In this study, twelve Wistar Rats were used and grouped into four groups of two females and one male. Group I rats served as the control.

From this study, lead exposure of Wistar rats at both prenatal and postnatal period of development has effect on the histology of the frontal cortex and motor activity of the rat pups. The histological study has shown normal neurons in the control group while degenerating cells exhibiting karyolysis, pyknosis, karyorrhexis, vacuolation were seen in the lead-treated groups. Group II and Group IV showed considerable deficit in their motor activity while Group III showed mild effect.

Conclusion: From this study, lead exposure of Wistar rats at both prenatal and postnatal period of development has effect on the histology of the frontal cortex as well as on their motor activity.

Keywords: Lead, Frontal cortex, Prenatal, Postnatal, Motor activity, Palmer grasp reflex.

INTRODUCTION

Lead (Pb) is a heavy metal well known from the history of mankind to the present day for its diverse uses, misuses, and toxicity. Even after the enactment of laws and regulations against the damaging use, still there are sporadic cases where the excess amount of Pb is being used. Once ingested orally in the food, from the environment, in mother’s milk to infants, or through placenta in fetus, the Pb is slowly absorbed into the gastrointestinal tract [1].

According to Apriku and Siminialayi (2013) [2], Pb is a heavy metal and environmental pollutant which occurs naturally as Pb oxide or Pb salts [3]. With its characteristic (dense, ductile, malleable, and corrosion-resistant) properties, the metal has wide industrial applications in modern society. Pb is being used in production of paints, batteries, water pipes, gun bullets, x-ray, and atomic radiation protection, eye cosmetics, base metal utensils, and also as additive in gasoline [4].

The frontal cortex of the brain (cerebrum) plays a vital role in speech and language production, some motor skills, comparing objects, forming memories, forming personality, and managing attention [5].

Pb is a highly neurotoxic agent affecting the developing central nervous system [6,7] through its interference of the cholinergic system and regulatory action of calcium on cell functions thereby disrupting many intracellular biological activities [8]. The alteration in this biochemical intercellular communication may precede neurobehavioral and neurological end points, usually observed in lead neurotoxicity [7].

Therefore, this study was conducted to find the effect of lead on the frontal cortex and motor activity of prenatally and postnatally exposed Wistar rats.

METHODS

Experimental animals

After obtaining ethical approval from the Ethics Committee on Animal Use and Care, Yusuf Maitama Sule University, Kano, a total of 24 (8 males and 16 females) adult Wistar rats from the animal house of the Department of Pharmacology, Bayero University, Kano, were used for the study. The animals were kept to acclimatize for 14 days in an animal house in the department of Human Anatomy, Yusuf Maitama Sule University, Kano. They were fed on a commercial pellet diet and allowed access to food and distilled water ad libitum.

Animal grouping

Two females with regular oestrous cycle were selected in the pro-oestrous stage and caged together with one male overnight. The 1st day of gestation was determined by the presence of sperms in the vaginal smear [9,10]. Pregnant female rats were then placed in different cages at 1st day of pregnancy. The animals were grouped as follows:

Group I (Control): This group consists of six rats, two males and four females. The normal control group will be fed food and distilled water ad libitum.

Group II (Prenatal exposure): This group is made up of six rats, two males, and four females. The female rats in this group were exposed to 500ppm of Pb acetate (BDH Chemicals Ltd, Product No.: 29021, Poole, England) in distilled drinking water of the mother from the 6th gestation day (GD) to GD21 [11,12].

Group III (postnatal exposure): Six rats, two males, and four females were used in this group. The female rats were exposed to 500 ppm Pb acetate in their distilled drinking water from postnatal day 1 (PND) to PND21.

RESULTS

The histological study has shown normal neurons in the control group while degenerating cells exhibiting karyolysis, pyknosis, karyorrhexis, vacuolation were seen in the lead-treated groups. Group II and Group IV showed considerable deficit in their motor activity while Group III showed mild effect.
Group IV (gestation through lactation exposure): Six albino Wistar rats, four females and two males were used in this category. The rats were exposed to 500 ppm lead acetate in distilled water of the mother from the 6th day of gestation (GD6) to parturition and continued up to PND 21 and stopped at weaning [13]. The time span of GD6 to PND 21 was chosen to allow for embryo implantation in utero, and to maximize the effect of developmental Pb exposure, given that [14] found higher blood Pb levels at the later stages of gestational exposure.

**Route of administration**

The different concentration of lead acetate was administered through drinking water. This is based on the method used by [15], which resulted in causing lead poisoning in the rat pups.

**Duration of lead exposure**

The duration of the experiment was different from the groups. Group I animals serves as control where the group II animals will be exposed to lead acetate from the GD 6 to parturition while group III animals were exposed from parturition up to the 21st day postnatally. The fourth group animals were exposed from GD6 to parturition and continued to PND 21. The fetuses in each group at the end of their period of Pb exposure were anaesthetized with ether (Trust Chemical Laboratories, Cat No. 67663, Hangzhou, China) and their whole brain was removed by opening the calvarium through the sagittal sutures.

**Histological tissue processing**

The harvested tissues were immediately fixed in Bouin’s solution (319.18 M, pH 2) for 24 h before the frontal cortices were isolated and subjected to routine histological processing based on the method of [16]. This was conducted in the Department of Human Anatomy, Yusuf Makama Sule University, Kano.

**Motor activity**

Palm grasp test: an object was placed in the hand of the pups with the fingers close and tightly grasping the object [17]. A stopwatch was used to record the time taken for the pups to hold to the object.

**Statistical analysis**

Data obtained from the motor activity was expressed as mean ± SEM using statistical package for service solution ([SPSS] IBM Corp, Version 21). Values of p<0.05 were considered statistically significant.

