. However, no aversive stimulus applied to animals upon re-entry into the dark compartment during testing. The method used was in line with the study of Rezazadeh, Ahmadifar and Manesh (15),

Elevated Plus Maze

The procedure for this test was carried out as previously described (16,17) and briefly described as follows; The animal was placed on an elevated maze of 32cm high having four open arms of 14.2cm (diagonally). The animal was placed in the center of the four arms and the stop watch started. The time taken for the animals to go through the four arms (both open and enclosed arm entries) was recorded as the Transfer latency (TL), the time (in seconds) taken by the animal to move from the open arm into any one of the covered arms with all its four legs. Entry into an arm was defined as the point when the animal places all four paws into the arm.

Statistical Analysis

The statistical analysis for this study was performed using Statistical package for Social sciences (SPSS) software version 23. The quantitative data were represented in the charts and graphs, while qualitative data from the behavioural study was represented in tables. The variation and the statistical significance of the differences between the groups were determined by Analysis of Variance (ANOVA) and Turkey post Hoc test.

Ethical Considerations

An approval for this study was sort from the center for Research ethics and management of the University of Port Harcourt prior to the commencement of the study.

Results

Table 1: *Effects of Cadmium and Concussion on cognitive function of wistar rats.*

Navigation Maze Task

Group	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Control group	25.20 ± 3.35	31.20 ± 3.35	34.00 ± 6.83	23.09 ± 6.96	31.20 ± 3.67
Cadmium group	67.54 ± 58.46	93.40 ± 52.04	80.43 ± 55.27	91.80 ± 52.28	78.83 ± 55.65
Concussion group	11.91 ± 9.06	134.89 ± 68.03	20.52 ± 8.15	74.09 ± 56.75	69.44 ± 57.69
Cad + Con group	7.06 ± 4.25	5.07 ± 6.25	6.35 ± 4.46	8.54 ± 7.38	9.49 ± 8.16
Epinephrinegroup	15.85 ± 5.62	28.40 ± 6.26	29.20 ± 7.73	24.07 ± 8.96	34.80 ± 8.14

Data are expressed as mean ± SEM, n=5 (time ± s)

Table 2: *Effects of Cadmium and Concussion on cognitive function of wistar rats.*

Barnes Maze Task

Group	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Control group	21.47 ± 9.11	75.20 ± 56.34	18.49 ± 6.55	72.20 ± 57.07	18.21 ± 7.14
Cadmium group	65.88 ± 58.57	74.88 ± 56.81	64.87 ± 58.84	67.50 ± 58.25	74.65 ± 56.92
Concussion group	30.30 ± 10.80	82.60 ± 54.63	72.22 ± 57.08	72.22 ± 57.08	70.08 ± 57.63
Cad + Con group	10.40 ± 4.82	19.60 ± 8.97	15.20 ± 8.14	15.20 ± 8.14	11.60 ± 5.31
Epinephrine group	15.40 ± 3.79	15.60 ± 3.14	23.80 ± 8.37	23.80 ± 8.37	20.00 ± 7.77

Data are expressed as mean ± SEM, n=5 (time ± s)

Table 3: *Effects of Cadmium and Concussion on cognitive function of wistar rats.*

Inverted Screen Test

Group	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Control group	14.52 ± 6.52	25.40 ± 8.77	23.20 ± 6.73	23.00 ± 8.49	23.80 ± 5.95
Cadmium group	11.60 ± 4.02	10.20 ± 3.35	11.40 ± 4.93	8.20 ± 3.04	7.40 ± 2.42
Concussion group	8.60 ± 3.97	8.80 ± 3.66	6.80 ± 2.81	6.80 ± 2.85	5.40 ± 2.29
Cad + Con group	5.80 ± 1.85	10.60 ± 2.97	9.20 ± 2.22	10.00 ± 2.00	8.80 ± 2.39
Epinephrine group	9.00 ± 1.76	7.40 ± 0.93	6.80 ± 1.32	7.60 ± 0.81	7.00 ± 1.00

Data are expressed as mean ± SEM, n=5 (time ± s)

Table 4: *Effects of Cadmium and Concussion on cognitive function of wistar rats.*

