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Effect of Lead Acetate on Norepinephrine and Serotonin Concentration in Albino Wistar Rats Induced Depression

Salisu Muhammad Highab[1*], Ismaila Raji[1], Iliya Jeremiah Makarau[2]

[1]Department of Pharmacology and Therapeutics,
Faculty of Basic Medical Sciences,
College of Medicine and Health Sciences,
Federal University Dutse,
Jigawa State, Nigeria.

[2]Department of Chemistry,
School of Science,
Federal College of Education Zaria,
Kaduna State, Nigeria.
Email: smhighab@gmail,com

Abstract
Depression is a mental disorder characterized by a pervasive and persistent low mood that is accompanied by low self-esteem and loss of interest in normally enjoyable activities. The aim of this study is to investigate the effect of lead acetate(LA) on norepinephrine (NE) and serotonin (5-HT)concentration in albino Wistar rats induced depression.The study employed thirty (30)albino Wistar rats 150– 200g, 3-4 month old randomly distributed into five (5) groups of six (6) rats each. The first group (negative control) was administered 1ml/kg of 0.9% saline once daily. The second (depressed, positive control)group was administered 100 mg/kg of methyl isobutyl ketone (MIK) once in one week, the third group was administered 100mg/kg of LA once daily for one week, the fourth group was administered 200mg/kg of LA once daily for one week, while the fifth group was administered 200mg/kg of LA once daily for one week and treated with 30mg/kg of Imipramine once daily for one week. All treatments were administered intraperitoneally for one week. Compared to the negative control group, the depressed group of rat, as well as the LA treated groups of rats had significantly ($p < 0.05$) lower levels of NE and 5-HT in both the brain and in serum. LA treatment significantly ($p < 0.05$) reduced brain NA and 5-HT when compared to the negative control group. These depletions of NA and 5HT were reversed by imipramine. In conclusion, lead acetate induced depression by decreasing brain and serum NE and 5-HTlevels, and this might be responsible for the behavioural and neurological abnormalities observed in this study.

Keywords: Albino Wistar rat, Imipramine, Lead toxicity, Norepinephrine, Serotonin,

INTRODUCTION
Lead toxicity or lead poisoning is a medical condition caused by increased levels of the heavy metal lead in the body. Symptoms include abdominal pain, headache, anaemia, irritability and in severe cases seizures, coma and death (Guidotti and Ragain, 2007). Lead is a health hazard to humans when inhaled or ingested. It disrupts many biological systems, particularly proteins (such as the red blood cells, necessary for oxygen transport in the body). Lead has also been associated with neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies (Patrick, 2006).

**Author for Correspondence*

Neurotransmitters play major roles in shaping everyday life and functions (Cherry and Kendra, 2014), and include glutamate, aspartate, D-serine, γ-aminobutyric acid (GABA), glycine; monoamines:dopamine (DA), norepinephrine (NE, noradrenaline; NA), epinephrine (adrenaline), histamine, and serotonin (5-HT) (Snyder and Innis, 1979; Robert, 2005).Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter. Biochemically derived from tryptophan (TRP), 5-HT is primarily found in the gastrointestinal (GI) tract, platelets, and the central nervous system (CNS) of animals, including humans (Young, 2007). Approximately ninety percent (90%) of the human body's total 5-HT is located in the entero-chromaffin cells in the GI tract, where it is used to regulate intestinal movements (Berger *et al*., 2009). The remainder is synthesized in serotonergic neurons of the CNS, where it has various functions such as the regulation of mood, appetite, and sleep, and some cognitive functions (memory and learning). Modulation of 5-HT at synapses is thought to be a major action of several classes of pharmacological antidepressants (Frazer and Hensler, 1999; Bianchi *at al.,* 2005; Lesurtel *et al*., 2006; Agabegi and Steven, 2008).