**RESULTS**

**Histological evaluation of the frontal cortex**

The H&E micrographs of Plate Ia and Plate Ib are representative sections of Group I at lower and higher magnifications, showing the normal cytoarchitecture of the frontal cortex of Wistar Rats with distinct pyramidal cells at both euchromatic and heterochromatic states.

Photomicrographs of Plates 2a and b are sections of frontal sections of rats administered 500 ppm of lead acetate from GD6 to parturition (Group II). The sections demonstrate showing areas of mild fibrosis and degenerating neurons (DN) at various stages; karyolysis (KL) and karyorrhexis (KH).

Plate 3a and b are photomicrograph of a section of Wistar rat's frontal cortex administered 500ppm of lead acetate from PND1 to PND21. They are characterised by neuropil vacuolation, perivascular vacuolation and DN; KL, pyknosis and KH.

The H&E micrographs of Plate 4a and b administered 500 ppm of lead acetate from GD6 to PND21. It featured swollen astrocyte (AST) or Alzheimer type II cells, discontinuous blood capillaries (DC), karyolytic (KL) and karyorrhexic neurons, and vacuolarized pyramidal neurons.

Plate 1: (a) Photomicrograph of a section of the frontal cortex of Wistar rat group I (control) showing normal architecture of the frontal cortex (H&E ×400). (b) Photomicrograph of a section of the frontal cortex of Wistar rat in Group I showing cortical pyramidal cells with their nuclei at both euchromatic and heterochromatic state (H&E ×1000)

Plate 2: (a) Photomicrograph of a section of frontal cortex of Wistar rat exposed to 500ppm of lead acetate from GD6 to parturition showing areas of mild fibrosis, degenerating neurons (H&E ×400). (b) Photomicrograph of a section of the frontal cortex of Wistar rat administered 500ppm from GD6 to parturition (prenatal group) showing a Karyolytic neuron, karyorrhexic as well as areas of mild fibrosis (H&E ×1000)

Plate 3: (a) Photomicrograph of a section of Wistar rat’s frontal cortex administered 500 ppm of lead acetate from PND1 to PND21. It is characterized by neuropil vacuolation and degenerating neurons; karyolysis, pyknosis, and karyorrhexis (H&E ×600). (b) Photomicrograph of a section of the frontal cortex of Wistar rat administered 500 ppm of lead acetate from PND1 to PND21 showing Karyorrhexic neuron, neuropil vacuolation (KH) as well as Perivascular Vacuolation (H&E ×1000)

Plate 4: (a) Photomicrograph of a section of the frontal cortex of Wistar rat administered 500ppm from GD6 to weaning, showing numerous astrocytes (AST), discontinued capillaries and karyolytic neurons (H&E ×400). (b) Photomicrograph of a section of the frontal cortex of Wistar rat administered 500 ppm from GD6 to weaning, showing numerous swollen astrocytes or Alzheimer type II cells and vacuolarized pyramidal neurons. (H&E ×1000)
Table 1: One-way analysis of variance of the time taken for the rats to hold on to the hanger

| Group     | Time (s)  | F value | P value |
|-----------|-----------|---------|---------|
| Group I   | 18.5±1.291* |         |         |
| Group II  | 12±2.160*    |         |         |
| Group III | 13.75±2.06*   |         |         |
| Group IV  | 12.5±1.291* | 11.503  | 0.0000*** |

Values are mean±SD. Similar superscripts indicate significant differences across the rows at P<0.05

DISCUSSION

The frontal cortex is responsible for problem solving, judgement, and motor function [18]. The cerebral cortex of lead-induced Wistar rats shows few degenerating cortical and pyramidal neurons as shown in this study. This is in agreement with the findings of [18].

The observation of Alzheimer type II AST has been previously reported by Hazell et al. (2006) and Nuhu, (2017) [19,20] which explained that increased activity of reactive oxygen species is the cause of Alzheimer type II AST.

Gears cytoplasm, swelling of the axon, degeneration of the nerve cells, vacuolization and necrosis of the brain cells, and glial cell reaction were also reported from other heavy metal studies [21,22].

The degenerative changes seen occur because neuronal mitochondrial activity is affected by lead, with disruptive effects on synaptic transmission in the brain. Lead picked up by mitochondria produced swelling and distortion of mitochondrial crista, uncoupled energy metabolism, inhibit cellular respiration, and altered calcium kinetics flow [19,23].

Lead intoxication in rats during the early postnatal period, with doses that approximate those in children, induced transient as well as persistent dysfunctions in exploratory behavior and motor skills [23]. These observed actions of lead may be related to impaired maturation of sensitive brain regions which develop postnatally. The present study has indicated a significant difference in the hand grasping ability among the lead treated groups at different stages of development and the control, with the highest time (18.5±1.291) observed in the control and the least (12±2.160) in the group exposed during gestation only (Table 1).

In a related study conducted by Grant et al. (1990) [24], motor activity was not affected except pivoting activity at day 14 of age in the group administered 250 ppm of lead acetate only. This may be due to the dosage of the administrated lead acetate.

It was also found in the Cincinnati lead study that prenatal and postnatal blood Pb levels were associated with poorer scores on assessments of bilateral coordination, upper-limb speed, and dexterity, and a composite index of fine-motor coordination [25].

CONCLUSION

In this study, lead toxicity has shown histological effects and motor impairment. Histopathological observations in lead-induced Wistar rat frontal cortex showed degeneration of cortical and pyramidal neurons causing scattered and shrunken neuronal cells with deeply stained cytoplasm, pyknotic nuclei and karyolytic nuclei. There was massive increase in vacuoles of affected neurons. The motor activity as demonstrated by the palmer grasp test has been impaired.

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