	Passive Avoidance Test				
Group	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
Control group	76.47 ± 56.14	81.69 ± 55.01	80.44 ± 55.31	79.89 ± 55.37	82.26 ± 55.17
Cadmium group	17.80 ± 10.02	18.80 ± 10.15	23.60 ± 11.21	8.23 ± 5.16	19.80 ± 9.53
Concussion group	124.80 ± 71.56	121.60 ± 72.83	69.43 ± 57.91	66.49 ± 58.46	67.22 ± 58.35
Cad + Con group	69.27 ± 58.01	125.00 ± 71.52	129.00 ± 70.02	132.20 ± 69.03	127.40 ± 70.55
Epinephrine group	129.55 ± 70.04	138.20 ± 66.63	70.82 ± 57.94	68.20 ± 58.27	69.96 ± 58.08

Data are expressed as mean ± SEM, n=5

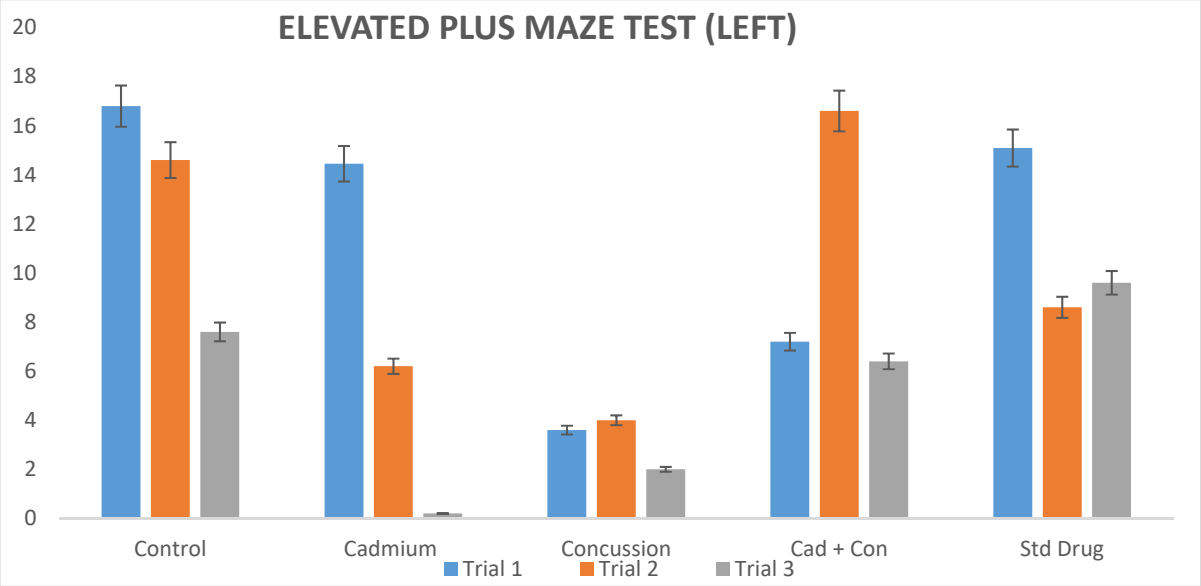

Figure 1: *Graphical representation of the result of the elevated plus maze test (left) on the effects of Cadmium and Concussion on cognitive function of wistar rats.*