Meanwhile, NE is synthesized from dopamine by dopamine β-hydroxylase (Katzung and Bertram, 2015). It is released from the adrenal medulla into the blood as a hormone, and as a neurotransmitter in the CNS and sympathetic nervous system where it is released from noradrenergic neurons. The noradrenergic neurons in the brain, when activated, exert effects on large areas of the brain, producing alertness and arousal, while also influencing the reward system (Drevets *et al*., 2002). Numerous differences have been found in elements of the NE system in post-mortem brains from depressed patients and healthy controls. Genetic studies also show that mice with genetically engineered functional enhancement of the NE system are protected from stress-induced depression-like behaviours (Rang, 2003).Experimental depletion of NE in the brain results in a return of depressive symptoms after successful treatment with NE antidepressant drugs (which specifically increase NE activity)and other therapeutic agents (Stahl, 2013). Therefore, the current study investigated the effect of LA induced depression on NE and 5-HT concentration in albino Wistar rats

MATERIALS AND METHODS

Experimental Animals

Wistar rats weighing between 150 – 200g, 3-4 month old 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 *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks. All experimental protocols were in accordance with the Ahmadu Bello University research policy (NIH publication number 85 - 23, revised 1996) and of regulations governing the care and use of experimental animals. The experiments were conducted in a quiet environment between the hours of 0900 and 1600.

Experimental Procedures

Thirty (30) Albino Wistar rats used were divided into five (5) groups of six (6) rats each, while the duration of the experiment was 14 days. Group 1 which is control group was given 1ml/kg of 0.9% saline once daily. Group 2 was injected intraperitoneally with 100mg/kg of MIK once in one week. Group 3 was given 100mg/kg weight of LA once daily for one week. Group 4 was given 200 mg/kg weight of LA once daily for one week. Group 5

was given 200 mg/kg weight of LA for one week and treated with 30mg/kg of imipramine (I) once daily for one week.

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

Determination of Serotonin and Norepinephrine
Serotonin was measured using serotonin ELISA kit and was quantitatively acylated. The subsequent competitive ELISA kit uses the microliter plate format. The antigen bounded to the solid phase of the microliter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes was removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction was monitored at 450 nm. Quantification of the serum and brain samples was achieved by comparing their absorbance with a reference curve prepared with the known standard concentration at 450nm.TheNorepinephrine level was measured by Spectrophotometric according to method of Hormozi and Khodaveisi (2009). The principle is based onreduction of Ag^{+} to silver nanoparticles (Ag-NPs) by NE in the presence of polyvinyl pyrrolidone (PVP) as a stabilizing agent. The Procedure include 1 ml of 0.01 M $AgNO_3$, 0.7 ml of 0.4 (g/L) PVP, 0.5ml of the supernatant of brain and serum sample at different concentrations and 1 ml of 0.001 M NaOH was prepared in 5ml volumetric flasks which was then mixed slowly, and a portion transferred within 7 min into a 1 cm spectrophotometric cell to record the absorbance at 440 nm. Quantification of the serum and brain samples was achieved by comparing their absorbance with a reference curve prepared with the known standard concentration.

Statistical Analysis of the Results
The data was analysed using analysis of variance (ANOVA). The difference between the various groups was compared using the Duncan Multiple Range Test. The result was expressed as mean ± standard deviation (SD) except where otherwise stated. ($p< 0.05$) was considered significant.

RESULTS AND DISCUSSION

Effect of Lead on Norepinephrine Concentration in Albino Wistar Rats Induced Depression
Compared with the negative control (normal, NRC) group, the depressed (positive) control (DPC), the NR + 100mg/kg of LA, NR + 200mg/kg of LA and NR + 200mg/kg of LA + I test groups showed significant ($p< 0.05$) reductions in the level of brain and serum NE. There was no significant reduction in the serum NE levels between the depressed and other test groups that did not receive imipramine. Addition of imipramine significantly ($p< 0.05$) reversed the depletion in NE levels in both the brain and serum of rat given LA treatment to levels close to those in the control group(Fig 1, Fig 2 and Fig. 3).

