Investigation of Sub-Chronic Exposure to Lead and Cadmium Contaminated Diets on BDNF, Acetylcholinesterase and fine motor functions in Albino Wistar Rats

Ukoro B., 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 - Heavy Metals toxicity has been verified as major hazard to human health which in turn has turn on view to be a global challenge with different diseases associated with their exposure. The current study investigated the sub-chronic exposure to Lead and Cadmium contaminated diets on basal ganglia functions in Albino wistar rats. 30 male and female rats with an average weight of 120g divided into 5 groups were used in this study. Group 1 served as the control group and was given normal animal feed and water ad libitum, Group 2 was administered with 40mg/kg of Cadmium (Cd), Group 3 was administered with 50mg/kg of Lead (Pb), Group 4 was administered with the combination of 25mg/kg of Cadmium and 25mg/kg of Lead, while Group 5 was induced by intra-peritoneal administration of 3mg/kg of Chlorpromazine (CPZ) to produce symptoms of Parkinsonism for comparative studies with the effects produced by Lead & Cadmium Toxicity. Administration of test substances lasted for 5 weeks while neurobehavioral activities was tested on a weekly bases using the Barnes Maze, Inverted screen test, Handgrip test, Beam walk test and the Rotarod test. The hand-held magnifier was also used to observe physical neuromuscular deficits. The activities of Acetylcholinesterase and Brain derived neurotropic factors (BDNF) were measured. The results of the study revealed significant ($P<0.05$) negative alterations of neurobehavioral activities which include impairment of neuromuscular activities as well as cognitive functions. The results also showed significant increase ($P<0.05$) in the level of Acetylcholinesterase and a significant ($P<0.05$) decrease in BDNF level in the brain which in turn could have caused alterations in neurotransmission. It was therefore concluded that exposure to heavy metals (Lead and Cadmium) could impair motor and cognitive functions, and also produce or aggravate the symptoms of Parkinsonism, Alzheimer's disease and other neurodegenerative diseases.

Keywords: Heavy metals, Toxicity, Neurodegeneration, Acetylcholinesterase, motor functions, Lead, Cadmium

1. Introduction

Heavy metals had demonstrated to be a significant danger to wellbeing and there are a few diseases related to exposure to these metals. Even though these metals do not have any biological role, remain present in the body and therefore become harmful for the human body and its proper functioning [1]. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment [2].

Although these metals have crucial biological functions in plants and animals, sometimes their chemical coordination and oxidation-reduction properties have given them an additional benefit so that they can escape control mechanisms such as homeostasis, transport, compartmentalization and binding to required cell constituents. These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity. Previous research has found that oxidative deterioration of biological macromolecules is primarily due to binding of heavy metals to the DNA and nuclear proteins [3,4].

The Basal ganglia (BG) is a collection of nuclei located deep beneath the cerebral cortex that is involved in learning and selection of rewarded actions. The Basal ganglia (BG) is an evolutionarily conserved complex network of excitatory and inhibitory neurons located in the deep brain of vertebrates that controls action selection [4]. The BG is comprised of the dorsal striatum, external and internal portions of the globus pallidus (GPe, GPi), subthalamic nucleus (STN) and substantia nigra. It is traditionally implicated in motor control since BG lesions are associated with movement disorders. The BG is a shared processing center involved in a broad spectrum of motor and cognitive control. A cortico-BG-thalamo-cortical neurocircuit loop is suggested to be the structure that provides this control [4]

Although there are several reports on the effects of heavy metals ingestion on various organs of the body, No special emphasis has been made on the effects of long term exposure to (Lead and cadmium) on Basal ganglia functions, [5] Hence this work is aimed at investigating sub-chronic exposure to heavy metals contaminated diet on Basal Ganglia Neural circuits in albino wistar rats.

2. Materials and methods

Experimental Animals

Animals weighing between 115–125g acquired from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt were used for this experiments. After weaning, animals received standard laboratory rat feeds and water *ad libitum*. Rats were housed in approved cages and kept on a regular 12 hours dark/light cycle. All animals received care in accordance with the Nigerian law on experimentation with laboratory animals which is based on the US National Institutes of Health guidelines.

Drug and Chemicals

Chlorpromazine (CPZ) was procured from Alpha Pharmacy and Stores Ltd. Ogbunabali Road, Old Port Harcourt Two, Port Harcourt.

Lead and cadmium were purchased from Joechem Chemicals, Choba Port Harcourt.

CPZ was used to induce animal model Parkinsonism like symptoms in order to compare the effects to that is produced by the ingestion of Lead and cadmium in the test groups.

GROUPS	DIVISION	TREATMENT
Group 1	Control (5 rats)	Distilled water
Group 2	Cadmium (5 rats)	40mg/kg of cadmium
		Test
Group 3	Lead (5 rats)	50mg/kg of Lead
		Test
Group 4	Lead and Cadmium (5 rats)	25mg/kg of Lead and 25mg/kg of Cadmium
		Test
Group 5	Chlorpromazine (CPZ) (5 rats)	3mg/kg of ZPZ
		30 minutes before Neurobehavoural test

Neurobehavioural Studies

Neuro-behavioral studies were conducted weekly following the treatment of test groups with Cadmium, Lead and Cadmium+Lead respectively. The test was conducted in 3 trials per week for the period of 4 weeks. The Neuro-behavioural test performed to ascertain the impact of sub-chronic exposure to heavy metals contamination includes Beam walk test, Hand grip test, inverted screen test and Rotarod Test.

