Comparative study of the effects of heavy metals contaminated diet and chlorpromazine induced neuroleptic effects on BDNF and cognitive functions in albino wistar rats

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ABSTRACT - There have been several reports on health risk associated with heavy metals exposure in both animals and human which poses a couple of challenge with different diseases associated with their exposure. This study investigated the Comparative study of the effects of heavy metals contaminated diet and chlorpromazine induced neuroleptic effects on BDNF and cognitive functions in albino wistar rats. 25 males and females rats with average weight of 120 was used for this work. The rats were grouped into 5 grouips according to the experimental design. 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 neuroleptic effects for comparative studies with the effects produced by Lead & Cadmium Toxicity on BDNF and cognitive functions in rats. Est substances were Administered for 4 weeks while various cognitive functions test were carried out on a weekly bases using the Barnes Maze and Navigational maze test. The activities of Brain derived neurotropic factors (BDNF) were measured. The results of the study revealed significant (P<0.05) negative alterations of cognitive functions activities which include impairment of explorative abilities, as well as motivation and time of task performance. The results also showed significant (P<0.05) decrease in BDNF level in the brain which in turn could have caused alterations in neurotransmission and impairment of cognitive functions. It was therefore concluded that exposure to heavy metals (Lead and Cadmium) could impair cognitive functions, and deteriorate BDNF level in rats, thereby affecting the formation, and also produce or aggravate the symptoms of Parkinsonism, Alzheimer's disease and maintenance of memory.

Keywords: Cognition, Toxicity, Neurobehaviour, Lead, Cadmium exposure,

1. Introduction

Brain-derived neurotrophic factor (BDNF), or abrineurin,is a protein (1) that, in humans, is encoded by the *BDNF* gene. (2,3) BDNF is a member of the Neurotrophin family of growth factors, which are related to the canonical nerve growth factor. Neurotropic factors are found in the brain and the periphery. BDNF was first isolated from pig brain in 1982 by Yves-Alain Barde and Hans Thoenen (4). Brain Derived Neurotrophic Factor (BDNF) is a key molecule involved in plastic changes related to learning and memory. The expression of BDNF is highly regulated, and can lead to great variability in BDNF levels in healthy subjects. Changes in BDNF expression are associated with both normal and pathological aging and also psychiatric disease, in particular in structures important for memory processes such as the hippocampus and parahippocampal areas. (5). Studies had it that heavy metals in the body may be implicated in everything from Alzheimer's and cognitive decline, behavioral problems, kidney dysfunction, Parkinson[1]s, epilepsy, and cardiovascular diseases.

Chlorpromazine is a standard antipsychotic drug recognized for its propensity to cause extrapyramidal symptoms, impaired memory and catatonia owing to blockade of striatal dopamine D2 receptors (7,8,9). Owing to the wide range of exposure to heavy metals in the environments, food materials and water, this research is aimed at investigating the effects of Lead and cadmium contaminated diet on Brain derived neurotropic factor and cognitive activities in rats.

2. Materials and methods

Animals

The 25 adult male and female albino Wistar rats, weighing between 120g and 130g were purchased from the Department Human Physiology, University of Port Harcourt. The animals were acclimatized and grouped according to their weights. 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.

Experimental design

The twenty-five albino Wistar rats after acclimatization were grouped into five groups with each group comprising of five rats. Group 1 served as the control group. Group 2 was administered 50mg/kg of Lead. Group 3 was administered with 40mg/kg. Group 4 was administered with a mixture of 25mg/kg of Lead and 25mg/kg of cadmium. Group 5 induced with chlorpromazine (3 mg/kg) 30 minutes before subjecting the animals to the various tests daily.