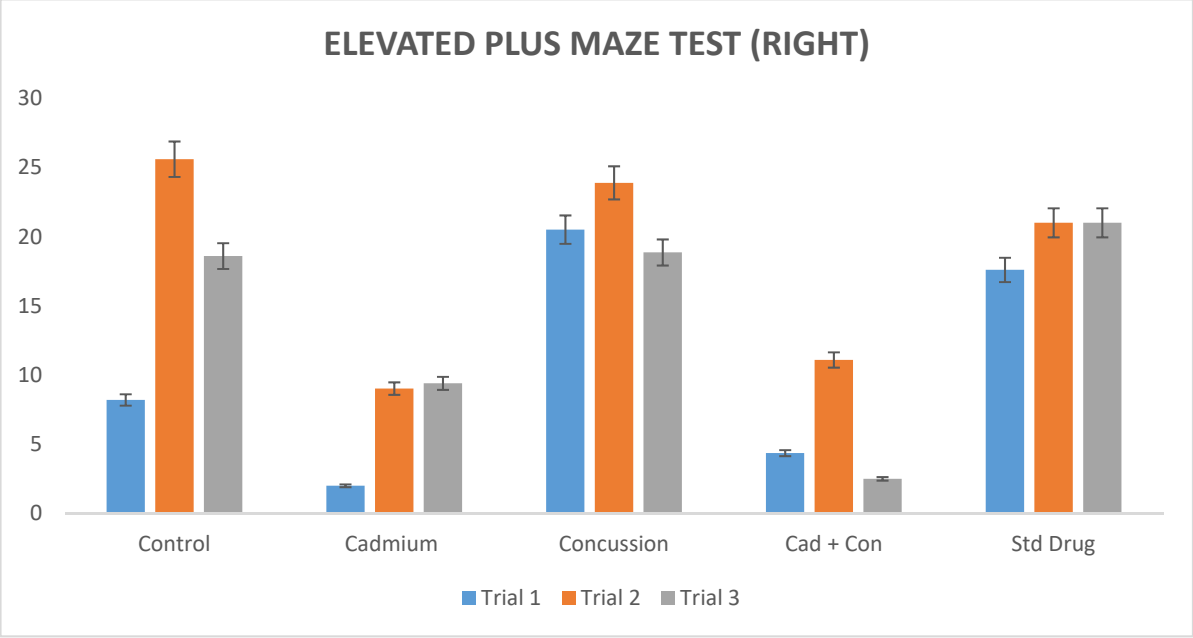

Figure 2: *Graphical representation of the result of the elevated plus maze test (right) on the effects of Cadmium and Concussion on cognitive function of wistar rats.*

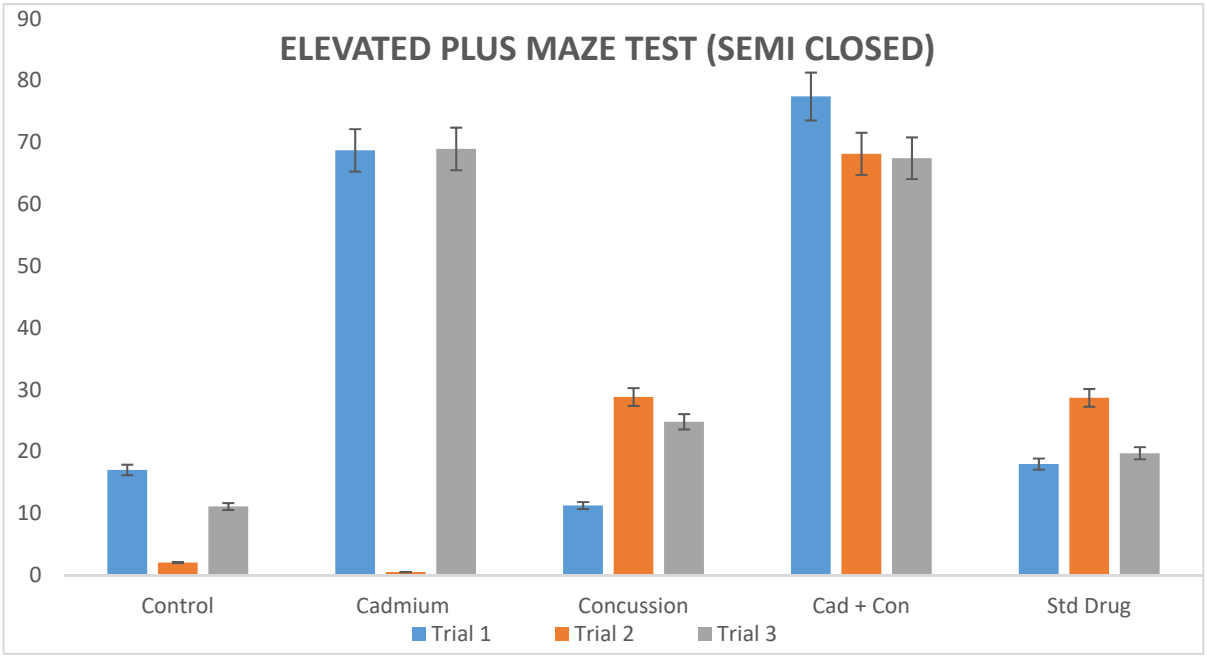

Figure 3: *Graphical representation of the result of the elevated plus maze test (semi-closed) on the effects of Cadmium and Concussion on cognitive function of wistar rats*