Rotarod test

The Rotarod, also identified as the Rotarod test originally described by Dunham and Miya (1957) [6] and modified by Crawley [7] was used for this study. It is custom as a rudimentary assessment tool for synchronization and balance in rodents and provides one measure of locomotors ability

Inverted Screen test (citation)

The Inverted screen test used for this work was based on the method developed by Kondziela 1964.[8] It is a test of muscle strength and endurance using all four limbs. It is a quick but insensitive gross screen, and the weights test described in this article will provide a finer measure of muscular strength. It is performed with rodent models of neuromuscular disorders to demonstrate neuromuscular impairment and motor coordination. It is an efficient and reliable outcome measure for the evaluation of effects of potential therapeutic compounds on muscle strength.

Climbing/Beam walk test

Fine motor coordination and balance can be assessed by the beam walking assay. The goal of this test is for the rodent to stay upright and walk across an elevated narrow beam to a safe platform. *Principle and procedure*

The Protocol used is established on those described by Southwell *et al [9]* & Carter [10]. Performance on the beam is quantified by measuring the time it takes for the mouse to traverse the beam and the number of paw slips that occur in the process.

Handgrip test

The grip strength test is a simple non-invasive method designed to evaluate mouse muscle force in vivo, by taking advantage of the animal's tendency to grasp a horizontal metal bar or grid while suspended by its tail.

Handgrip Protocols

The bar or grid is devoted to a force transducer, and the force produced during the pull on the bar can be repeatedly measured at intervals (e.g., weekly) throughout a given experimental period.. The modified method as described by [11] was employed for this work. The new forelimb grip strength test was modified from the conventional test by rotating the system vertically.

Collection of samples/preparation of mixture homogenates

The rats were anesthetized in a di-ethyl ether saturated chamber. The rats were then dissected and brain tissues harvested. Tissues were flushed in super cold PBS (0.02 mol/L, pH 7.0-7.2) to eliminate abundance blood altogether and gauged prior to homogenization. The tissues were then minced into little. The subsequent suspension was exposed to two freeze-defrost cycles to additionally break the cell layers. From that plug headlong, the were centrifuged for around 5 minutes at 5,000 x g. the supernatant was eliminated and assay was done as quickly as possible.

Estimation of Acetylcholinesterase

Mind AChE action was measured spectrophotometrically in 0.1 M sodium phosphate Triton X-100 0.1 g% pH 7.5 by an adjustment of Ellman's technique (Ellman et al, 1961), as portrayed by Silva Filho and associates (Silva et al, 2014). The explicitness of AChE for substrates (P%) was determined by the proportion of AChE movement when utilizing propionylthiocholine as substrate isolated by AChE action when utilizing acetylthiocholine: P%, [(activity with ProScol/action with AceScol) x 100] (%) [12]

Statistical Analysis

Data across the groups were evaluated using one (ANOVA). Thereafter, the post-hoc test of multiple comparisons (Newman Keuls test) remained used to experiment the individual groups contrary to each other." Confidence level was at 95% and P-value <0.05 was well thought-out significant.

3. RESULTS AND DISCUSSION

TABLE 1 Performance time in beam walk test following exposure of rats to heavy metals contaminated diet in rat

	Week1 time (s)			**Week2 time (s)**			**Week3 time (s)**			**Week4 time (s)**		
Groups	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
Group1 (Control)	34.60 ±6.74	51.40 ±18.20	52.20 ±9.44	52.20 ±10.42	60.00 ±9.12	66.80 ±16.89	60.00 ±9.12	34.60 ±6.74	51.40 ±18.29	52.20 ±49.4	51.40 ±18.29	52.20±25.97
Group2 (Cadmium only)	92.00 ±11.00*	107.20±10.78*	77.60 ±6.11	99.40 ±12.99*	82.40 ±7.08	103.40±13.10	112.00±7.42	125.40±48.34*	191.± 44.67*	216.60±27.85*	128.00±24.16	191.20 ±44.67*
Group3 (Lead only)	161.00±21.00*	154.60±18.80*	145.20±22.99*	157.00±36.63*	168.80±34.11*	147.00±2.93*	300.00±00*	300.00 ±0.00*	240.00±26.83*	234.40 ±32.70*	300.00 ±0.00*	298.00 ±2.00*
Group4 (Lead+ Cadmium)	212.6 ± 25.00*	228.80±24.64*	228.00±19.45*	242.80±24.03*	270.60± 17.78*	117.00±1.34*	263.80±63.29*	300.00 ±0.00*	253.40±28.55*	300.00 ±0.00*	300.00 ±0.00*	274.20 ±23.61*
Group5 (Chlorpromazine)	192.00±18.74	188.20±29.93	150.32±17.49*	232.80±25.73*	233.40±28.07*	212.60±25.00*	250.80±13.21*	300.00 ±0.00*	264.00±24.00*	234.40 ±32.73*	300.00 ±0.00*	300.00 ±0.00*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control

TABLE 2 Pattern of task performancein the inverted screen test following long term exposure to heavy metals contaminated diet in rat