Details of Experimental design

GROUPS	DIVISION	TREATMENT	PROCEDURE AND DURATION
Group 1	Control (5 rats)	Normal feed and water	Stress free, used as experimental control group
Group 2	Cadmium (5 rats)	50mg/kg of cadmium	The rats were administered orally with 50mg/kg of cadmium for 4 weeks.
		Test	The rats were subjected to Neurocognitive task using Barnes maze test, Navigational maze, and Inverted screen test. After 4 weeks of exposure, the animals were sacrificed and midbrain tissues were homogenized for BDNF Assay.
Group 3	Lead (5 rats)	40mg/kg of Lead	The rats were treated with 40mg/kg of Lead for 5 weeks
		Test	The rats were subjected to Neurocognitive task using Barnes maze test, Navigational maze, and Inverted screen test. After 4 weeks of exposure, the animals were sacrificed and midbrain tissues were homogenized for BDNF Assay.
Group 4	Lead and Cadmium (5 rats)	25mg/kg of Lead and 25mg/kg of Cadmium	The rats were treated with 25mg/kg of Lead and 25mg/kg of Cadmium for 5 weeks
		Test	Neurocognitive task using Barnes maze test, Navigational maze, and Inverted screen test. After 4 weeks of exposure, the animals were sacrificed and midbrain tissues were homogenized for BDNF Assay.
Group 5	Chlorpromazine (CPZ) (5 rats)	3mg/kg of ZPZ	The rats were induced
		30minutes before Neurobehavoural test	Induced Parkinson's experimental model. Through Intraperitoneal injection of CPZ (3 mg/kg) 30 minutes before subjecting the animals to the various tests daily. Animals were sacrificed after 4 weeks, and brain tissues were harvested and homogenized and assayed for BDNF

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.

Neurocognitive tests

Barnes Maze

The Barnes maze test was which was first designed by Dr. Carol Barnes in 1979 was used for this work. It is a research method used in psychological laboratory research to determine the learning and spatial memory in an experimental animal. The basic role of Barnes maze is to determine the ability of a mouse or rat to learn and remember the sight of the target zone using a configuration of distal visual cues placed around the experimental area.(Harrison. *e tal* (12)

Navigational Maze

Maze navigation tests are utilized in the assessment of exploration, path planning, and navigation which rely on learning and memory capacities to form cognitive maps. (Maze Engineers, (13)

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 mixture. 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.

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. Result and Discussion

Table 1 Result of Barnes Test following exposure of test groups to heavy metals contaminated diet.

BARNES	**Time(s)**			
GROUPS	Week 1	Week 2	Week 3	Week 4
Grp 1 Control	23.00±13.76	21.00±8.05	21.60±4.00	19.8±6.01
Grp2 (Cadmium)	*83.00±12.33	98±14.81	103.00±16.15	108.00±12.00
Grp 3 (Lead)	158.75±68.06	166±67.45	*178.25±20.58	188.21±21.15
Grp 4 (Lead + Cadmium)	115.50±83.42	*155.75±45.41	173.75±72.97	198.00±32.23*
Grp 5 (Chlorpromazine)	113.00±57.00*	188.50±61.50	*175±67.25	*212.00±51.02

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

Barnes maze test showed significant ($p \leq 0.05$) decrease in task performance from weak 2 after exposure to heavy metal contaminated diet.

Table 2 Pattern of Navigation after exposure of test groups to various test substances

NAVIGATIONAL MAZE Time(s ± sem)				
GROUPS	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP 1 (control)	25.20±6.41	20.80±4.55	19.60±3.47	16.60±3.47
GROUP 2 (Cadmium)	130.00±8.71	143.80±37.22	173.60±16.12	300.00±2.00
GROUP 3 (Lead)	142.40±2.21	165.40±2.20	186.50±4.89	300.00±2.20
GROUP 4 (Lead +Cadmium)	106.60±41.13	155.40±39.19	184.60±68.95	300.00±2.20
GROUP 5(starvation)	139.00±14.26	160.40±8.93	3000.00±7.84	300.00±2.20

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

Table 3 showing result of Neurochemical markers; Acetylcholinesterase and BDNF following long term exposure of test groups to heavy metals contaminated diet in rats

	BDNF (ng/ml)
Group1(control	14.23 ± 0.25
Group2(cadmium)	7.18 ± 0.08*
Group3(lead)	7.12 ± 0.07*
Group4(Lead+Cadmium)	8.52 ± 0.027*
Group5(CPZ)	10.38 ± 0.46