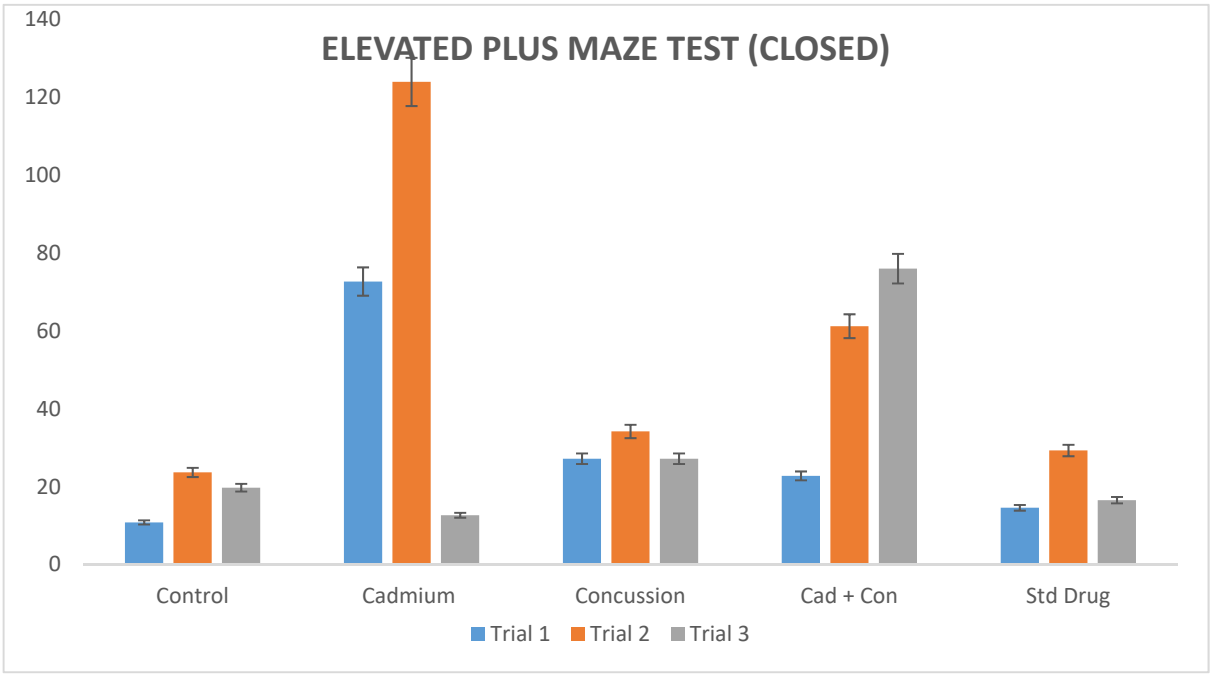

Figure 4: *Graphical representation of the result of the elevated plus maze test (closed) on the effects of Cadmium and Concussion on cognitive function of wistar rats during day four.*

Discussion

The effect of exposure to environmental pollutants and toxins such as heavy metal on the brain and nervous system has been the subject of many researches on recent times. According to Kumar *et al*. (18), the interactions between an organism and its environment are known to influence and evoke neurobehavioral changes. It has since been proven that the mechanism of their action revolves around Cytotoxicity which leads to necrosis and apoptosis, oxidative stress and lipid peroxidation, immunotoxicity and neurotoxicity (19, 20, 21, 22). Another issue of concern to the public health with serious consequence on the nervous system especially in the area of cognition is repetitive concussion as a result of several voluntary or involuntary activities as well as accidents. It has been said to be able to affect attention and concentration, cognitive processing speed/efficiency, learning and memory, working memory, executive function and verbal fluency (23,24). Hence, the aim of this study was to determine the effects of administration of Cadmium and repetitive concussion on cognitive functions in adult

Wistar Rats, using the navigational task, Barnes-Maze test, passive avoidance model, inverted screen model and elevated plus maze tasks.

The findings of the study based on the completion of navigational task showed that exposure to cadmium and repetitive concussion in the animals had only a short term negative impact on navigational performance of the animals while a combination of the two had no noticed negative effect on the navigational performance of the rats as a result of the time taken to complete the navigational task when compared with the control group. In contrast to what was observed in this study, despite being a known neuro-toxicant, cadmium administration did not affect cognitive functioning of the experimental animals used in the study of Wang and Du (25). However, in the study of Wang *et al*. (26), it was observed that Cd exposure in animals for 20 weeks, impaired hippocampus dependent spatial working memory in male mice.

