Rauvolfia vomitoria remediates neurodegenerative deficiencies in hippocampus of wistar rats treated with lead nitrate

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Abstract

Background*: Rauvolfia vomitoria* is a useful medicinal plant primarily used for the treatment of hypertension and mental disorder. In Nigeria, it is used traditionally for the management of psychiatric issues. This study was designed to evaluate the histological assessment of ethanolic leaf extract of *Rauvolfia vomitoria* on the hippocampus of lead nitrate treated rats. Thirty (30) female adult Albino Wistar rats were used for the experiment. They were randomly divided into six groups of five animals each.

Methods: The animals were weighed before and after the experiment. Group 1 was given feed and water for 28 days. Group 2 was treated with 100mg/kg body weight of lead nitrate ($PbNO_3$) within 14 days period. Group 3 was treated with 212.5mg/kg body weight of *Rauvolfia vomitoria* leaf extract within 14 days period. Group 4 was treated with 100mg/kg of lead nitrate ($PbNO_3$) and 212.5mg/kg of *Rauvolfia vomitoria* leaf extract within 14 days period respectively. Group 5 was treated with 100mg/kg body weight of lead nitrate ($PbNO_3$) and 425mg/kg body weight of *Rauvolfia vomitoria* leaf extract within 14 days period respectively. Group 6 received 100mg/kg body weight of lead nitrate ($PbNO_3$) and 850mg/kg body weight of *Rauvolfia vomitoria* leaf extract within 14 days period respectively via oral route using a cannula. After the last day, the rats were euthanated by chloroform inhalation and the hippocampus harvested, and subsequently preserved in 10% buffered formalin, processed and stained with Hematoxylin and eosin dye. Light microscope was used and hispathological observations were made.

Results: Histologically, group 1 revealed normal molecular layer, pyramidal cell layer and polymorphic cells layer. Group 2 showed atrophied neurons in the pyramidal cell layer and vacuolated glial cell in polymorphic cell layers. Group 3 revealed hypertrophied pyramidal neurons in the pyramidal cell layer and vacuolated glial cells in polymorphic cell layer. Group 4 revealed atrophied neurons throughout pyramidal cell layer. Group 5 showed normal pyramidal neurons. Group 6 showed normal pyramidal neuron throughout the pyramidal cell layer.

Conclusion: This study shows that the plant extract (*Rauvolfia vomitoria*) contains neuroprotective potentials that can remediate the neuronal changes and effects associated with lead nitrate ($PbNO_3$).

Keywords: *Rauvolfia vomitoria*, lead nitrate ($PbNO_3$), hippocampus

1. Introduction

Rauvolfia vomitoria is a medicinal plant widely distributed in Asia and other West African countries. It grows to 15m high and has oval and shiny leaves in whorls and a conglomerate of inconspicuous white or greenish flower producing red berries. In the Yoruba speaking region of Nigeria, the plant is popularly known as 'Asofeyeje" meaning bearing fruits for the birds. It is called "Akanta" in Igbo, "Penpe" in Ashanti, "Wada" in Hausa, "Akata" in Bini and "Utoenyin" in Efik [1]. The plant is a natural medicinal herb which has been used for years for the management of hypertension and mental disorders [2]. Lead nitrate commonly occurs as a colourless crystal or white powder, and unlike most other lead (II) salt, is soluble in water and was first identified in 1597 by the Chemist Andreas Libavius, who called the substance plumbum dulce, meaning "sweet lead", because of its taste. It is being produced as a raw material for making pigment such as chrome yellow (lead (II) chromate, Pb(ro4) and chrome orange (basic lead (II) chromate, Pb_2(ro5) and Naples yellow [3]. Hippocampus from the Greek word "Seahorse" is one of the components of the brain of humans and other vertebrates. Human and other mammals have two hippocampi, one in each side of the brain [4]. It is part of the limbic system, and plays important roles in information consolidation from short-term memory to long-term memory and in spatial memory that enables navigation [5]. It is found in the allocortex, with neural projection into the neocortex in humans [6]. In humans, it contains two main interlocking parts. The hippocampus proper also called the Ammon's horn and the dentate gyrus [4]. They are located in the medial temporal lobes of the cerebrum. In the lateral view of the human brain, the frontal lobe is at the left, the occipital lobe at the right, and the temporal and parietal lobe have largely been removed to reveal one of the hippocampi underneath [7].

