Histomorphological assessment of ethanolic extract of *Rauwolfia vomitoria* on the cerebellum of lead nitrate treated albino wistar rats

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Abstract

Despite the development of more researched and formulated orthodox medicines, herbal medicines continue to be well patronized for persons across the world. *Rauwolfia vomitoria* is one of the most prominent herbs commonly associated with psychiatric management because of its antipsychotic and sedative properties. Lead (II) nitrate, an inorganic compound with the chemical formula $Pb(NO_3)_2$ is known to have neurotoxic effects capable of causing neurological changes. The present study sort to investigate the histomorphological impacts of *Rauwolfia vomitoria* leaf extract on the cerebellum of lead nitrate $Pb(NO_3)_2$ treated albino Wistar rats. Thirty (30) adult Wistar rats were divided into 6 groups (n=5). Group 1 was given feed and water *ad libitum* for 28 days. Group 2 received 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days, group 3 received 212.5mg/kg body weight of *Rauwolfia vomitoria* leaf extract for 14 days, group 4 received 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days and 212.5mg/kg body weight of *Rauwolfia vomitoria* leaf extract for 14 days, Group 5 was administered with 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days, then 425mg/kg body weight of *Rauwolfia vomitoria* leaf extract for 14 days and group 6 received 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days and 850mg/kg body weight of *Rauwolfia vomitoria* leaf extract for 14 days. The animals were sacrificed and their brain harvested and routinely processed using Haematoxylin and Eosin method and viewed under the light microscope. Distortion of Purkinje cell layer and hyperplasia of granules in granular cell layer was found in the group administered with 100mg/kg body weight of lead nitrate only (Group 2)), denoting brain injury. Similar results was found of group administered with *Rauwolfia vomitoria* only (Group 3), whereas severe distortion of Purkinje cell layer was seen of group administered with lead nitrate followed by high dose (850mg/kg) of the extract (Group 6). However, groups administered with lead nitrate and moderate dose of *Rauwolfia vomitoria* (Group 4 and Group 5) was found to have similar (intact) cyto-architecture with the control group suggesting normal neural function. In conclusion, distortion of the cytoarchitecture of the cerebellum could be associated with the toxic effect of the 100mg/kg Lead nitate and high dose (850mg/kg body weight) of *Rauwolfia vomitoria* constituents such as alkaloids, reserpine in the leaf extracts and this may result in functional changes that may be detrimental to the mental health of the animals.

Keywords: lead nitrate, cerebellum, *Rauwolfia vomitoria*, neurological changes and purkinje cell layer

1. Introduction

Humans have used natural products such as plants, animals, microorganisms and marine organisms in medicine to alleviate and treat diseases. The use of natural products as medicine must of course have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants which led to vomiting, diarrhea, coma or other toxic reactions- perhaps even death [1]. According to fossil records, the human use of plants as medicines may be traced back to atleast 60,000 years ago [2]. *Rauwolfia vomitoria* is a shrub found mainly in West Africa and Asia. The roots, leaves and stem are used in medicine. Some of its health functions include killing of cancer cells, decrease of blood pressure, anti-convulsion and it is known for enhancing brain functions [3]. Lead is a persistent environmental occupational toxic metal, and its poisoning remains a health threat [4]. Exposure to lead $Pb(NO_3)_2$ causes acute health effects. Headaches, reduced memory, disturbed sleep and mood as well as personality change are the most common effects on exposure to the chemical [5]. Inhaling lead nitrate can irritate the nose and throat and contacts can irritate the eyes and skin. Lead nitrate may cause damage to the blood cells causing anaemia as well as kidney and brain damage [6]. It is worthy of note that lead accumulates in the body due to consistent and repeated exposure and it takes a long period of time for the body to get rid of it [7]. The cerebellum is the part of the brain responsible for coordinating voluntary movements. It is also responsible for a number of functions including motor skills such as balance coordination, vision and posture [8]. The cerebellum accounts for approximately 10% of the brain volume and 50% of the total number of neurons in the brain. Historically, the cerebellum has been considered a motor structure because cerebella damage leads to impairment in motor control and posture. Motor commands are not initiated in the cerebellum; rather, the cerebellum modifies the motor commands of the descending pathways to make movements more adaptive and accurate [9]. As a result of the close relationship between the cerebellum and movement, the most common signs of a cerebellar disorder involve a disturbance in muscle control. Other symptoms and signs may include difficulty with walking and mobility, slurred speech, abnormal eye movements and headaches [10]. The main symptoms of