	Week1 time (s)			**Week2 time (s)**			**Week3 time (s)**			**Week4 time (s)**		
Groups	Trial1	Trial 2	Trial3	Trial1	Trial2	Trial 3	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3
Group1 (Control)	35±32.40	33.60 ±6.54	31.60 ±7.61	34.00 ±5.89	33.60 ±6.54	31.60 ±7.61	32.40 ±3.35	35.20 ±5.37	31.60 ±7.61	34.00 ±5.89	33.60 ±6.54	31.60 ±7.61
Group2 (Cadmium only)	33.80 ±11.12	32.40 ±9.26	30.20 ±13.07	23.60 ±3.04	20.20 ±0.66	19.40 ±5.69	19.20 ±7.45	16.00 ±2.38*	16.00 ±6.72*	14.40 ±2.13*	12.80 ±1.88*	20.60 ±5.50
Group3 (Lead only)	30.20 ±7.85	47.40 ±8.89	24.80 ±8.35	12.40 ±7.44	13.20 ±9.52	17.40 ±6.92	14.60 ±1.60*	13.80 ±2.86*	3.04± 1.36*	7.20± 1.09*	10.60 ±2.15*	11.00 ±2.16*
Group4 (Lead+Cadmium)	29.40 ±9.09	37.00 ±4.79	23.00 ±3.46	20.20 ±2.72	14.00 ±4.39*	18.80 ±1.85	17.60 ±1.43*	19.00 ±3.67*	3.34± 1.49*	11.80 ±1.35*	10.60 ±0.92*	7.20± 0.58*
Group5 (Chlorpromazine)	36.20 ±8.59	37.00 ±9.01	13.80 ±4.63	16.80 ±6.88*	11.00 ±0.54*	8.80± 2.09*	14.60 ±1.12*	15.40 ±0.87*	13.60 ± 0.81*	13.80 ±1.85*	8.20± 1.28*	8.20± 1.11*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

TABLE 3 Endurance and coordination time on Rotarod test following long term exposure to heavy metals contaminated diet in rat

	Week1 time (s)			**Week2 time (s)**			**Week3 time (s)**			**Week4 time (s)**		
Groups	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**
Group1 (Control)	17.80±6.36	18.20± 4.24	19.60± 2.40	29.80±3.92	25.20± 1.65	32.20± 7.39	24.00±1.89	26.00±1.58	36.40± 5.82	26.80±4.28	38.20±4.36	26.80± 1.74
Group2 (Cadmium only)	19.40± 2.44	26.60± 4.54	17.80± 6.36	18.20±4.24*	18.00± 2.82	10.00± 1.26*	11.80±2.63*	12.80±2.43*	8.40±1.28*	10.00±2.52*	9.20±1.42*	10.00± 2.52*
Group3 (Lead only)	12.00±0.707	18.20± 1.98	14.40± 1.96	13.60±0.812*	8.40±0.812*	12.40± 1.07*	13.80±1.42*	13.80±1.56*	11.60± 1.20*	9.60±0.92*	10.20±0.58*	8.40±1.12*
Group4 (Lead+Cadmium)	18.40±2.63	18.20± 4.71	20.40± 6.37	20.60±5.37*	26.40± 7.44	16.80± 1.71*	13.20±1.01*	12.80±1.11*	13.00± 1.73*	12.80±2.43*	9.40±1.16*	7.00±0.54*
Group5 (Chlorpromazine)	23.40±8.06	27.00± 6.08	16.80± 2.24	12.20±1.98*	15.20± 1.52	12.40± 1.83*	13.80±2.17*	10.40±2.87*	7.80±1.20*	14.20±2.57*	11.00±1.78*	5.60±0.74*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Table 4 Showing result from the hand grip test following long term exposure to heavy metals contaminated diet in rat

	Week1 time (s)			**Week2 time (s)**			**Week3 time (s)**			**Week4 time (s)**		
Groups	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**	**Trial1**	**Trial2**	**Trial3**
Group1 (Control)	81.00± 16.58	125.40 ±3.60	122.00 ±2.0	95.00± 25.96	123.40 ±3.65	95.00± 25.96	133.40± 9.55	122.00± 2.00	95.00± 25.96	132.60± 9.84	122.00 ±2.00	122.00 ±2.00
Group2 (Cadmium only)	56.20± 6.66*	23.80± 3.72*	14.80± 2.15*	11.60± 1.28*	23.80± 3.72*	13.20± 1.65*	12.80±1 .11*8	31.40±7 .46*	15.40± 2.488*	12.40±1 .968*8	11.60± 2.978*	8.40±1 .128*
Group3 (Lead only)	39.60± 6.53*	29.60± 4.80*	11.60± 0.67*	11.60± 1.69*	25.40± 4.57*	10.80± 1.49*	7.80±1. 11*	10.20±2 .13*8	12.40± 2.40*	10.60±2 .56*	8.40±1 .508*	7.80±0 .58*
Group4 (Lead+Cadmium)	48.80± 2.59*	51.80± 3.67*	12.20± 1.35*	14.20± 1.93*	33.00± 8.64*	21.00± 8.17*	13.00±2 .7088*8 8	11.80±1 .9588*	13.00± 2.608*	11.00±1 .8188*8 8	9.60±1 .698*	8.60±2 .038*
Group5 (Chlorpromazine)	37.60± 7.45	25.60± 5.69*	11.40± 0.81*	14.60± 1.32*	27.80± 7.55*	24.20± 7.28*	16.60±2 .618*	14.20±2 .57*8	10.00± 1.64*8	10.20±2 .808*	8.60±0 .978*	8.60±1 .72*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.Table 5. Showing result of Neurochemical markers; Acetylcholinesterase and BDNF following long term exposure of test groups to heavy metals contaminated diet in rats

	Acetylcholinesterase (U/ml)	**BDNF** (ng/ml)
Group1(control)	37.433 ± 2.21	14.23 ± 0.25
Group2(cadmium)	101.02 4± 1.10*	8.18 ± 0.09*
Group3(lead)	105.534 ± 1.10*	7.12 ± 0.07*
Group4(Lead+Cadmium)	93.357 ± 3.31*	8.62 ± 0.027*
Group5(CPZ)	113.652 ± 3.31*	10.38 ± 0.46

From table 4.6, Result from Acetylcholinesterase and Brain derived neurotropic factor (BDNF) were estimated and analysis of data from the result reveals that the level of ACTHe was significantly ($p \leq 0.05$) increased in all test groups when compared to the control. This pattern of variation was relatively opposite for BDNF as the level was significantly ($p \leq 0.05$) reduced in the test groups when compared to the control groupComparative Analysis