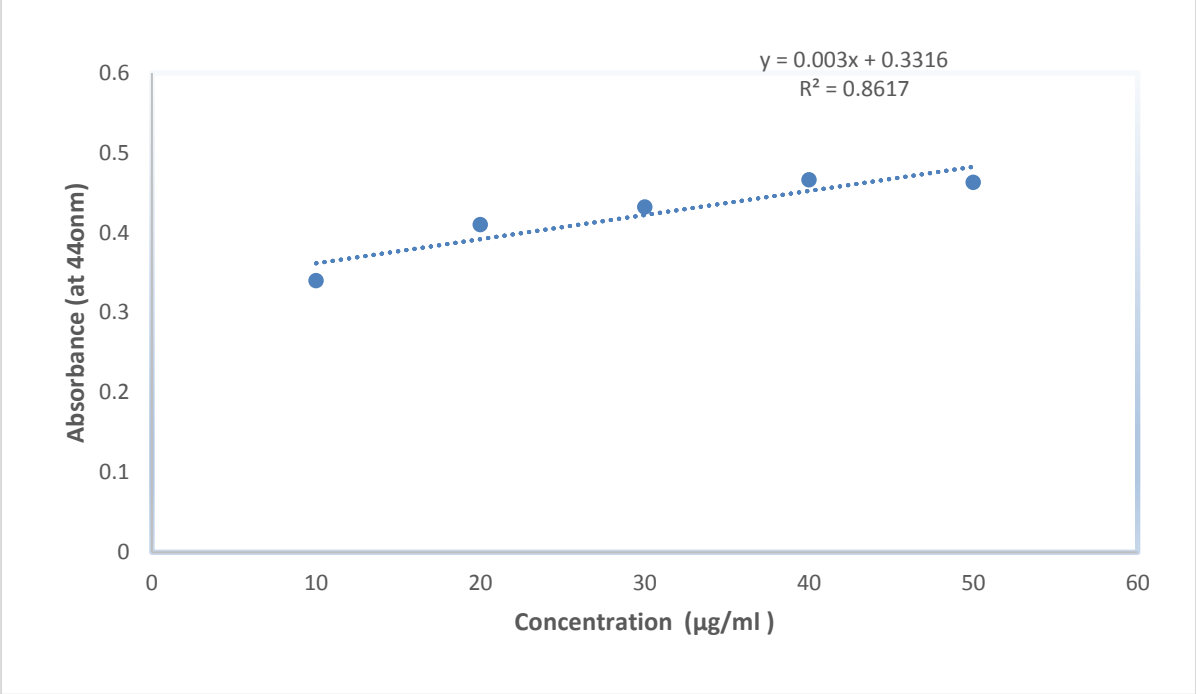


Fig 1: Showing Standard Curve for Nor-Epinephrine

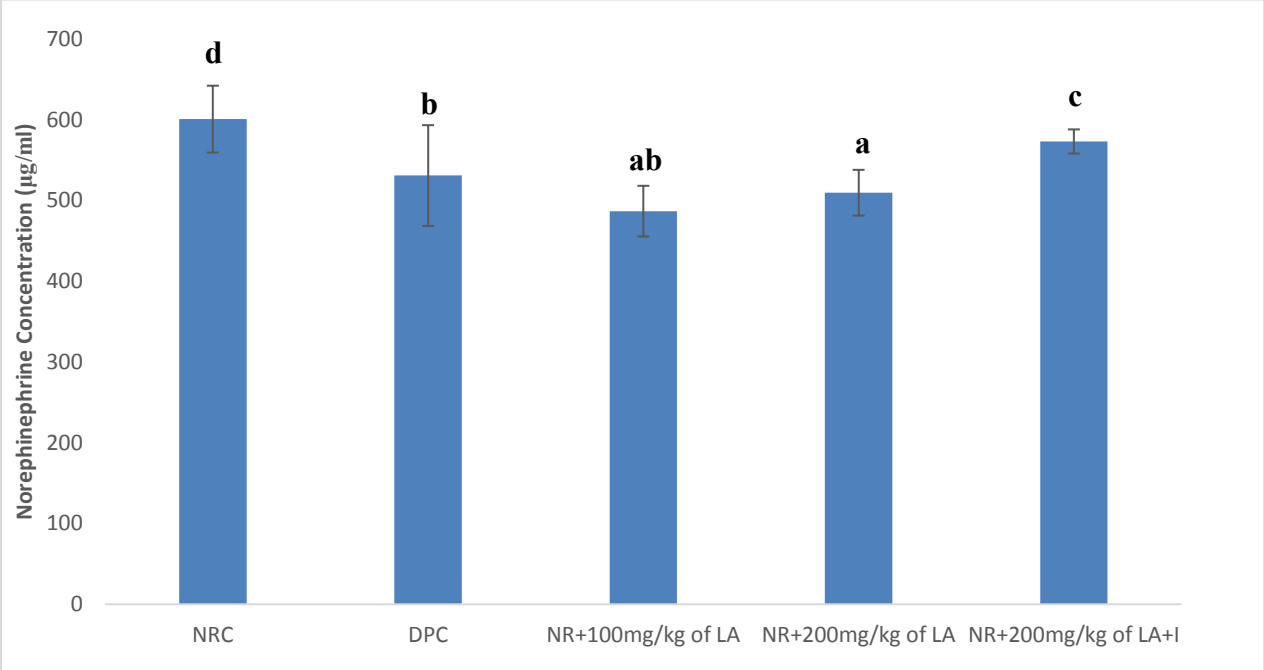


Fig2 :Effect of Lead on NE Concentration in the Brain of Albino Wistar Rats Induced Depression. Values are presented as means ± SD,^{abcd} = $p < 0.05$ compared to NRC and DPC. One way ANOVA followed by Duncan Multiple Range Test. NRC-Normal control, DPC-Depressed control, LA-lead acetate,I-Impramine.