cerebellum dysfunction are ataxia which is a loss of muscle coordination and control. Ataxia can be caused by several factors including genes, stroke, tumors, head injury, multiple sclerosis, cerebral palsy, chicken pox and other viral infections. Sometimes ataxia is reversible when the underlying cause is treatable. A generic mutation causes genetic or hereditary ataxia. There are several different mutations and types with rare disorders of which the most common type, Friedreich's ataxia affects 1 in 50,000 people [11].

2. Materials and Methods

2.1 Experimental Animals

Thirty (30) adult Wistar rats weighing between 195 and 225g were used for this study. The Wistar rats were obtained from the Faculty of Basic Medical Sciences animal house, University of Uyo. They were transferred to Faculty of Pharmacy animal house, University of Uyo where they were acclimatized for two weeks before administration. They were housed in wooden cages with adequate space to enhance mobility and good ventilation. Saw dust was used as beddings in the cage and were replaced with clean ones every two days. The Wistar rats were allowed twelve hours light and twelve hours dark cycle at room temperature. The animals were fed with standard rat pelletized diet (vital feed growers, green cereals Nigeria LTD) and given water *ad libitum*. The rats were divided into six groups (n=5). Group 1 was the control group and group 2,3,4,5 and 6 were the experimental groups. All animals were treated in accordance with the "Guide for the care and use of laboratory animals", prepared by the national academy of science and published by the national institute of health [12].

2.2 Preparation of lead nitrate $Pb(NO_3)_2$

Lead nitrate was gotten from the laboratory of the chemistry department, University of Uyo, Uyo. The chemical was weighed using an electric weighing balance in the histology laboratory using a beaker. The lead nitrate weighing 1g was dissolved in 20ml of distilled water to get the stock concentration of 50mg/ml.

2.3 Preparation of the leaf extract of *Rauwolfia vomitoria*

Rauwolfia vomitoria leaves were obtained from the city of Uyo, Akwa Ibom state. They were identified and authenticated by a botanist in the University of Uyo herbarium with Voucher number UUPH 46(e) and were processed in the Faculty of Pharmacy animal house, University of Uyo. The leaves were picked, washed and sliced after which they were air-dried and grounded with a manual grinder. They were macerated and soaked in ethanol for 72 hours and were filtered and left in the water bath to concentrate. The stock concentration of the extract was determined 50mg/ml and refrigerated till used.

2.4 Experimental design and protocol

The animals were assembled into six (6) groups of five (5) rats per group. Group 1 (control group) were given food and distilled water only throughout the duration of the study. Group 2 were administered with 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days, group 3 were administered with 212.5mg/kg body weight of *Rauwolfia vomitoria* extract for 14 days, group 4 were administered with 100mg/kg body weight of lead nitrate $Pb(NO_3)_2$ for 14 days and 212.5mg/kg body weight of *Rauwolfia vomitoria* extract for 14days, group 5 were administered with 100mg/kg body weight of lead nitrate for 14 days and 425mg/kg body weight of *Rauwolfia vomitoria* extract for 14 days and group 6 were administered with 100mg/kg body weight of lead nitrate for 14 days and 850mg/kg body weight of *Rauwolfia vomitoria* for 14 days.

2.5 Animal sacrifice and organ collection

The animals were anaesthesized through chloroform fumes after which they were sacrificed. The brain of each rat was dissected and the cerebellum obtained and weighed. They were rinsed in normal saline to clear all blood stains before being fixed in 10% buffered formalin prior to tissue processing. The tissues were stained in H&E and viewed under a light microscope.