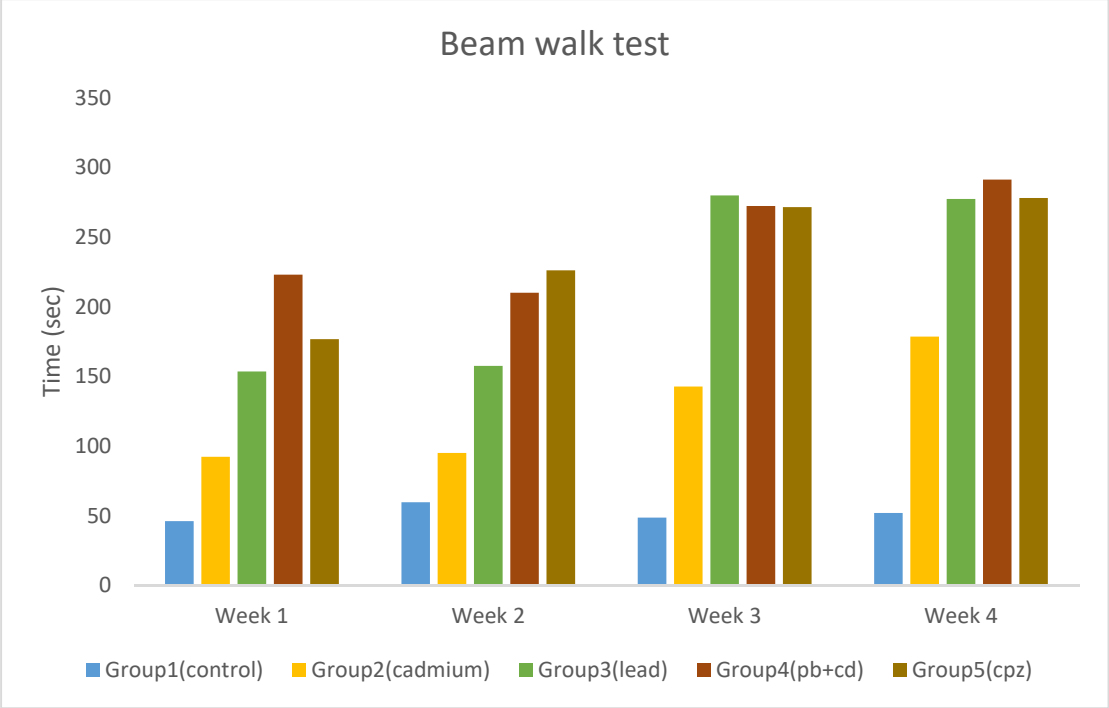

Fig 1. Comparative analysis of pattern of performance in the beam walk test across the groups within the 4 weeks of exposure of test groups to heavy metals contaminated diet

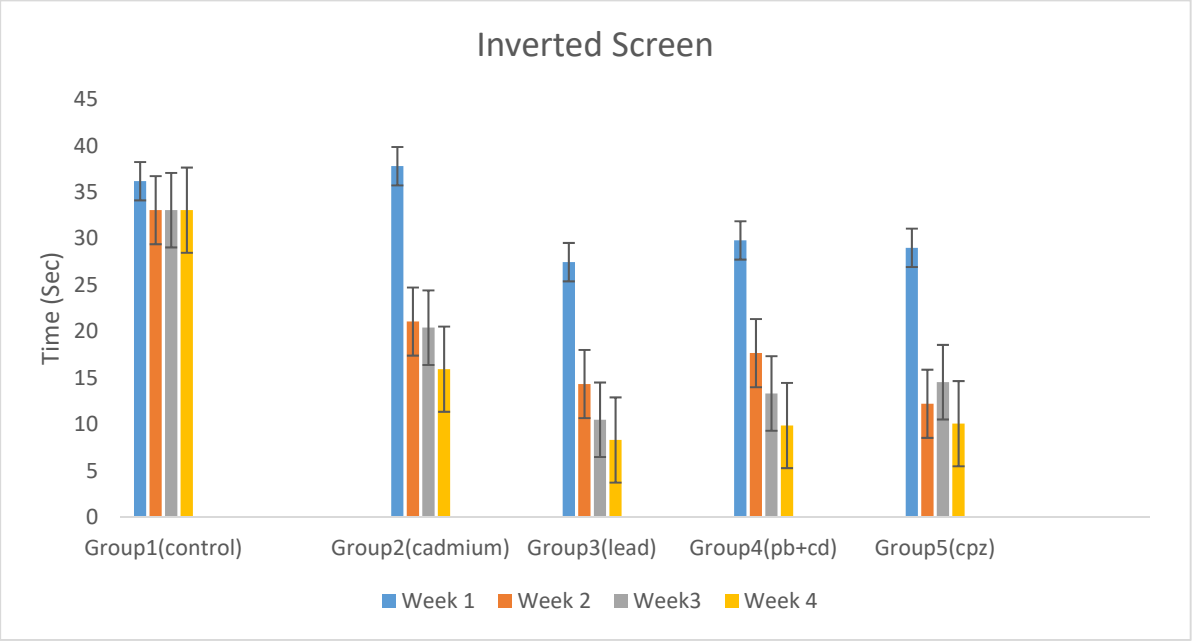

Fig 2. Comparative analysis of performance pattern in the Inverted screen test across the groups within 4 weeks of exposure of test groups to heavy metals contaminated diet