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

Comparative studies

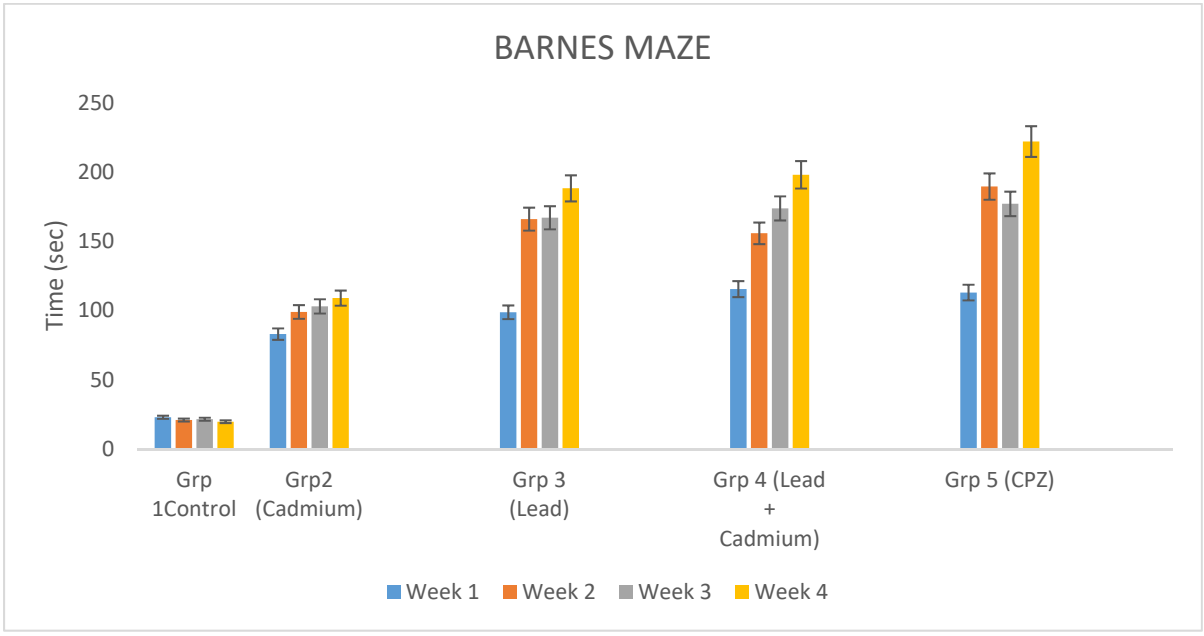

Fig .1 Comparative study of performance pattern in the Barnes maze test across the groups within 4 weeks of exposure of test groups to heavy metals contaminated diet

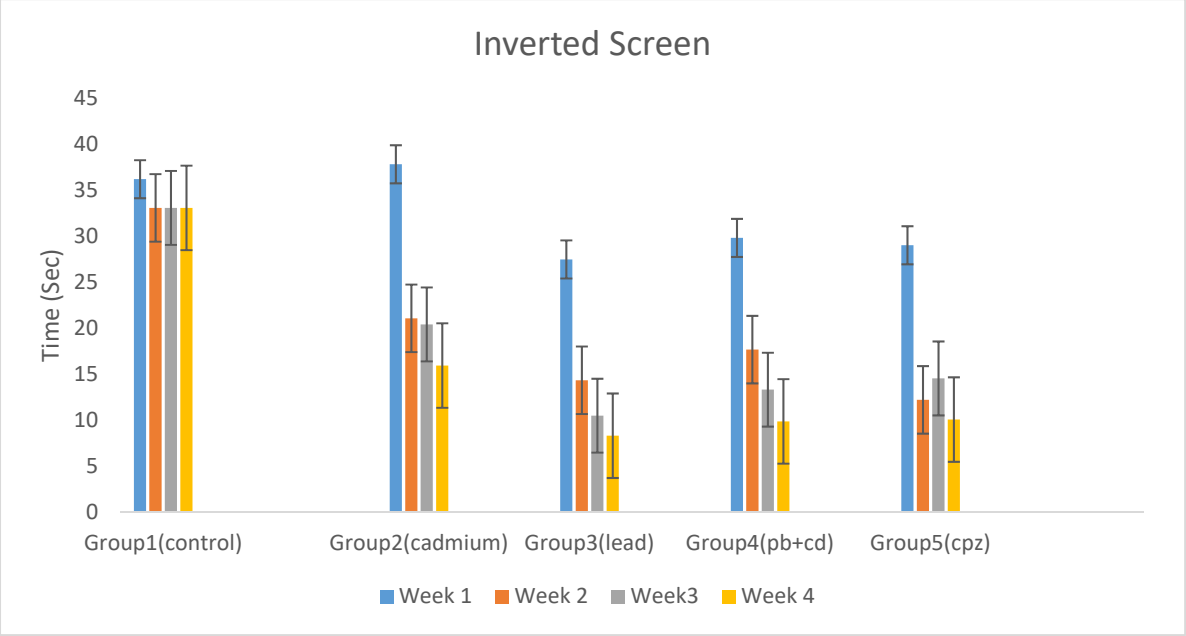

Fig .2 Comparative study of performance pattern in the Inverted screen test across the groups within 4 weeks of exposure of test groups to heavy metals contaminated diet

