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Research Article

Effect of Lead Poisoning and Antidepressant Drug on the Cerebral Cortex of the Wister Rats

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Abstract

The aim of this experiment is to investigate the effect of lead poisoning and antidepressant drug on the cerebral cortex of the wistar rats. The study employed thirty (30) wistar rats (150 - 200g) divided equally into five (5) groups: the first group (Negative control) was administered 1 ml/Kg of 0.9% saline once daily; second group depressed group (positive control) was administered 100 mg/kg of methyl isobutyl ketone once in one week; third group was administered 100 mg/kg of lead acetate (LA) once daily for one week; fourth group was administered 200 mg/kg of LA once daily for one week and fifth group was administered 200 mg/kg of LA once daily for one week and treated with 30 mg/kg of Imipramine once daily for one week. All treatments were administered intraperitoneally for one week while the fifth group was for two weeks. Behavioural test was carried out on the LA induced depression using Forced Swim Test (FST) and Open Field Test (OFT). The brain tissues were examined for histopathological parameters. The result indicated that there was significant ($p < 0.05$) increase in the duration of immobility in groups that received 100 mg/kg and 200 mg/kg of lead acetate when compared with the depressed control group. Activities of the rats in Open Field were also reduced. LA caused alterations and degeneration of the pyramidal and the cortical cells respectively and the effects was seen to have increased as LA dose increases. Therefore, it is concluded that histopathological observations in the 100 mg/kg and 200 mg/kg of LA of the brain showed degeneration of the cortical and pyramidal cells causing scattered shrunken neuronal cells with deeply stained cytoplasm, pyknotic nuclei, neuronal chromatolysis and the reduction in the staining ability of some neuronal cells.

Keywords: Lead Poisoning; Antidepressant Drug; Wistar Rat; Cerebral Cortex

Introduction

Lead is a pervasive and persistent environmental pollutant that can be detected in almost all phases of environment and biological systems. Lead constitutes the most abundant non-essential element in the humans, due to its dispersion in ambient air, many foods, drinking water, and dust. Humans have used lead since ancient times. However, the quantity of lead used in the 20th century far surpasses the total consumption in all previous eras. This is mainly because of the industrial applications especially the consumption of vast quantities of lead as an anti-knock agent in gasoline [1].

Lead toxicity or lead poisoning is a medical condition caused by increased levels of the heavy metal lead in the body. Symptoms of lead toxicity include abdominal pain, headache, anemia, irritability and in severe cases seizures, coma and death. Lead is a health hazard to humans if it is inhaled or ingested, interfering with the production of red blood cells it has the potential to disrupt many biological systems, particularly proteins because it forms complexes with important functional chemical groups including carboxyl (-COOH), amine (-NH) and thiol (-SH). Thus, many enzymes are potential targets. Several researches indicated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies.

In Nigeria, the deadly lead poisoning started five years ago in the year 2009 in Zamfara State (ICCON, 2014). This lead poisoning in Zamfara has led to the death of at least 734 children below the age of five, out of 5,395 kids within the age bracket, have been confirmed killed by lead poisoning between 2010 and March 2013 (This Day Newspaper, 2014). Adults are also affected with lead poisoning and have led to reproductive problems, high blood pressure, and nervous disorder and memory problems. All these problems and disorders are comorbid diagnoses to depression.

The brain is the magnificent and most complex organ in a vertebrate's body. It is an organ that serves as the centre of the nervous system in all vertebrate and most invertebrate animals. The brain is located in the head, usually close to the primary sensory organs for such senses as vision, hearing, balance, taste, and smell. It is made up of more than 100 billion nerves that communicate in trillions of connections called synapses (WebMD, 2015). The brain is made up of many specialized areas that work together: The cortex which is the outermost layer of brain cells. Thinking and voluntary movements begin in the cortex [2]; the brain stem is between the spinal cord and the rest of the brain. Basic functions like breathing and sleep are controlled there; the basal ganglia are a cluster of structures in the centre of the brain. The basal ganglia coordinate messages between multiple other brain areas [3]; the cerebellum

is at the base and the back of the brain. The cerebellum is responsible for coordination and balance. The brain is also divided into several lobes: The frontal lobes are responsible for problem solving, judgment and motor function. The parietal lobes manage sensation, handwriting, and body position. The temporal lobes are involved with memory and hearing. The occipital lobes contain the brain's visual processing system. The brain is surrounded by a layer of tissue called the meninges. The skull helps protect the brain from injury.