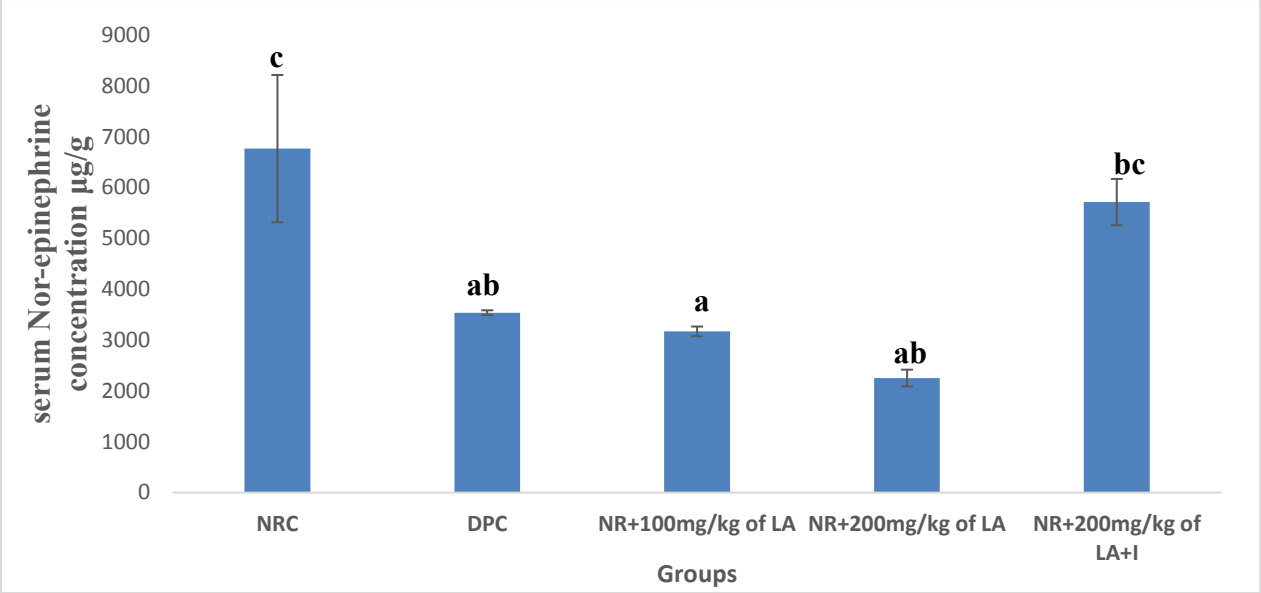


Fig 3:Effect of Lead on Nepinephrine Concentration in the Serum of Albino Wistar Rats Induced Depression. Values are presented as means ± SD, ^{abcd} = $p < 0.05$ compared to NRC and DPC. One way ANOVA followed by Duncan Multiple Range Test. NRC-Normal control, DPC-Depressed control, LA-lead acetate,I- Imipramine.