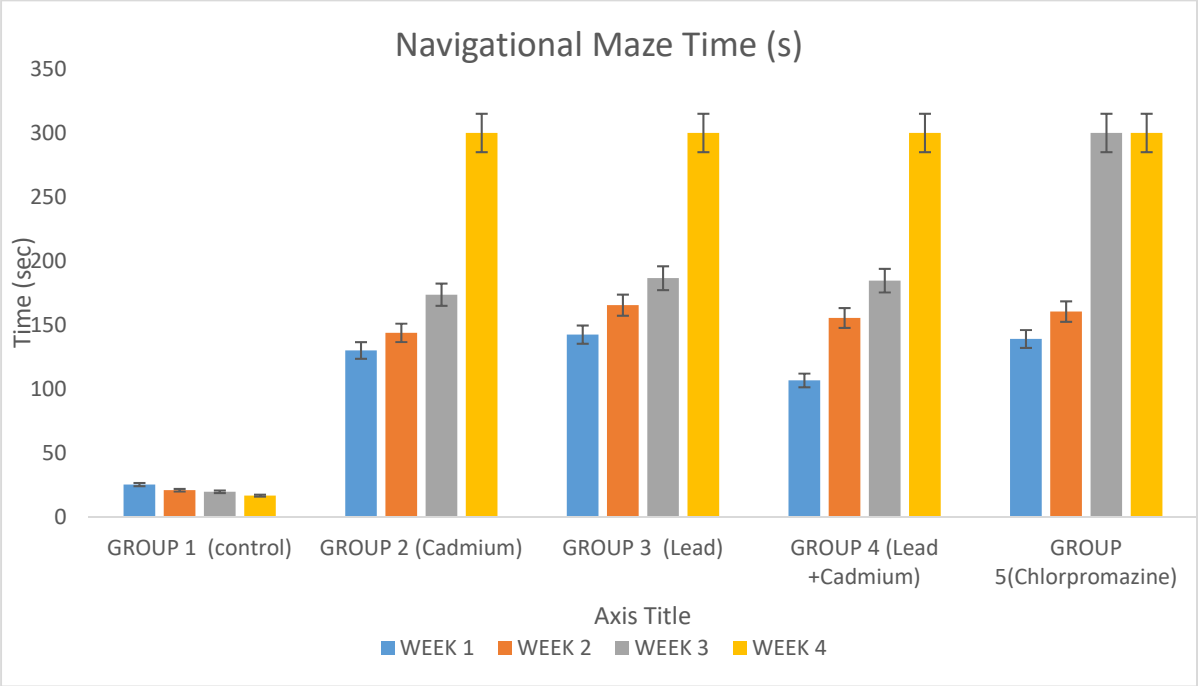

Fig 3 Comparative analysis of explorative pattern in the Navigational maze test across the groups within 4 weeks of exposure of test groups to heavy metals contaminated diet

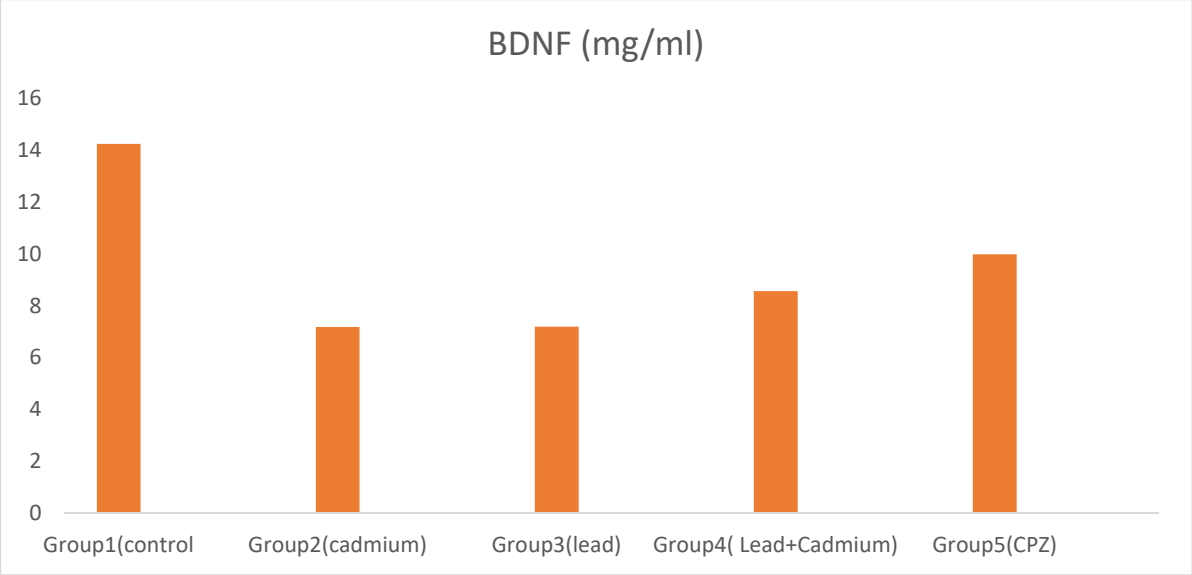

Fig 4 Comparative analysis of the mean values of the level of BDNF following 4 weeks of exposure of test groups to heavy metals contaminated diet