Many structures of the forebrain appear to be involved in depression, although it is not certain if disturbances to these brain areas cause depression, or if they are simply affected in the course of the disease. The brain areas involved include the frontal and temporal lobes of the forebrain, the basal nuclei, and parts of the limbic system including the hippocampus, amygdala and the cingulate gyrus. The cerebral cortex controls thinking, and it is likely that abnormalities in this part of the forebrain are responsible for the negative thoughts that are typical of depression. The hypothalamus and the pituitary gland may also play a role in depression, as they are involved in hormonal control, and increased levels of some hormones may play a role in maintaining a depressed state.

If the body is placed under stress, the hypothalamus-pituitary-adrenal axis becomes activated. The hypothalamus produces corticotrophin releasing factor (CRF) which is hypothesised to play a role in the precipitation of certain forms of depression. CRF stimulates the pituitary gland to secrete adrenocorticotropic hormone, which in turn stimulates the adrenal glands to release cortisol. Cortisol depresses mood and approximately 50% of people with severe depression have raised cortisol levels. In the brainstem, the raphe nuclei and the locus coeruleus are involved in the transmission of signals to other parts of the brain and are likely to be involved in depression. An imbalance or deficiency of the neurotransmitters, serotonin, noradrenaline and dopamine are implicated in depression, although it may be a change in receptor function, and not neurotransmitter concentration that causes depression. The present study investigate the effect of lead poisoning and antidepressant drug on the cerebral cortex of the wistar rats.

Materials and Methods

Materials

Chemicals and Reagents

Norepinephrine, Silver Nitrate, Methyl Isobutyl Ketone, Sodium hydroxide, Poly vinyl pyrolidine including the lead acetate were purchased from Sigma Chemical Company St Louis U.S.A and Serotonin ELISA kit was obtained from ImmuSmol SAS, 15 rue de l'Amiral Prouhet, 33600 Pessac, France with Shipping note No. L1502250635000209. All other chemicals were of analytical grade.

Animals

Wistar rats weighing between (150 - 200g) were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The rats were kept and maintained in well ventilated cages under standard laboratory conditions and maintained at ambient temperature and relative humidity respectively. Light and dark cycles were maintained at 12h each. They were maintained on grower's mash (Vital feeds Nigeria Ltd.) and provided with water at *ad libitum.* They were allowed to acclimatize to the laboratory conditions for two weeks.

Methods

Experimental Animals

Thirty (30) Wistar rats used were divided into five (5) groups with each group having six rats each, while the duration of the experiment was fourteen (14) days. Group 1: The control group was given 1 ml/Kg of 0.9% saline once daily. Group 2: This group was injected intraperitoneally with 100 mg/kg of methyl isobutyl ketone once in one week. Group 3: This group was given 100 mg/kg weight of lead acetate once daily for one week. Group 4: This group was given 200 mg/kg weight of lead acetate once daily for one week. Group 5: This group was given 200 mg/kg weight of lead acetate for one week and treated with 30 mg/kg of imipramine once daily for one week.

Induction of Depression

Lead acetate (LA) was prepared diluted with distilled water at stock solution form which the measured dose of 100 mg/kg and 200 mg/kg body weight of rats was induced by the intraperitoneal administration of lead acetate once daily for one week.

Determination of Depression

Depression was confirmed using Open Field Test (OFT) and Forced Swim Test (FST).