Looking at the results obtained from the Barnes-maze test on the spatial working and reference memory of the Wistar rats, it was observed that administration of cadmium and exposure to repetitive concussion alone had short term (instant) effect on the cognitive performance of spatial working memory, spatial reference memory (short and/or long term) and cognitive flexibility of the animals with respect to the control group. However, combination of the two had no noticeable effect on cognitive performance of spatial memory of the animals with respect to the control group, as a result of the continuous reduction in the escape latency time recorded in the study. This result did not totally agree with that of a population wide study conducted in china by Emsley *et al*. (27) which did not detect an association between cognitive score and water cadmium levels. In contrast with the finding of this study, a small study by Hart, Rose, & Hamer (28), which evaluated occupationally exposed workers from a refrigerator coil manufacturing plant, found that workers with higher urinary cadmium levels over time, had worse performance in tests of attention/psychomotor speed, and memory. In another similar study of 89 workers (42 exposed and 47 control), urinary cadmium was significantly associated with poor visuomotor performance (symbol digit substitution and simple reaction time tests) (29). These effects may be attributable to longer and greater level of exposure to cadmium among the workers.

The inverted screen test analyses the muscle (grip) strength of the experimental animals using all four limbs by taking advantage of the animal's tendency to grasp the wire mesh in an inverted position. The data generated from the study showed that the combined exposure to cadmium and repetitive concussion brought about a continuous decrease in the duration of the inverted screen test, while those in groups two (cadmium) and three (concussion) showed no sign of any significant effect in the grip strength of the animals when compared with those in groups 1 (control) and 5 (epinephrine). This data implies that the combined exposure to cadmium and repetitive concussion leads to a progressive loss of motor skills.

Passive avoidance test has been instrumental in ascertaining learning and memory in wistar rats as a result of changes in the step-through latency. As stated in the study of Roediger *et al*. (30), the passive-avoidance task is a hippocampal and amygdala-dependent test which evaluates long-term (24 h) emotional memory, based on contextual-fear conditioning and instrumental learning to avoid an inescapable electrical shock, and longer retention latencies indicate a better learned experience. In the test, the animals upon exposure to the first trial are expected to acquire the information that entry into dark chamber results in painful experience of electric shock, and the cognitive ability of the animals was reflected by avoidance of the entry (31). It was seen from the experimental data that the groups administered with cadmium as well as those exposed to repetitive concussion developed a significant impairment in acquisition, consolidation and recall of a passive avoidance response as seen in the decline in the step-through latency. However, a combination of the two in the experimental animal showed an opposite result as seen in the steady increase in step-through latency which demonstrates an improvement in short and long-term memory and learning function of the brain. The results of the present study show that administration of cadmium as well as exposure to concussion in rats induces a significant learning and memory disturbance in passive avoidance paradigm. In another study where the experimental animals were stressed, the passive avoidance test revealed impaired memory retention while an earlier study by Lehotzky *et al*. (32) found that prenatal exposure to Cd significantly retarded the acquisition of the conditioned escape response in rats. Some more recent studies have shown that Cd exposure also decreases the step-down latency in inhibitory avoidance task in rats (33,34,35).

Conclusion

This study was carried out to ascertain the effect of administration of cadmium and exposure to repetitive concussion on cognitive functions of adult male wistar rats. The findings show that repetitive concussion can be implicated in the cases of short term impairment in spatial working memory, spatial reference memory, cognitive flexibility, as well as impairment in acquisition, consolidation and recall, as well as a state of anxiety. In the same vein, administration of cadmium could be said to demonstrate its neurocognitive effect according to this study in the area of short term impairment in spatial working memory, spatial reference memory and cognitive flexibility as well as impairment in the acquisition, consolidation and recall. A combination of the two factors showed a progressive loss of motor skills and anxiety.

References

[1] Ajao, D. O., Pop, V.,& Kamper, J. E. (2012). Traumatic brain injury in young rats leads to progressive behavioral deficits coincident with altered tissue properties in adulthood. J Neurotrauma., 29, 2060 – 2074.

[2] Cummings JL (1993) Frontal-subcortical circuits and human behavior. Archives of Neurology 50(8): 873–880.

[3] Alvarez, J. A., &Emory, E. (2006). Executive function and the frontal lobes: A meta-analytic review. Neuropsychology Review, 16, 17–42.