4. Discussion

The Barnes Maze test was conducted to assess spatial learning and memory after the administration of the heavy metals, Lead and cadmium. The results of the Barnes Maze test showed significant increase ($P \geq 0.05$) in the time spent by the test groups to locate escape opening to the darkbox when compared with the time spent by the control group. There was significant ($P \geq 0.05$) increase in time for the animals in test groups to locate escape holes. The control group was observed to have used Serial search pattern, while the random search pattern was observed in the test groups. In most cases, the animals in the test group could not successfully complete the task. The effects were more in Lead group, followed by Lead+cadmium group, and least in cadmium only group. However the effects of heavy metals were comparable to those in group 5 that was induced with chlorpromazine, the effects became fully manifested at week 3 and 4 of exposure. This spatial navigation alteration is an evidence of possible pathological changes in the hippocampus or other cortical areas of the brain part that are involves in learning, memory and motor response. (14).

Table 2, above showed result from the Navigational maze test. "Navigational maze test is a test of cognitive motor functions for rats that relies on cues to navigate from start locations to the exit end through a labyrinth" (15). In other words, Navigational maze task is used in behavioural neuroscience to study spatial learning and memory.

In the navigational maze test involving 4 weeks trials, demonstrated significant alterations in the performance of group 2, 3, 4 and 5 from week 1 after exposure to week 4. The time taken to perform the navigation is a clear reflection of cognitive astuteness of the animal in challenging situations. Hence inability of test animals to complete the navigation or poor navigational pattern is an indication of cognitive astuteness or impairment. Tomas, (16) reported that lead is a potent inhibitor of the NMDA receptor, a protein known to play an important role in brain development and cognition. "His study demonstrated that lead exposure decreased the amount of NMDA receptor gene and protein in a part of the brain called the hippocampus. This change is associated with impairments of nerve communication in the brain and of learning."

Lower visuospatial memory scores have been consistently documented [17], suggesting that lead exposure disrupts visuospatial skills and the ability to remember visual stimuli. Occupational lead exposure is also associated with lowered visual memory scores, specifically delayed recall of a complex figure [18]

As presented in Table 4.3 The Brain Neurotrophic factor was measured along the Neurobehavioral test conducted, As shown in table 4.3, 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, BDNF a Neurotrophin (NT) which at the brain level is synthesized in the hippocampus, stria terminalis, amygdala, septum, nuclei of solitary, and cortex regulate survival & differentiation of neuronal population which include both sensory and motor neurons. (19). It has been reported that alterations of BDNF expression are implicated in the development of CNS diseases like Parkinson's disease, Alzheimer's disease and other depression related diseases (20) BDNF is also postulated as an essential unit of cellular mechanisms that aids formation and maintenance of memory through promotion of synaptic consolidation (21), Several other studies have shown that application of BDNF as therapy prevents the degeneration of neurons after Axotomy/other neural injuries and further suggested that BDNF is a potential

therapeutic agent for human neurodegenerative diseases. (22). From our current study, Lead and cadmium toxicity significantly altered the level of BDNF, and other neurobehavioral activities, hence metal toxicity poses debilitating effects on learning and memory and also associated with the development of neurodegenerative diseases.

Conclusion

Exposure to heavy metals contaminated diet has proven to cause alterations in cognitive functions, motor coordination and balance. It was ascertained that 4 weeks exposure to these metals also significantly altered the activities of "Brain derived neurotropic factor" an enzyme which is postulated as an essential unit of cellular mechanisms that aids formation and maintenance of memory through promotion of synaptic consolidation.

It was further detected that the activities of Lead and cadmium contaminated diet produces neuroleptic effects which are comparable to those caused by the effects chlorpromazine (CPZ) induction in rats. Therefore long term exposure to heavy metals contaminated diet poses delirious effect on BDNF and other Neurochemicals that enhances the effective functions of Neuro-activities as related to learning and memory.

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