Open Field Test (OFT)

Open Field Test was carried out in a square wooden arena (80 cm × 80 cm × 40 cm high) with white smooth polished floor divided by black lines into 16 equal squares. The test was performed under white light in a quiet room. Each of the rats was placed at the same corner square and observed for 5 min. The floor and walls was cleaned after testing each rat. The following parameters were recorded during the 5 min observation period; latency: time taken by each animal till it starts moving in the arena, ambulation frequency: number of squares crossed by the animal, rearing frequency: number of times the animal stood stretched on its hind limbs with or without forelimb support and the number of fecal pellet [4-6].

Forced Swim Test

The forced swim test was performed according to the method described Porsolt., *et al.* [7] where each rat was placed for 5 minutes in a cylindrical water tank (70 cm high, 40 cm diameter) where, water level was about 40 cm and water temperature maintained at 23 - 25°C. The tank was emptied and washed with fresh water flush between each rat to remove any traces of urine or faeces. Two sessions was conducted; an initial 15 minutes training session (pre-test session) followed 24 hour later by a 5 minutes test session. During the test session, the immobility time, swimming and climbing times was observed. The total duration of the immobility was measured during the 5 minutes test. Upon removal from the water, rats were towel dried and finally returned to the cage. This was carried on the 6th and 7th day respectively.

Histopathological Analysis

Histological studies of the brain was carried out using the Haematoxylin and Eosin (H and E) Staining Protocol based on the principle that the oxidation product of haematoxylin is haematin, and is the active ingredient in the staining solution.

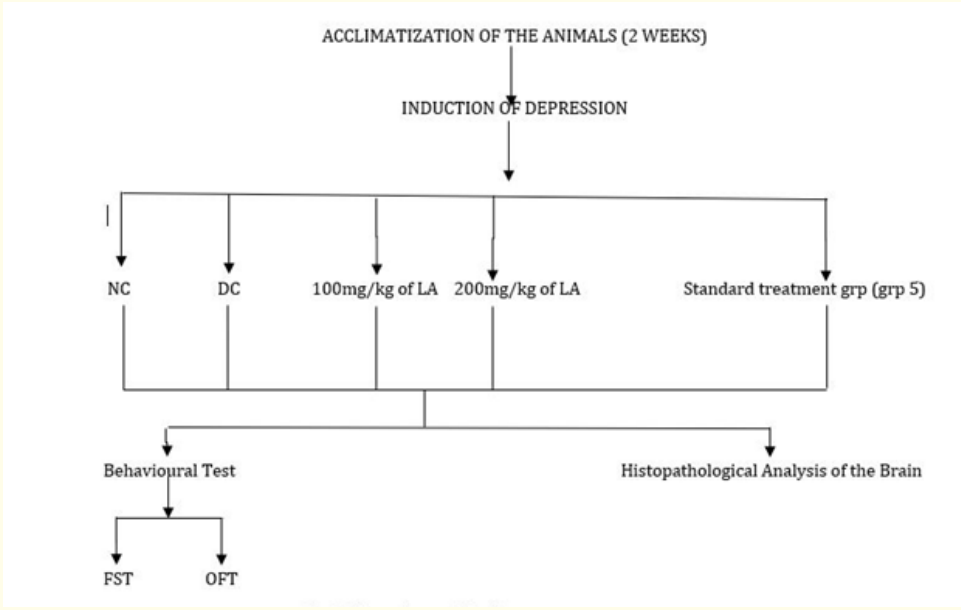


Figure : Experimental design.

Key: FST: Force Swim Test; OFT: Open Field Test; NR: Normal Control; DC: Depressed Control; LA: Lead Acetate

Result

Effect of Lead on Albino Wistar Rats Subjected to Forced Swim Test

The duration of immobility of rats after induction of depression with lead acetate was recorded as shown in table 1; there was a significant increase in the duration of immobility of the rats in groups that depression was induced when compared to the normal rats. A significant ($p < 0.05$) increase was also recorded in the duration of immobility when the group that received 100 mg/kg of Lead acetate and 200 mg/kg of lead acetate were compared with the depressed control group, also there was a significant ($p < 0.05$) reduction in the duration of immobility when the group that received 100 mg/kg of lead acetate was compared with the group that received 200 mg/kg of lead acetate.