[4] Miller EK and Cohen JD (2001) An integrative theory of prefrontal cortex function. Annual Review of Neuroscience 24: 167–202.

[5] Giza, C. C., J. S. Kutcher, S. Ashwal, J. Barth, T. S. D. Getchius, G. A. Gioia, G. S. Gronseth,K. Guskiewicz, S. Mandel, G. Manley, D. B. McKeag, D. J. Thurman, and R. Zafonte.2013. Evidence-Based Guideline Update: Evaluation and Management of Concussion inSports. Report of the Guideline Development Subcommittee of the American Academy of Neurology. American Academy of Neurology.

[6] Harmon, K. G., J. A. Drezner, M. Gammons, K. M. Guskiewicz, M. Halstead, S. A. Herring,J. S. Kutcher, A. Pana, M. Putakian, and W. O. Roberts. 2013. American Medical Societyof Sports Medicine position statement: Concussion in sport. British Journal of SportsMedicine 47(1):15-26.

[7] McCrory P, Meeuwisse W, Johnston K, et al. Consensus Statement on Concussion in Sport: the 3rd International Conference on Concussion in Sport held in Zurich, November 2008. Br J Sports Med 2009; 43(1): i76–90.

[8] Ropper AH, Gorson KC. Clinical practice. Concussion. N Engl J Med 2007;356:166–72.

[9] Sarkar, A., Ravindran, G., & Krishnamurthy, V. (2013). A Brief Review On The Effect Of Cadmium Toxicity: From Cellular Toorgan Level. International Journal of Bio-Technology and Research, 3(1), 17-36

[10] Sharma, H., Rawal, N., Mathew, B. B. (2015). The Characteristics, Toxicity And Effects Of Cadmium. International Journal of Nanotechnology and Nanoscience, Vol. 3, 2015, 1-9

[11] Smith DH, Wolf JA, Lusardi TA, Lee VM, Meaney DF. High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. Journal of Neuroscience. 1999;19(11):4263–4269.

[12] Casalino, E., Calzaretti, G., Sblano, C., Landriscina, C., 2000. Cadmium-dependent enzyme activity alteration is not imputable to lipid peroxidation. Arch. Biochem. Biophys. 383, 288–295.

[13] Wozniak DF, Hartman RE, Boyle MP, Vogt SK, Brooks AR, Tenkova T, Young C, Olney JW, Muglia LJ (2004) Apoptotic neurodegeneration induced by ethanol in neonatal mice is associated with profound learning/ memory deficits in juveniles followed by progressive functional recovery in adults. Neurobiol Dis 17:403– 414.

[14] Langford-Smith A, Langford-Smith KJ, Jones SA, Wynn RF, Wraith JE, Wilkinson FL, et al. Female mucopolysaccharidosis IIIA mice exhibit hyperactivity and a reduced sense of danger in the open field test. PloS one. 2011; 6(10):e25717. Epub 2011/10/27.

[15] Rezazadeh, M., Ahmadifar, M., Manesh, M. A. (2018). The Study of Effect of Amphetamine on Passive Avoidance Learning in Wistar Male Rats. Advances in Applied Physiology, 3(1): 1 – 7

[16] Adeyemi, O. O., Akindele, A. J., Yemitan, O. K., Aigbe, F. R., &Fagbo, F. I. (2010). Anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of Securidacalongepedunculata Fresen. Journal of Ethnopharmacology, 130(2), 191 – 195.

[17] Xiang, X., Huang, W., Haile, C. N., Kosten, T. A. (2011). Hippocampal GluR1 associates with behavior in the elevated plus maze and shows sex differences. Behav Brain Res 222:326–331.

[18] Kumar RS, Narayanan SN, Kumar N, Nayak S. Exposure to enriched environment restores altered passive avoidance learning andameliorates hippocampal injury in male albino Wistar rats subjected to chronic restraint stress. Int J App Basic Med Res 2018;8:231-6.

[19] Jadhav, S.H.; Sarkar, S.N.; Patil, R.D.; Tripathi, H.C. Effects of subchronic exposure via drinking water to a mixture of eight water-contaminating metals: A biochemical and histopathological study in male rats. Arch. Environ. Contam. Toxicol. 2007, 53, 667–677.