Effect of Lead on Serotonin Concentration in Albino Wistar Rats induced Depression

There was a significant (p<0.05) reduction in the brain 5-HTlevel in the treated groups when compared with the normal (negative) control group. The treated group that received 200mg/kg of LAhad lowest 5-HT level (Fig 5). All test groups, except that with the addition of imipramine had significantly (p < 0.05) lower serum levels of 5-HT. There was no significant difference in serum levels of 5-HTbetween the (negative) control group and the imipramine group that received 200mg/kg LA. (Fig 6).

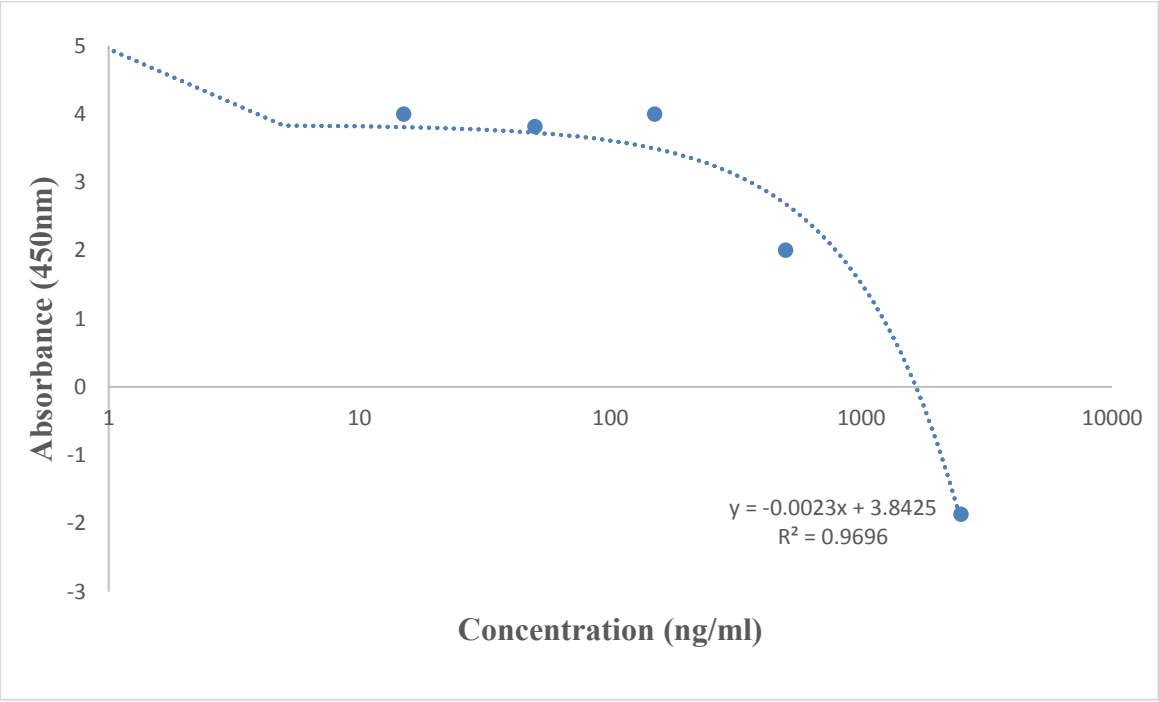


Fig 4: Showing Standard Curve for Serotonin

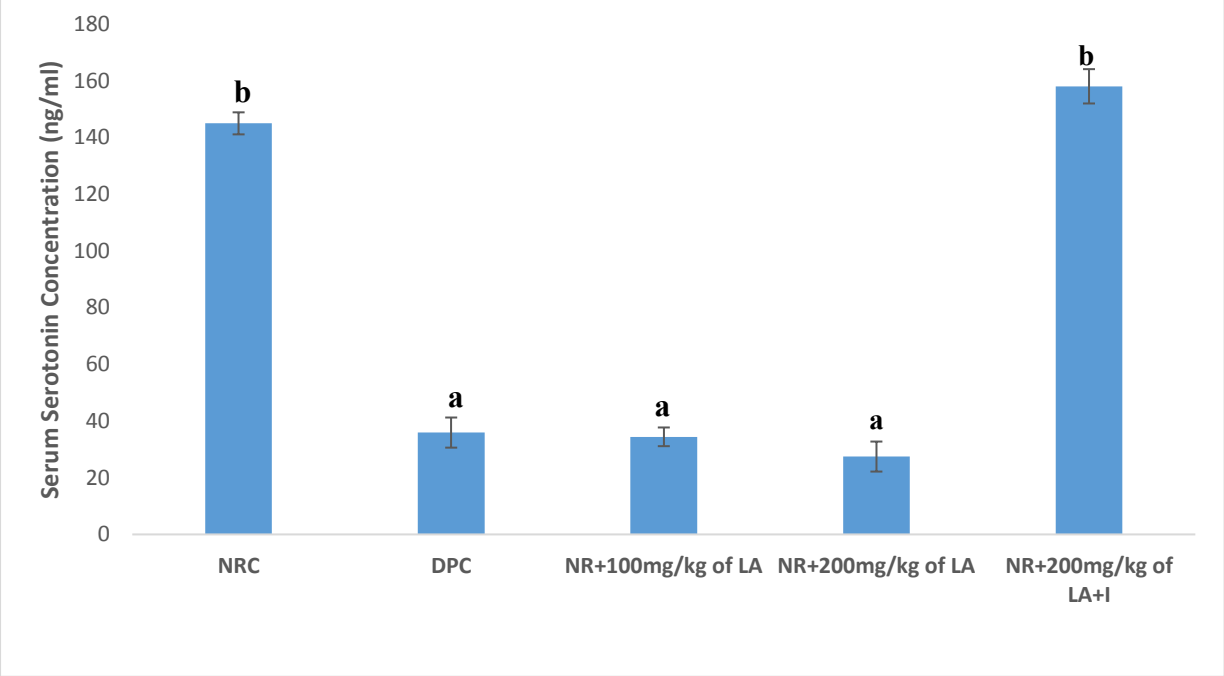


Fig 5: Effect of Lead on Serotonin Concentration in the Brain of Albino Wistar Rats Induced Depression.Values are presented as means ± SD, ^{ab} = $p < 0.05$ compared to NRC and DPC. One way ANOVA followed byDuncan Multiple Range Test. NRC-Normal control, DPC-Depressed control, LA-lead acetate,I- Imipramine.