2. Methodology

2.1 Animal care and protocol

Thirty adult female Wistar rats within the weight range of 99 and 225g were procured for this research. They were procured from the animal house of the College of Health Sciences, University of Uyo. They were transferred to the animal house of the Department of Pharmacology, University of Uyo and acclimatized for two weeks. The animals were housed in wooded cages with adequate space to enable free locomotion and good ventilation. They were allowed twelve-hour light and

twelve hours dark cycle at the normal room temperature obtainable in the test environment and were fed with standard rat pelletized diet (vital feed Growers, Green cereals Nigeria Ltd). The rats were further grouped into six (6) groups consisting of five rats each. Group 1 was designated as control while group 2, 3, 4, 5 and 6 were the test groups. All animals were treated in accordance with "guide for the care and use of laboratory animals" developed by the National Academy of Science and made available online by the National Institute of Health [8].

2.2 *Rauvolfia vomitoria* leaf extract preparation

Rauvolfia vomitoria leaves were procured within Uyo metropolis. The leaves were washed with clean water, chopped and cut into small pieces, air dried then grind with the aid of a manual grinder. Maceration of the leaves in ethanol took 72 hours, afterwhich were sieved and taken to hot water bath to concentrate. 50 mg/ml was determined to be the stock concentration of the extract. The end product was kept in a refrigerator for use.

2.3 Constitution of lead nitrate ($PbNO_3$)

Lead nitrate was gotten from a chemical shop in Uyo metropolis, stored in a chemical bottle and well corked. It weighed 1g with the use of an electric weighing balance. The stock concentration was obtained by dissolving 1g of the chemical in 20ml of distilled water.

2.4 Experimental plan

The rats were grouped into six (6) groups of five (5) rats per group.

Group 1: The control group were given food and distilled water solely.

Group 2: Orally given 100 mg/kg body weight of lead nitrate ($PbNo_3$) within 14 days period.

Group 3: Orally given 212.5 mg/kg body weight of *Rauvolfia vomitoria* leaf extract within 14 days period.

Group 4: Orally given 100 mg/kg body weight of lead nitrate ($PbNo_3$) and 212.5 mg/kg body weight of *Rauvolfia vomitoria* leaf respectively within 14 days period.

Group 5: Orally given 100mg/kg body weight of lead nitrate ($PbNo_3$) and 425 mg/kg body weight of *Rauvolfia vomitoria* leaf extract respectively within 14 days period.

Group 6: Orally given 100 mg/kgbody weight of lead nitrate ($PbNo_3$) and 850 mg/kg body weight of *Rauvolfia vomitoria* leaf extract respectively within 14 days period.

2.5 Histological Tissue Processing

The organs were harvested and stored in 10% buffered formalin, afterwhich they were processed for light microscopy.

3. Results

Hippocampus from control revealed normal histological features with three basic layers; molecular cell layer, pyramidal cell layer and polymorphic cell layer (figure 1). Sections of animals orally given 100mg/kg body weight of lead nitrate revealed atrophied neurons throughout the pyramidal cell layer and vacuolated glial cells in polymorphic cell layer (figure 2). Sections of animals orally given 212.5mg/kg body weight of *Rauvolfia vomitoria* leaf extract revealed hypertrophied pyramidal neurons, vacuolated glial cells in the polymorphic cell layer (figure 3). Sections of rats given 100mg/kg body weight of lead nitrate and 212.5mg/kg body weight of *Rauvolfia vomitoria* respectively showed atrophied neurons throughout the pyramidal cell layer (figure 4). Sections of animals given 100mg/kg body weight of lead nitrate and 425mg/kg body weight of *Rauvolfia vomitoria* respectively showed near normal pyramidal neurons (figure 5). Hippocampus of animals given 100mg/kg body weight of lead nitrate and 850mg/kg body weight of *Rauvolfia vomitoria* leaf extract respectively showed normal pyramidal neurons throughout the pyramidal cell layer (figure 6).

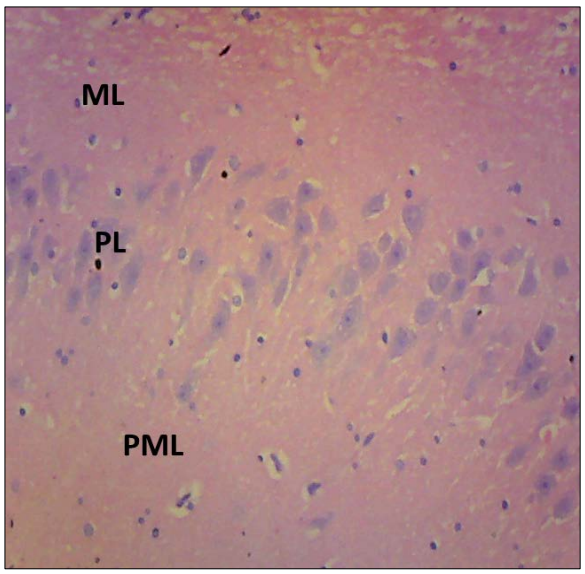

Fig 1: Section of hippocampus of Albino rat showing the three basic layers, molecular cell layer (ML), pyramidal cell layer (PL) and polymorphic cell layer (PML) (H&E X100**)**