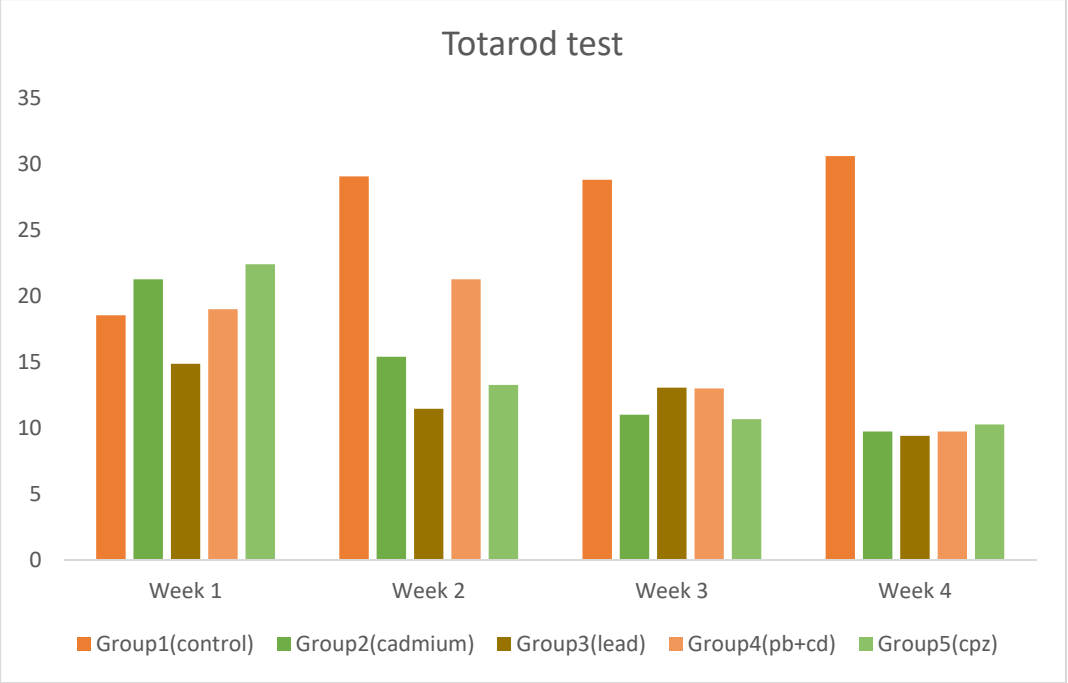

Fig 3. Comparative analysis of performance pattern in the Rotarod test across the groups within 4 weeks of exposure of test groups to heavy metals contaminated diet

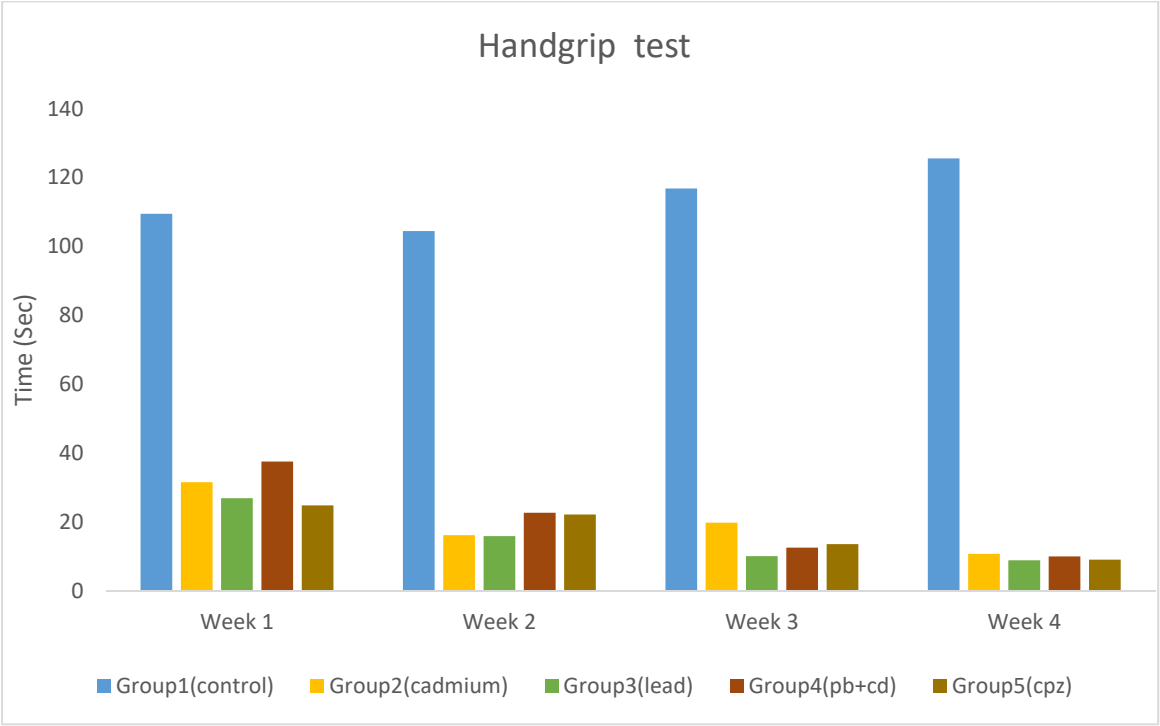

Fig 4. Comparative analysis of pattern of performance in the Hand grip test across the groups within the 4 weeks of exposure of test groups to heavy metals contaminated diet