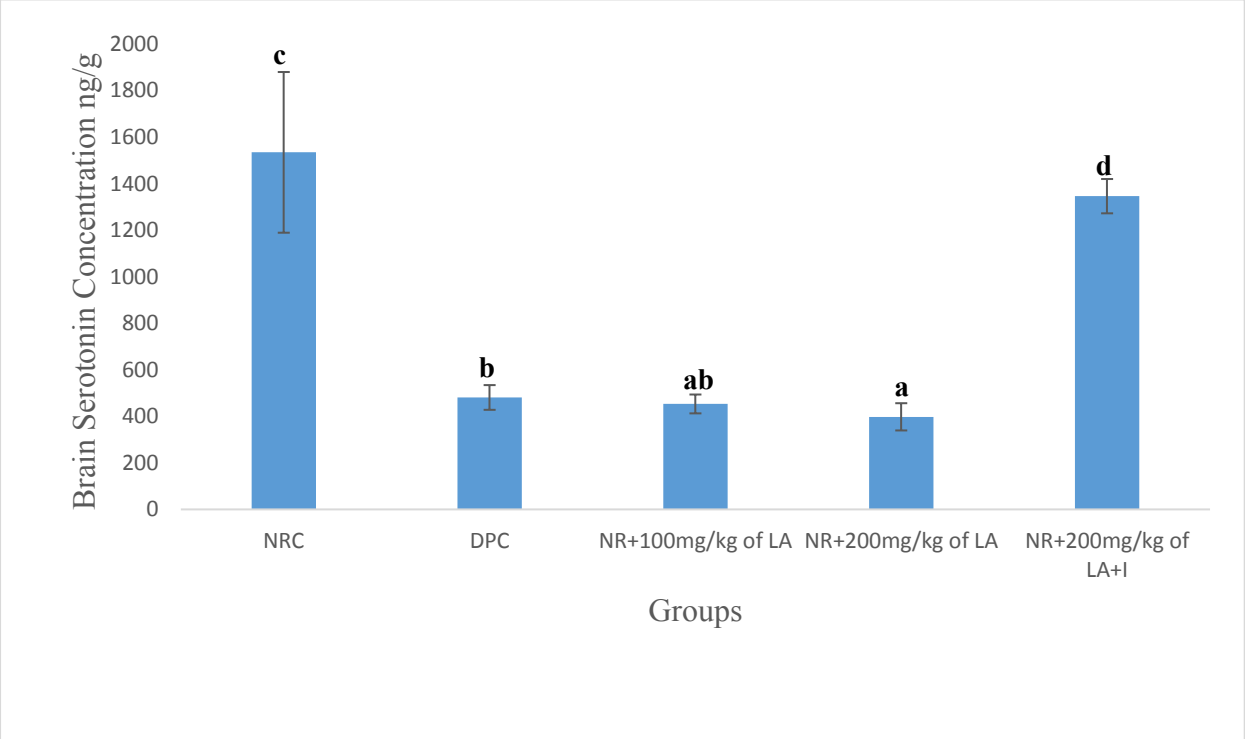
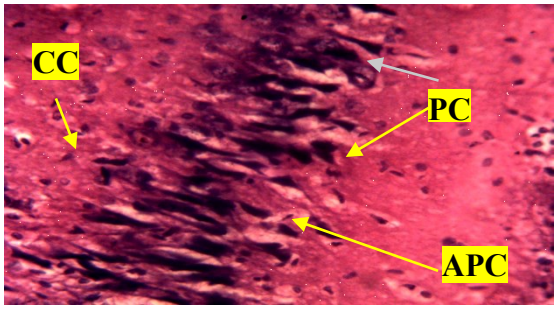


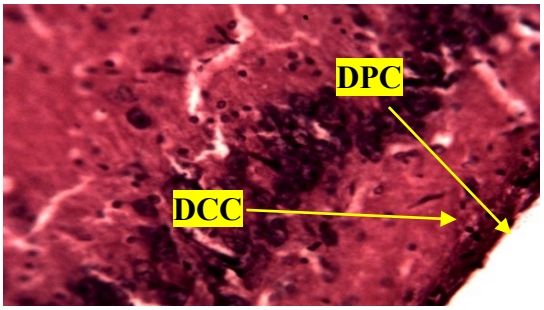
Fig 6: Effect of Lead on Serotonin Concentration in the Serum of Albino Wistar Rats Induced Depression. Values are presented as means ± SD, ^{abcd} = $p < 0.05$ compared to NRC and DPC. One way ANOVA followed byDuncan Multiple Range Test. NRC-Normal control, DPC-Depressed control, LA-lead acetate,I- Imipramine.

Effect of on the Histopathology of the Cerebral Cortex of the Brain

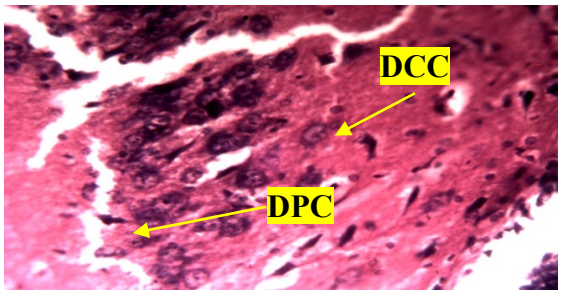
The histopathological changes are presented on Plates 1: the normal control cerebral cortex of the brain showing part of the hippocampal layers with normal architectural structure without any degenerating cells (A). The Depressed control group, cerebral cortex of the rats revealed few degenerating cortical and pyramidal cell (B). The cerebral cortex of the rats induced with 100mg/kg and 200mg/kg of LA revealed fewer degenerating pyramidal and cortical cells (C) and (D) and the treatment with imipramine revealed more improvement by reducing degree of degeneration of pyramidal and cortical cells (E).