Groups	Duration of Immobility (Seconds)
Normal Control	80.67 ± 4.16^{a}
Depressed Control	91.00 ± 1.00^{b}
Normal + 100 mg/kg of Lead Acetate	138.00 ± 1.00^{d}
Normal + 200 mg/kg of Lead Acetate	131.00 ± 2.00^{c}
Normal + 200 mg/kg of Lead Acetate + I	87.67 ± 3.51^{b}

Table 1: The Effect of Lead acetate on Albino Wistar rats when subjected to Forced Swim Test.

Values are expressed as means ± SD (n = 6); abcdP < 0.05, as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison.

Key: NRC: Normal Control; DPC: Depressed Control

NR + 10 0mg/kg of LA – Normal + 100 mg/kg of Lead Acetate

NR + 200 mg/kg of LA – Normal + 200 mg/kg of Lead Acetate

NR + 200 mg/kg of LA + I – Normal + 200 mg/kg of Lead Acetate + 30 mg/kg of Imipramine

Effect of Lead Acetate on Albino Wistar Rats Subjected to Open Field Test

Open field test was performed to confirm depression. As shown in table 2, there was a significant ($p < 0.05$) reduction in the ambulation frequency when the normal control group is compared with the depressed control group, group that received 100 mg/kg and 200 mg/kg of lead acetate. As shown in table 2, there was a significant ($p < 0.05$) reduction in the rearing frequency when the normal control group is compared with the depressed control group and with the rest of the treatment groups. Also, as shown in table 2, there is a significant ($p > 0.05$) difference in the number of faecal pellet of the normal control rats group when compared with depressed control group and group that received 200 mg/kg of lead acetate.

Group (n = 6)	Ambulation Frequency (count/5min)	Rearing Frequency (count/5min)	Number of Faecal Pellet
NRC	53.25 ± 3.30^{c}	25.50 ± 2.38^{c}	4.75 ± 0.96^{c}
DPC	23.50 ± 4.66^{b}	8.75 ± 2.99^{a}	2.33 ± 0.96^{b}
NR+100 mg/kg of LA	10.00 ± 5.29^{a}	12.00 ± 2.00^{a}	3.00 ± 0.58^{b}
NR+200 mg/kg of LA	22.80 ± 6.76^{b}	8.00 ± 0.82^{a}	1.25 ± 0.50^{a}
NR+200 mg/kg of LA+I	58.00 ± 1.00^{c}	17.67 ± 3.06^{b}	4.33 ± 0.58^{bc}

Table 2: Effect of Lead acetate on Albino Wistar rats when subjected to Open Field Test.

Values are expressed in mean ± SD (n=6); abcP<0.05, as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison.

Key: NRC: Normal Control; DPC: Depressed Control

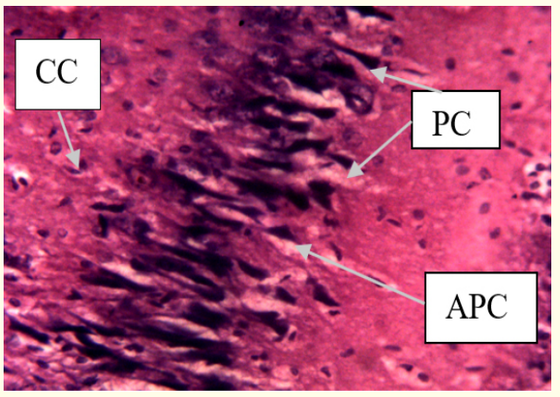
NR + 100 mg/kg of LA – Normal + 100 mg/kg of Lead Acetate