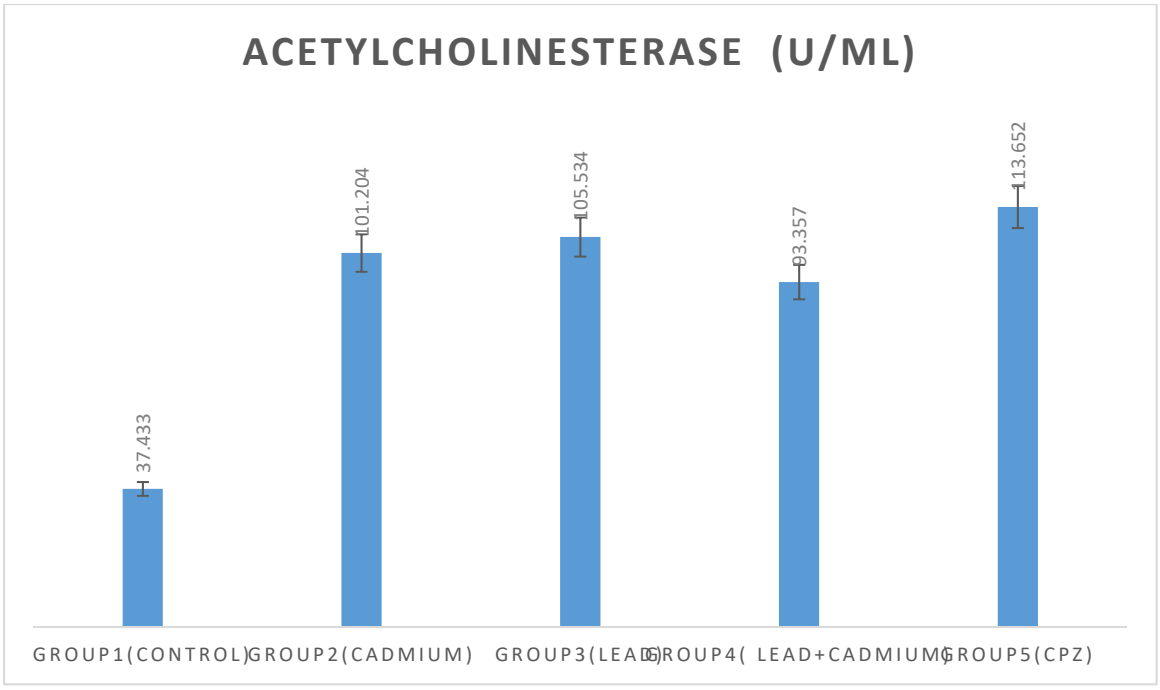

Fig 5. Comparative analysis of the mean values of the level of brain acetylcholinesterase following 4 weeks of exposure of test groups to heavy metals contaminated diet

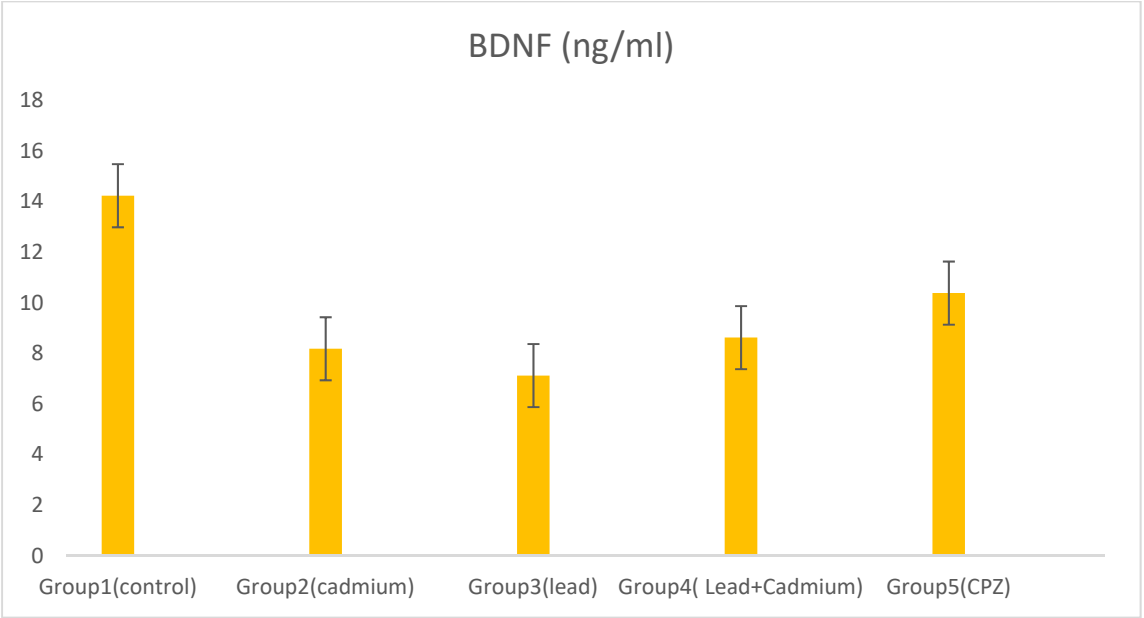

Fig 5. Comparative analysis of the mean values of the level of brain BDNF following 4 weeks of exposure of test groups to heavy metals contaminated diet

Discussion

Following exposure to heavy metals contaminated diets, Analysis of the result from the Neurobehavioral test and biomarkers as shown in tables and charts above are discussed as follows:

Table 4.1 shows result from the beam walk test after 4 weeks of exposure to heavy metals contaminated diet in albino rats across the groups. In each week, 3 trials of the beam walk test was conducted, it is to determine the time it took the animal to transverse through the beam within a given time, it also screened for motor coordination and balance of the animals across the beam. From the result, Group 2, 3, and 4 which were administered with 40mg/kg of cadmium, 50mg/kg of lead, and 50mg/kg (25mg of Lead, and 25mg of Cadmium) respectively, showed significant ($P \geq 0.05$) increase in the time taken to transverse through the beam. This increment was higher in group 4 which were administered with a mixture of Lead and cadmium.