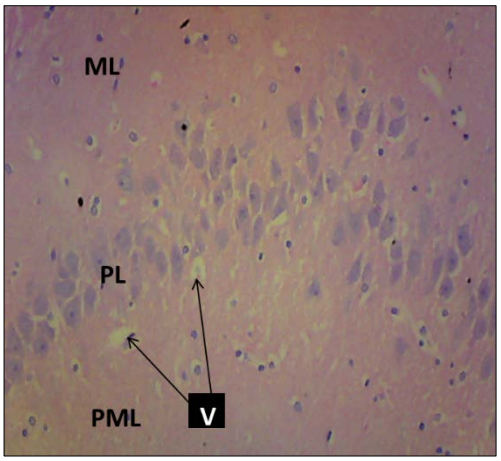

Fig 2: Section of hippocampus of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days showing atrophied (short arrows) neurons throughout the pyramidal cell layer (PL) and vacuolated (V) glial cells in polymorphic cell layer (PML) (H&E X100).

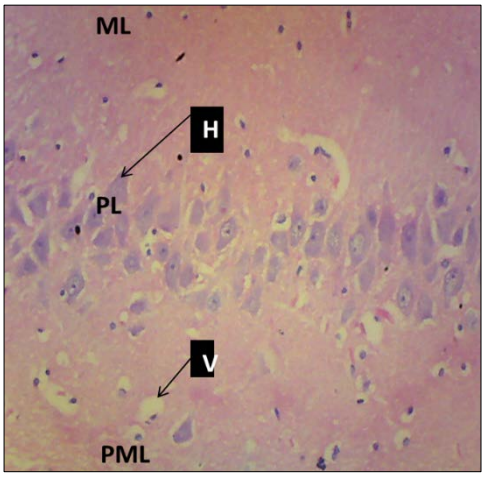

Fig 3: Section of hippocampus of Albino rat administered with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing hypertrophied (H) pyramidal neurons in the pyramidal cell layer (PL) vacuolated (V) glial cells in the polymorphic cell layer (PML) (H&E X100).

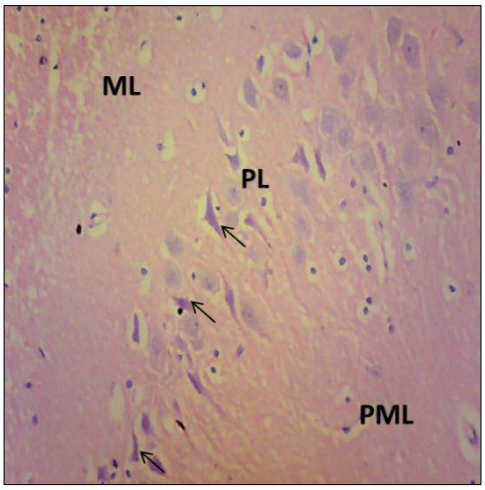

Fig 4: Section of hippocampus of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing atrophied (arrows) neurons throughout the pyramidal cell layer (PL) (H&E X100).

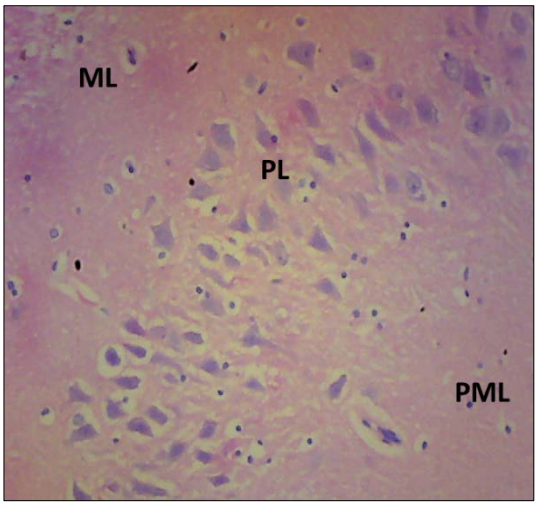

Fig 5: Section of hippocampus of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 425mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing near normal pyramidal neurons (H&E X100).