3. Results

The histological section of the cerebellar cortex of the Wistar rats in the control group (group 1) showed three layers; from the superficial to the deep surface were molecular cell layer, Purkinje cell layer and granular cell layer (Fig.1). The histomorphological section of the cerebellar cortex of Group 2 animals administered with 100mg/kg lead nitate $Pb(NO_3)_2$ showed distortion of Purkinje cell layer and hyperplasia of granules in the granular layer (Fig.2). The histomorphological cerebellar section of Group 3 animals administered with 212.5mg/kg body weight of *Rauwolfia vomitoria* showed slightly distorted Purkinje cell layer (Fig.3). The histomorphological cerebellar section of Group 4 Wistar rats administered with 100mg/kg body weight of Lead nitrate for 14 days and 212.5mg/kg body weight of *Rauwolfia vomitoria* for 14 days showed intact purkinje cell layer (Fig.4). The histomorphological cerebellar section of Group 5 Wistar rats administered with 100mg/kg body weight of Lead nitrate for 14 days and 425mg/kg body weight of *Rauwolfia vomitoria* for 14 days also showed intact Purkinje cell layer (Fig.5). The histomorphological cerebellar section of Group 6 Wistar rats administered with 100mg/kg body weight of Lead nitrate for 14 days and 850mg/kg body weight of *Rauwolfia vomitoria* for 14 days showed severe distortion of the Purkinje cell layer (Fig.6).

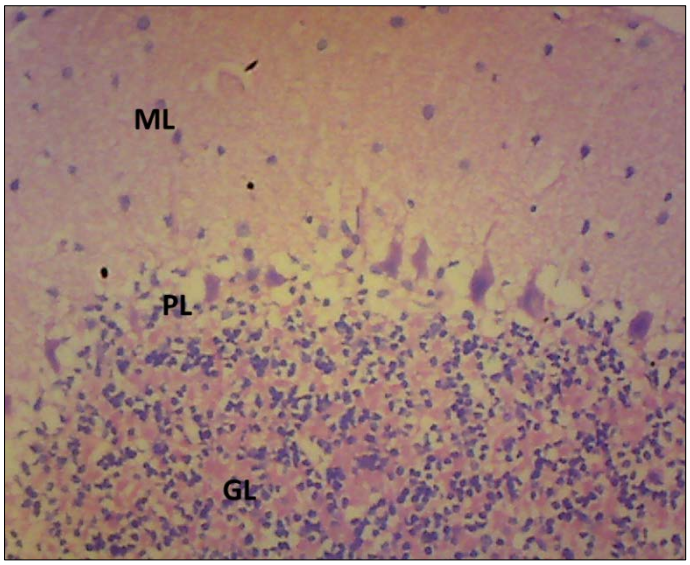

Fig 1: Section of Cerebellum of Albino rat showing the molecular cell layer (ML), Purkinje cell layer (PL) and granular cell layer(GL) (H&E X100)

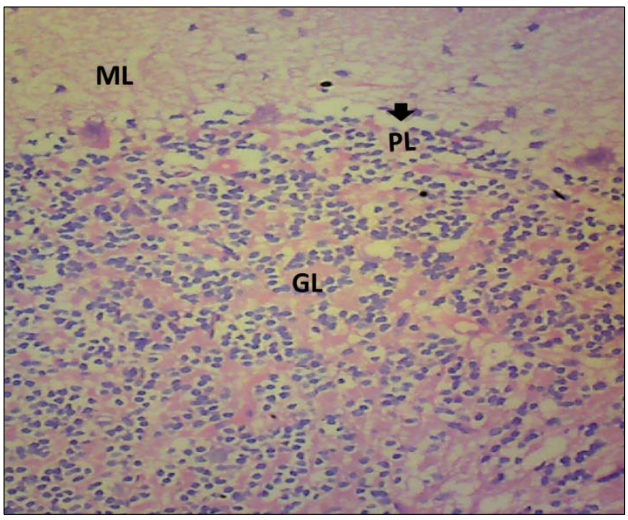

Fig 2: Section of cerebellum of Albino rat administered with 100mg/kg body weight of Lead nitrate for 14 days showing distorted Purkinje cell layer (PL) and hyperplasia of granules in the granular cell layer (GL) (H&E X100)