During the week two of the beam walk test, Group 2 (Cadmium Only Group) only showed significant increase in trial one of the test. Group 3 (Lead group) and group 4 (Lead+Cadmium group) were shown to have significant increase in the latency of the performance across all trials when compared with the control group. At week 3 of the beam walk test, group 2 (Cadmium group) only showed significant increase in trial 2 and 3 while all other groups showed significant increase in all the trials. At Week four of this experiment, all test group showed significant increase in all the trials. Observation of the animals during the period of task performance showed a significant imbalance and struggle for the animals to transverse the beam especially at the 3rd and 4th week after exposure to the contaminant. Sluggishness and deficiency of normal and purposive locomotion has been described by [13] as a typical sign of motor disorder in PD, the most common disease affecting the basal ganglia.

As shown in table 4.2, the result of the inverted screen was obtained following 4 weeks of exposure to heavy metals and the reference drug. The four limb hang test was performed with rat models of neuromuscular disorders to demonstrate neuromuscular impairment and motor coordination anomalies. After one week of exposure, group 2 (cadmium group), group 3 (Lead group) and group 4 (Lead+Cadmium group) revealed a decrease in the time expended in performing the task, however this decrease were not statistically significant ($P \geq 0.05$) when the result was compared to the control group. In week two of the test, the latency of task performance were decreased in Group 2, and 3, although not significant when compared to the control group. Furthermore, the administration of (Lead+Cadmium) in group 4 resulted in a significant ($P \geq 0.05$) decrease in the latency of performance in trial 2 of the week, and insignificant decrease in the other trials. Week 3 and 4 of the exposure triggered a remarkable deterioration in task performance and at all the trials across the groups. The animals at this period were markedly characterized with visible tremor at the limbs, weakness of muscles and lack of explorative motivation. According to the results, all the test groups at this point, showed significant ($P \geq 0.05$) decrease in time of task performance. The result from this phase of the work demonstrated a comparable result with the group induced with the Chlorpromazine (CPZ) which is known to produce symptoms of Parkinson's disease (at a particular dose concentration in rodents. [14]. "*In vitro* studies have shown that lead can be neurotoxic to dopamine cells and impede dopaminergic neurotransmission. Few epidemiologic studies have assessed the role of lead in PD" [15]. According to a 1997 report in *Neurology*, epidemiological studies at the Henry Ford Health System [16] found that more than 20 years of occupational exposure to lead plus copper or lead plus iron increased the risk of developing Parkinson's disease. Occupational exposure to lead was estimated by an industrial hygienist based on occupational histories. "A subsequent study by the same group found that an estimated lifetime exposure to lead alone — using occupational history, blood lead, and tibial and calcaneal lead — was also associated with increased risk of PD. Parkinson's disease (PD) is a neurodegenerative issue portrayed by the sign of motor side effects, which are essentially ascribed to the loss of dopaminergic neurons in the substantia nigra pars compacta" (SNc) [16]. It is settled that the degeneration of the monoaminergic frameworks caused a disorder of the neuronal action in the subthalamic nucleus (STN), a basal ganglia structure assuming a key role in the pathophysiology of PD. In reality, the standard example of the neuronal activity of the STN gets sporadic and bursty in animal models of PD [17] and in PD patients.

Assessment of motor coordination and balance was equally carried out using the Rotarod test to ascertain the locomotive ability in the test groups after exposure to heavy metals and the result was recorded in table 4.3 above. During the first week of exposure, motor balance and coordination were slightly affected in all the test groups, although the variations were not statistically significant ($P \geq 0.05$) when compared to the control group. At the second week of exposure, Group 2 (cadmium group) and group 4 (Lead+Cadmium) showed significant deterioration in trial 1 and trial 3 respectively, while group3 (Lead group) had significant deterioration in all the trials. Moreover, the result of week 3 and week 4 in the Rotarod test was marked by a high variations in all the trials across all the test groups as significant ($P \geq 0.05$) decrease in task performance was recorded, it was evident that at week 3 and four of this test, the animals lost total coordination and high deterioration of balance.

Table 4.4 also showed the result from the Handgrip test following exposure to heavy metal contamination in rats. The handgrip test was conducted to evaluate the muscular strength in the test animals which is an essential step for the research of neuromuscular disorders in rats. The disorder associated with the ingestion of heavy metals started manifesting from week 1 of exposure as seen in table 4.4 above. On close observation under the hand held magnifier lens, muscle weakness was characterized by tremor in the forelimbs, the endurance capacity was also used to estimate the neuromuscular disorder by the latency of the animal to hold onto the grip. According to the results, Group1,2,3, and 4 were significantly ($P \geq 0.05$) decreased in all the trials and across all the groups during week 1, week2, week3, and week 4 respectively, however the decrease in muscular strength were more noticeable in week 3 and week 4 into the work.

As shown in table 4.6, the activities of Acetylcholinesterase was measured from brain tissue homogenates of the rats exposed to heavy metals (Lead & Cadmium) contaminated diet. From the result, both Lead and cadmium in their singular form or in combination caused significant increase in the level of Acetylcholinesterase after the period of exposure under our experimental condition. There has been several literatures postulating that metals inhibits the activities of Acetylcholinesterase, there are also conflicting reports where increased activities are

noticed, increased Acetylcholinesterase activities may be due to initial inhibition of Its synthesis during acute exposure to heavy metals [18]

ACHe has been associated with Alzheimer's disease (AD), which is a progressive neurological disease, the most common form of dementia, characterized by memory and other intellectual abilities loss, it is connected with the loss, or malfunction of cholinergic neurons in the brain with decreased level of Acetylcholine [19]

The Brain Neurotrophic factor was measured along the Neurobehavioral test conducted, as shown in table 6, the concentration of BDNF in brain tissue homogenate showed that Lead and Cadmium toxicity significantly ($P \leq 0.05$) reduced the level of BDNF in all the test groups, ([20]. From our study, Lead and cadmium toxicity significantly altered the level of BDNF, Acetylcholinesterase and other neurobehavioral activities, hence metal toxicity has debilitating effects on neurodegenerative disease and also associated with the development of such diseases like PD, AD, Myasthenia Gravis, and others.