[20] Lin, C.Y.; Hsiao, W.C.; Huang, C.J.; Kao, C.F.; Hsu, G.S.W. Heme oxygenase-1induction by the ROS–JNK pathway plays a role in aluminum-induced anemia. J. Inorg. Biochem. 2013, 128, 221–228.

[21] Freitas Fonseca, M.; De Souza Hacon, S.; Grandjean, P.; Choi, A.; Rodrigues Bastos, W. Iron status as a covariate in methylmercury-associated neurotoxicity risk. Chemosphere 2014, 100, 89–96.

[22] Hernández-García, A.; Romero, D.; Gómez-Ramírez, P.; María-Mojica, P.; Martínez-López, E.;García-Fernández, A.J. In vitro evaluation of cell death induced by cadmium, lead and their binary mixtures on erythrocytes of Common buzzard (Buteo buteo). Toxicol. Vitro 2014, 28, 300–306.

[23] Guskiewicz KM, Bruce SL, Cantu R, et al. National Athletic Trainers' Association Position Statement: Management of sports-related concussion. J Athl Train. 2004;39:280–297.

[24] Covassin, T., Elbin, R. J. (2010). The cognitive effects and decrements following concussion. Open Access Journal of Sports Medicine, 1: 55 – 61

[25] Wang B, Du Y. Cadmium and its neurotoxic effects. Oxidative Med Cell Longev. 2013;2013:898034.

[26] Wang, H., Zhang, L., Abel, G. M., Storm, D. R., Xia, Z. (2018). Cadmium Exposure Impairs Cognition and OlfactoryMemory in Male C57BL/6 Mice. Toxicological sciences, 161(1), 87–102

[27] Emsley CL, Gao S, Li Y, Liang C, Ji R, Hall KS, Cao J, Ma F, Wu Y, Ying P, Zhang Y, Sun S, Unverzagt FW, Slemenda CW, Hendrie HC: Trace element levels in drinking water and cognitive function among elderly Chinese. Am J Epidemiol 2000, 151:913–920.

[28] Hart RP, Rose CS, Hamer RM: Neuropsychological effects of occupational exposure to cadmium. J Clin Exp Neuropsychol 1989, 11:933–943.

[29] Viaene MK, Masschelein R, Leenders J, De Groof M, Swerts LJ, Roels HA: Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. Occup Environ Med 2000, 57:19–27.

[30] Roediger, H.L., Agarwal, P.K., McDaniel, M.A., & McDermott, K.B. (2011). Test-enhanced learning in the classroom: Long-term improvements from quizzing. Journal of Experimental Psychology: Applied, 17(4), 382-395. doi:10.1037/a0026252.

[31] Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Farbood Y. 2013. Central and peripheral antinociceptive effects of ellagic acid in different animal models of pain. Eur J Pharmacol. 707: 46–53.
[32] Lehotzky, K., Ungvary, G., Polinak, D., and Kiss, A. (1990). Behavioral deficits due to prenatal exposure to cadmium chloride in Cfy rat pups. Neurotoxicol. Teratol. 12, 169–172.
[33] Goncalves, J. F., Nicoloso, F. T., da Costa, P., Farias, J. G., Carvalho, F. B., da Rosa, M. M., Gutierres, J. M., Abdalla, F. H., Pereira, J. S., Dias, G. R., et al. (2012). Behavior and brain enzymatic changes after long-term intoxication with cadmium salt or contaminated potatoes. Food Chem. Toxicol. 50, 3709–3718.
[34] Abdalla, F. H., Schmatz, R., Cardoso, A. M., Carvalho, F. B., Baldissarelli, J., de Oliveira, J. S., Rosa, M. M., Nunes, M. A. G., Rubin, M. A., & da Cruz, I. B. M. (2014). Quercetin protects the impairment of memory and anxiogenic-like behavior in rats exposed to cadmium: Possible involvement of the acetylcholinesterase and Naþ, Kþ-ATPase activities. Physiol. Behav. 135, 152–167.
[35] da Costa, P., Goncalves, J. F., Baldissarelli, J., Mann, T. R., Abdalla, F. H., Fiorenza, A. M., da Rosa, M. M., Carvalho, F. B., Gutierres, J. M., de Andrade, C. M., et al. (2017). Curcumin attenuates memory deficits and the impairment of cholinergic and purinergic signaling in rats chronically exposed to cadmium. Environ. Toxicol. 32, 70–83.