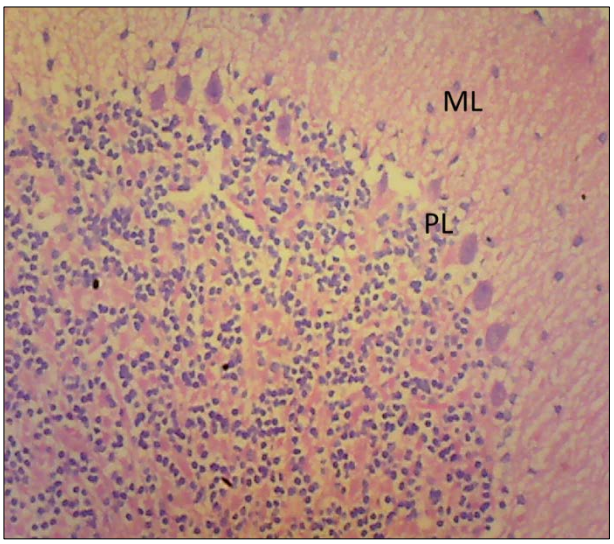

Fig 4: Section of cerebellum of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing intact Purkinje cell layer (PL) (H&E X100)

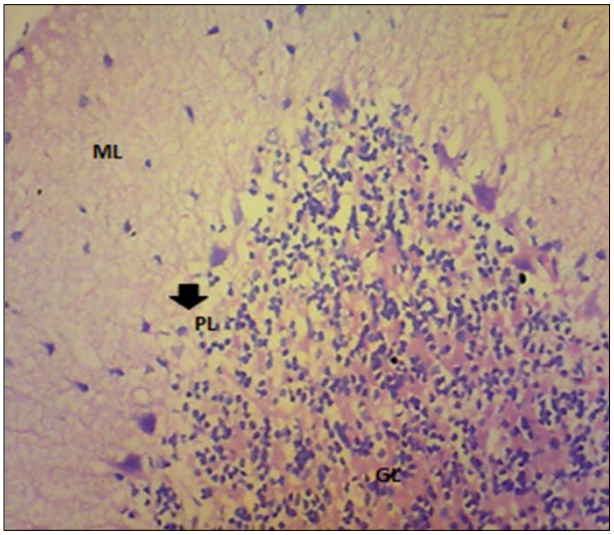

Fig 3: Section of cerebellum of Albino rat administered with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing slightly distorted Purkinje cell layer (PL) (H&E X100)

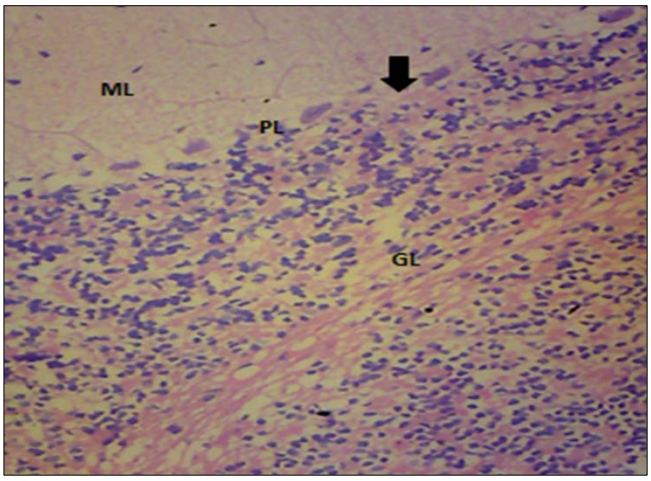

Fig 5: Section of cerebellum of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 425mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing intact Purkinje cell layer (PL) (H&E X100)