Conclusion

Long term exposure to heavy metals contaminated diet has proven to cause alterations in Neurobehavioral and other motor functions such as neuromuscular activities, coordination and balance. It was ascertained that sub-chronic exposure to these metals also significantly altered the activities of Acetylcholinesterase by increasing the level in the brain, although the mechanisms to which this increase occurred was not studied. It was further detected that the activities of Lead and cadmium toxicities significantly reduced the level of the Neurotrophin "BDNF" which is an important enzyme that helps in the survival of neurons which are already in existence and also encourages the formation, differentiation and growth of new synapses and neurons. Lead and cadmium contaminated diet produces symptoms comparable to diseases, such as Parkinson's disease, Alzheimer's disease, Myasthenia Gravis and other neurodegenerative diseases and so, long term exposure to lead and cadmium can initiate, or aggravate neurodegenerative diseases, and so, exposure to heavy metals contaminated diet heavily threatens the functions of Basal ganglia neauraxis.

REFERENCES

[1] Krishna AK, Mohan KR. (2016) Distribution, correlation, ecological and health risk assessment of heavy metal contamination in surface soils around an industrial area, Hyderabad, India. Environment and Earth Science. 2016; 75:411. DOI: 10.1007/s12665-015-5151-7

[2] Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, et al. (2005) Lowlevel environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113: 894-899.

[3] Flora SJS, Mittal M, Mehta A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J Med Res 128: 501–523

[4] Grillner S, Robertson B, Stephenson-Jones M (2013). The evolutionary origin of the vertebrate basal ganglia and its role in action selection. J Physiol. 15;591(22):5425–31. Pmid:23318875

[5] Nobuo YANAGISAWA (2018). Functions and dysfunctions of the basal ganglia in humans. Proc Jpn Acad Ser B Phys Biol Sci. 2018 Aug 3; 94(7): 275–304.doi: 10.2183/pjab.94.019 PMCID: PMC6117491

[6] Dunham N. W., Miya W, (1957) A note on a simple apparatus for detecting neurological deficit .onlinelibrary.wiley.com › doi › jps.3030460322 https://doi.org/10.1002/jps.3030460322.

[7] Crawley, J. N. 2003. Behavioral phenotyping of rodents. Comp. Med. 53:140-146

[8] Kondziela W. Eine neue (1964) method zur messung der muskularen relaxation bei weissen mausen. Arch. Int. Pharmacodyn.; 152:277–284

[9] Southwell AL, Ko J, Patterson PH. (2009) Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci. ;29:13589–13602

[10] Carter RJ, Morton J, Dunnett SB. (2001) Motor coordination and balance in rodents; Curr Protoc Neurosci.; Chapter 8 [PubMed] [Google Scholar]

[11] Hikari Takeshita, Koichi Yamamoto,a, Satoko Nozato, Tadakatsu Inagaki, Hirotsugu Tsuchimochi,2 Mikiyasu Shirai,2 Ryohei Yamamoto,3 Yuki Imaizumi,1 Kazuhiro Hongyo, Serina Yokoyama, Masao Takeda, Ryosuke Oguro, Yoichi Takami, Norihisa (2017); Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice

[12] Oliveira MM, Silva MV, Bastos V, Fernandes FC, Bastos JC.(2007) Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. Marine Environmental Research. 63(4):303–12. ISI: 000245700800001. Pmid: 17118441

[13] Nobuo YANAGISAWA. (2018): Functions and dysfunctions of the basal ganglia in humans. Proc Jpn Acad Ser B Phys Biol Sci. 94(7): 275–304. doi: 10.2183/pjab.94.019

[14] Souravh Bais et al, NS Gill, N Kumar (2015). Neuroprotective effect of Juniperus communis on chlorpromazine induced Parkinson disease in animal model. Chinese Journal of Biology

[15] Gorell JM, Johson CC, Richardson RJ, et al. (1997) Occupational exposure to metals as risk factors for Parkinson's disease. Neurology ; 48:650–658.

[16] Ehringer H, Hornykiewicz O (1960). Verteilung von noradrenalin and dopamin im gehirn des menschen und ihr verhalten bei erkrankungen des extrapyramidalen systems. *Klin Wschr* 38: 1126–1239 [Google Scholar]

[17] Delaville C., Navailles S., Benazzouz A. (2012b). Effects of noradrenaline and serotonin depletions on the neuronal activity of globus pallidus and substantia nigra pars reticulata in experimental Parkinsonism. Neuroscience 202, 424–433. 10.1016/j.neuroscience.2011.11.024 [PubMed] [CrossRef] [Google Scholar]

[18] Henn Birgit Claus Henn Suzanne Mc Master Stephanie PadillaStephanie Padilla (2006) Measuring Cholinesterase Activity in Human Saliva Journal of Toxicology and Environmental Health Part A 69(19):1805-18 Follow journal DOI: 10.1080/15287390600631458

[19] Monisha J, Tenzin T, Naresh A, Blessy BM, Krishnamurthy NB. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology. 2014; 7(2):60-72

[20] Silva A, Naia L, Dominguez A, Ribeiro M, Rodrigues J, Vieira OV, et al. (2015) Overexpression of BDNF and full-length TrkB receptor ameliorate striatal neural survival in Huntington's disease. Neurodegenerative Diseases. 15(4):207-218