NR + 200 mg/kg of LA – Normal + 200 mg/kg of Lead Acetate

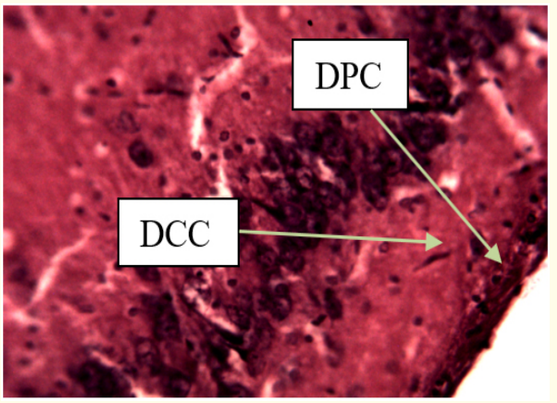
NR + 200 mg/kg of LA + I – Normal + 200 mg/kg of Lead Acetate + 30 mg/kg of Imipramine

Histopathological Analysis of the Cerebral Cortex of Brain

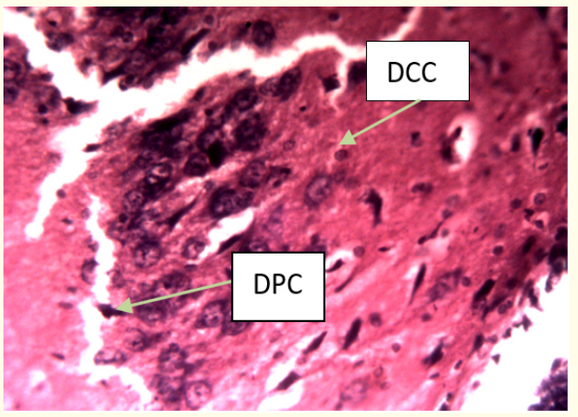
The histopathological changes are presented on plates 1; the normal control cerebral cortex of the brain showing part of the hippocampal layers shows normal architecture without any degenerating cells (A). The Depressed control cerebral cortex of the rats shows few degenerating cortical and pyramidal cell (B). The cerebral cortex of the rats induced with 100 mg/kg and 200 mg/kg of Lead acetate shows fewer degenerating pyramidal and cortical cells (C) and (D) and the treatment with imipramine shows reduced degree of degeneration of pyramidal and cortical cells (E).



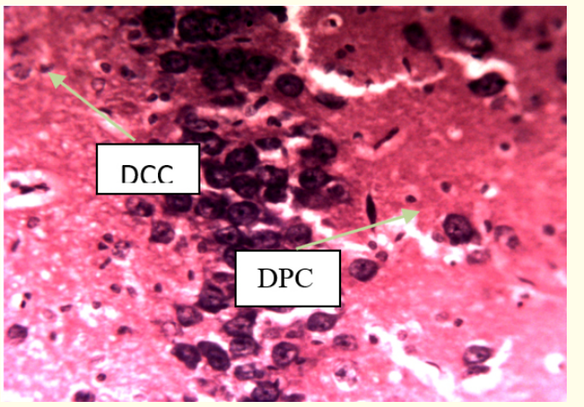
A: Normal control



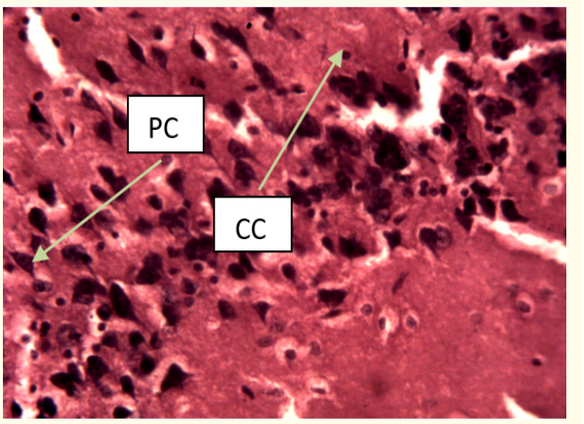
B: Depressed Control



C: 100mg/kg of lead acetate group



D: 200mg/kg of lead acetate group



E: Standard drug treatment group

Plates 1: A section of the cerebral cortex of the Brain showing part of the hippocampal layers stained with H & E technique, 250 × magnification: Cerebral cortex of the brain showing part of the hippocampal layer from A: Normal Control (NC) group showing normal architecture; B: Fewer degenerating pyramidal and cortical cells; C: Few degenerating pyramidal cells and cortical cells; D: few degenerating pyramidal and cortical cells; E: pyramidal and cortical cells appearing to go to the normal condition but with few degenerating cells.