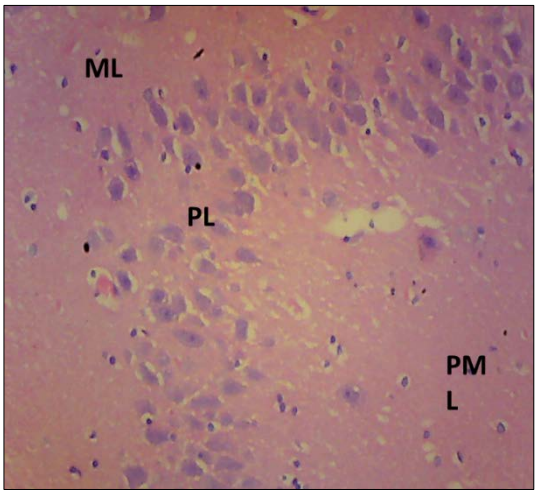

Fig 6: Section of hippocampus of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 850mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing normal pyramidal neurons throughout the pyramidal cell layer (P) (H&E X100)

4. Discussion

For centuries, lead nitrate ($PbNO_3$) poisoning had posed a serious environmental threat for human health in all societies [9]. Children become more vulnerable to lead nitrate ($PbNO_3$) exposure than adults for many potential reasons including their exposure to lead nitrate ($PbNO_3$) favoured by the habit of eating unhealthy food occupied with the evidence that a child's intestine absorbs lead nitrate much faster than that of an adult [10]. Among the negative effects of lead nitrate are the disruption of peripheral and central nervous systems, blood and skeletal systems [11]. Consequently, it constitutes a significant public health catastrophe despite efforts to minimize its level in the environment [12]. Neurological damage induced by lead toxicity is a well-known condition that has been documented to be a baseline for several mental disorder and retardation, behavioural problems, nerve damage, Alzheimer's disease, parkinson's disease and possibly schizophrenia [13]. Lead nitrate affects neuronal functioning on the rat brain.

phytochemical analysis of *Rauvolfia vomitoria* reveals that the plant has high reserpine and ajmaline concentrations. These alkaloids have various pharmacological properties including anti-malarial antitumor and anti-diabetic efficacy [14]. The plant is very useful in the treatment of mental illness, the root is added to gin and given to mentally ill persons [15]. Sections of animals given 100mg/kg of lead nitrate revealed atrophied neurons in the pyramidal cell layer and vacuolated glial cell in polymorphic cell layers. This finding is in accordance with a study which discovered that a chronic low level of lead nitrate exposure may inhibit neurogenesis and affect the differentiation/ maturation of the newly generated neurons in the hippocampus during the development [16]. Hippocampus of animals given 100mg/kg body weight of lead nitrate and 212.5mg/kg body weight of *Rauvolfia vomitoria* leaf extract revealed atrophied neurons throughout the pyramidal cell layer. This is in tandem with the report of Sanders *et al.* (2009) [17] which says that lead nitrate interferes with the neuronal functioning on rat brain. Animals given 100mg/kg body weight of lead nitrate and 425mg/kg body weight of *Rauvolfia vomitoria* leaf extractrespectively showed normal pyramidal neurons which reveals that *Rauvolfia vomitoria* leaf extract may have a neuroprotective potential and ameliorate some of the threats posed by lead nitrate [18-19]. Animals given 100mg/kg body weights lead nitrate and 850mg/kg body weight of *Rauvolfia vomitoria* leaf extract respectively revealed normal pyramidal neuron throughout the pyramidal cell layer. The improvement in the pyramidal cell layers may be due to the elevation in the concentration of *Rauvolfia vomitoria* and hence increase in its ameliorating effect. This finding is in support with a report documented by Oguche *et al* [20] 2016 which states that administration of *Rauvolfia vomitoria* leaf extract ameliorated the degenerative changes in the hippocampus caused by mercuric chloride toxicity. Cytoplasmic vacuolization develops spontaneously due to exposure to bacterial or viral pathogens as well as to various natural and artificial low-molecular weight compounds [21]. Cytoplasmic vacuolization of mammalian cells may also be transient or irreversible. Transient vacuolization is observed only during the exposure to an inducer and reversibly affect the cell cycle and migration [22]. Many of the most known inducers of transient vacuolization are weak basic amine containing lipophilic compounds. The accumulation of charged forms of weak bases increases the intra-organellar osmotic pressure. Thus, osmotic effects associated with disturbed ionic balance in the organelles rather than the impact on proteins controlling cellular functions enhance the action of transient vacuolization inducers [23]. In contrast, irreversible vacuolization marks cytopathological conditions resulting to cell death as long as the cytotoxic stimulus is present, irreversible vacuolization has been shown for a variety of natural and synthetic compounds of different chemical structure including medical drugs and industrial pollutants [22]. The vacuolation evident in figures 2 and 3 are the onset of cellular degeneration. This finding agrees with a similar report which opines that irreversible vacuolation accompanies cell death [21].

5. Conclusion

Results of this experiment therefore suggest that the ethanolic leaf extract of Rauvolfia vomitoria has the potentials of remediating the neuronal changes and effects associated with lead nitrate ($PbNO_3)_2$. This effect could be attributed to high antioxidant activity of *Rauvolfia vomitoria*.

Conflict of interest

The authors declare that no conflict of interest exist

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