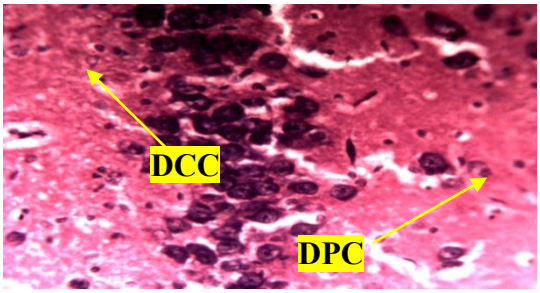
A: Normal control



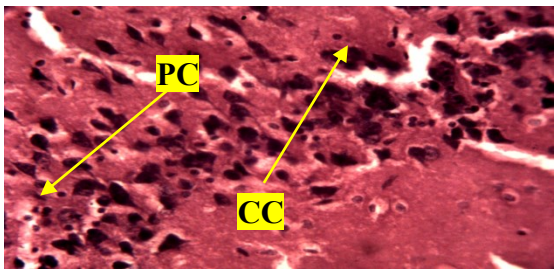
B: Depressed Control



C: 100mg/kg of LA group



D: 200mg/kg of LA group



E: 200mg/kg of LA + 30mg/kg of I

Key:
CC: Cortical Cell
PC: Pyramidal Cell
APC: Axon of Pyramidal Cell
DPC: Degenerating Pyramidal Cell
DCC: Degenerating Cortical Cell
LA: Lead Acetate
I: Imipramine

Plates1: 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.

DISCUSSION

Methyl isobutyl ketone (MIK) produced a reduction in brain NE levels, and a significant (p <0.05) reduction in serum NE levels. It significantly (p <0.05) reduced both brain and serum 5-HT levels. Lead acetate significantly (p <0.05) decreased brain and serum levels of NE and

5-HTin a time and dose-dependent fashion when compared to the controls, while imipramine significantly (p <0.05)restored brain and serum levels of these neurotransmitters. The decreased neurotransmitter levels with LA are in agreement withthe previous works of Waggas, (2012) and Abbas *et al*., (2015).In the sympathetic nervous system, lead exposure alters the concentration ofNE and dopamine (Gill *et al*., 2003). Previous studies showed that lead (Pb)induced an increase in spontaneous transmitter release, either by an increase in intra-neuronal ionized calcium, or by the stimulation of Pb or Ca^{2+}-activated molecules mediating transmitter release (Nachshen, 1984 and Minnema *at al*.,1988). Lead is reported to exert its neurotoxic effect by interfering with Ca^{2+}calmoduline mediated neurotransmitter release, which is eventually responsible for behavioural impairment (Bouton *et al*., 2001). Also the decrease of 5-HT observed in both brain and serum of the lead-induced depression may be due to the increased degradation of TRP in the liver by the enzyme pyrolase, leading to the decreased transportation of TRP from plasma to brain, or it could be that the activity of enzyme required for the synthesis decreased by the administration of Pb. Also, the strong correlation between Pb administration and decrease 5-HT level may be due to a disruption of 5-HT storage pools leading to the increased degradation of 5-HT inside the neurons by monoamines (Sajitha *et al*., 2010).

The brains of the (MIK) depressed, the 100mg/kg body weight LA depressed and the 200mg/kg body weight LA depressed rats revealed slight changes in the form of scattered shrunken neuronal cells with deeply stained cytoplasm and pyknotic nuclei in addition to neuronal chromatolysis, revealing some degenerative changes as shown in Plate 1. Lead acetate treatment reduced the staining ability of some neuronal cells, which was improved by imipramine, suggesting that the degree of degeneration was reduced by imipramine. This corroborates a previous report by Amal and Mona (2009), which stated that lead administration of different dose levels caused pyknosis of neurons associated with focal gliosis in addition to focal cerebral haemorrhage. The histopathological finding is in concert with changes in neurotransmitters levels observed in the brain and serum of the rats in the study, and is in agreement with the work reported by Sharifi *et al*., (2002) and Abbas *et al*., (2015).

CONCLUSION

Lead acetate induced depression by decreasing the brain and serum norepinephrine and also the serotonin levels. An effect that may be responsible for the neurological abnormalities observed in this study, and by inference, behavioural changes observed with lead toxicity.

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