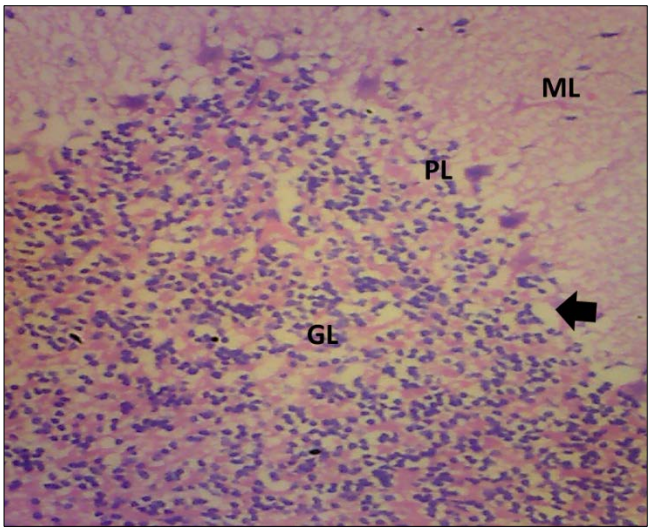

Fig 6: Section of cerebellum of Albino rat administered with 100 mg/kg body weight of Lead nitrate for 14 days and 850mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing severe distortion (arrow) of the Purkinje cell layer (PL) (H&E X100)

4. Discussion

This study investigated the histomorphological impacts of ethanolic leaf extract of *Rauwolfia vomitoria* on the cerebellum of lead nitrate $Pb(NO_3)_2$ treated Wistar rats. Histomorphological study of the cerebellar sections of group 2 animals which showed distortion of the Purkinje cell layer and hyperplasia of granules in the granular cell layer suggests a brain (cerebellar) injury. A study by Balzano *et al.*, (2018) [13] showed that distortion of the Purkinje cell layer disrupts the synaptic connections between the Purkinje cells and the intracerebellar nuclei. Similar histomorphological results were seen from group 3 animals, having slightly distorted Purkinje cell layer. This result seems to agree with the results of Okon *et al.*, (2018) [14] which found irregularly shaped Purkinje cell bodies, reduction in the number and size of Purkinje cells and slight reduction in cell population in the granular cell layer as a result of similar dose of *Rauwolfia vomitoria* extract. The result of sections administered with lead nitrate agrees with the study of Sharma *et al.*, (2019) [15] which concluded that though the exact mechanism of lead toxicity is not clear, the study showed that lead nitrate toxicity can be detrimental to mental health as one of its effects is atrophy of neural tissue. Photomicrograph of Group of animals administered with moderate dose (212.5mg/kg and 425mg/kg) of *Rauwolfia vomitoria* after administration of 100mg/kg body weight of lead nitrate shows similar cytoarchitecture with the control group. This suggests that moderate dose of *Rauwolfia vomitoria* has an ameliorative potential on neurotoxic effect of lead nitrate. The present result aligns with an experimental study by Okon *et al.*, (2020) [16] where *Rauwolfia vomitoria* was found to have the potentials of ameliorating the neurodegenerative effects caused by Sida Acuta on the pyramidal cells. Furthermore, *Rauwolfia vomitoria* at a high dose (850mg/kg) had neither ameliorative nor neuroprotective potential owing to the photomicrograph results of group 6 in this study. Sections of the group 6 animals show neurotoxic effect of severe distortion of Purkinje cell layer suggesting severe brain injury. The cerebellum maintains balance of posture and co-ordinates the timing and force of muscle groups to produce limb or body movements. Reports have also shown that the cerebellum is important for learning, and is also involved in certain cognitive functions [17, 18].

5. Conclusion

From the present research, it could be concluded that distortion of the cytoarchitecture of the cerebellum could be associated with the toxic effect of the 100mg/kg Lead nitate and high dose (850mg/kg body weight) of *Rauwolfia vomitoria* constituents which are alkaloids, reserpine etc in the leaf extracts and this may result in functional changes that may be detrimental to the mental health of the Wistar rats.

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