Key: CC: Cortical Cell; PC: Pyramidal Cell; APC: Axon of Pyramidal Cell; DPC: Degenerating Pyramidal Cell; DCC: Degenerating Cortical Cell

Discussion

Lead (Pb) is one of the major heavy metals known to be toxic to mammal with physiological problem such as mental retardation, learning disabilities, low birth weight and behavioural problems being associated with lead poisoning [8]. In this present study, lead-induced wistar rats have shown behavioural impairment in the behavioural studies and this was confirmed through a depression model called forced swim test. This depression model of behavioural studies has shown a significant increase in the duration of immobility time in lead-induced male

wistar rats at both doses. While the wistar rats that received treatment with imipramine shows reduced immobility time significantly to 87.67 seconds. This result correspond to the substance induce depression work done by Shabinum and co. [9] where they use Methylmercury to induce depression and they found out that low level of Methylmercury induces alterations in depression like behaviour.

OFT is normally used to prove that the reduction of immobility time was not caused by the stimulation of motor activities. Many researchers use it to complement FST to avoid false positive results that can be obtained in the forced swim test [10]. In this present study, there was significant reduction in the ambulation frequency, rearing frequency and faecal pellets in the open field test when compared to the normal control group. This further suggest that the lead-induced wistar rats where depressed and this behavioural change was also observed to be dose dependent depending on the degree of exposure, while the group that was treated with standard drug (imipramine) try to reverse the condition. This findings where similar to that reported by NourEddine., *et al.* [11] and Pachauri., *et al* [12].

The histopathological study of structure and chemical composition of tissues of animals are related to their function. The primary aim is to understand how tissues are organized at all structural levels, including the molecular and macromolecular, the entire cell and intercellular substances and the tissues and organs. Compared to other organ systems, the nervous system is often referred to as the most sensitive to lead induced toxicity [13]. Lead toxicity is reported to cause brain damage and even death at higher levels [14]. Cerebral cortex is a sheet of neural tissue which is the outer-most part of the cerebrum of a mammalian brain. Its key roles are in memory, attention, perceptual awareness, thought, language and consciousness [15]. From the histopathological results in this present study, the brain of the depressed and lead-induced depressed wistar rats showed slight changes in the histopathological structure of brain, with scattered shrunken neuronal cells with deeply stained cytoplasm and pyknotic nuclei in addition to neuronal chromatolysis, revealing some degenerative changes. While the degree of degeneration was reduced in the imipramine treated wistar rats. These findings were in agreement with the reports of Amal and Mona [16], Sharifi., *et al*. [17] and Abbas., *et al* [18] stated that lead administration of different dose levels caused pyknosis of neurons associated with focal gliosis in addition to focal cerebral haemorrhage [19].

Conclusion

In the present study, lead acetate was able to cause depression, and this was determined in the behavioural test (FST and OFT). In the Force Swim Test, increase the duration of immobility (s) was significantly observed in lead-induced wistar rats.

In the Open Field Test, there was significant decrease in Ambulation frequency, rearing frequency and the number of fecal pellet in lead-induced wistar rats.

The histopathological observations in lead-induced wistar rat brains showed degeneration of the cortical and pyramidal cells causing scattered shrunken neuronal cells with deeply stained cytoplasm, pyknotic nuclei, neuronal chromatolysis and the reduction in the staining ability of some neuronal cells. This effect was shown to have been corrected in imipramine treated wistar rats.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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