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Comparative Study of Neuronal Degenerative Potentials of Ethanolic Root Bark and Leaf Extracts of Rauwolfia Vomitoria on the Cerebellum of Adult Wistar Rats

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Abstract
Rauwolfia vomitoria has been used for centuries in India and Africa for treatment of a variety of disorders including snake bites and sting, insomnia and insanity. Neuronal degenerative potentials of ethanolic root bark and leaf extracts of Rauwolfia vomitoria on the cerebellum of adult wistar rats was investigated. Thirty wistar rats weighing between 170-240g were divided into six groups, each consisting of five rats. Groups A served as the normal control that received distilled water while group B served as the olive oil control that received 0.5mls of olive oil. Experimental groups C and D received 200mg/kg, 300mg/kg of ethanolic root bark extracts while groups E and F received 200mg/kg, 300mg/kg of the leaf extract orally respectively for seven days. In this study, the treatment groups showed a dose-dependent degree of silver impregnation of the cell bodies and axons. The sections of the cerebellar cortex of the treated group C, D, E and F showed various degrees of neurodegenerative changes highlighted by the silver stain impregnation which was more intense in groups C and D that received 200mg/kg and 300mg/kg ethanolic root-bark than in the groups E and F that received 200mg/kg and 300mg/kg of ethanolic leaf extract. These changes may result in cerebellar dysfunction
Keywords: Ethanolic extracts, Root-bark extract, Leaf extract, Rauwolfia vomitoria

1. Introduction

The increasing widespread use of herbal medicine has prompted the WHO to promote the integration of traditional medicine and complementary and alternative medicines into the national health care systems of some countries (WHO, 2005). Herbal medicines, also called botanical medicines or phytomedicines, refer to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (WHO, 2008). In Nigeria, many herbal products are used for treating illnesses. For instance, *Ocimum gratissimum* is used for treating diarrheal diseases (Ilori et al., 1996). The seeds of Citrus parasidi are effective in treating urinary tract infections that are resistant to the conventional antibiotics (Oyelami et al., 2005); pure honey healed infected wounds faster than eusol (Okeniyi et al., 2005); dried seeds of *Carica papaya* L. is effective in the treatment of intestinal parasitosis (Okeniyi et al., 2007); the analgesic and inflammatory effects of Garcinia kola is known to enhance its use for osteoarthritis treatment (Adegbehingbe et al., 2008); *Rauwolfia vomitoria* is a common herb used traditionally for psychiatric management in Nigeria (Akpanabiatu et al., 2006). Its extracts have anti-inflammatory effect (Kweifio-Okai et al., 1995), antipyretic effect (Amole and Onabanjo, 1999), anti-diabetic effect (Campbell et al., 2006) and anti-cancer effect (due to the β-carboline alkaloid, alstonine)(Bemis et al., 2006). Sharma (2004) reports that the roots of *Rauwolfia* is good for the treatment of snake bites; insect stings; nervous disorders; mania; epilepsy; intractable skin disorders such as psoriasis, excessive sweating, itching; hypertension; sedative; uterine contration in child birth and gynecological ointment for the treatment of menopausal disorders. *Rauwolfia vomitoria* have been found to have adverse effects on the central nervous system and also affects the cerebellar cyto-architecture (Eluwa et al., 2009). There is, however, limited literature on the effects of ethanolic root-bark and leaf extracts of *Rauwolfia vomitoria* on axons and neurofibrils in the cerebellum of adult wistar rats, hence this research to investigate the effects of ethanolic root-bark and leaf extracts of *Rauwolfia vomitoria* on the cerebellar axons and neurofibrils of adult Wistar rats.

2. Materials and methods

Thirty (30) female Wistar rats weighing 170g – 240g were obtained from the Department of Physiology, University of Calabar. They were kept in the animal house of Anatomy Department to acclimatize for two weeks under standard conditions of temperature 27^{o}C – 30^{o}C, photoperiod 12hour dark and 12-hour natural light cycle. The animals were fed with rat chow from Agro Feed Mill Nigeria Ltd and had access to drinking water *ad libitum*. After the acclimatization period, they were randomly divided into 6 groups (n=5).

2.1 *Extracts preparation*

The root-bark and leaves of *Rauwolfia vomitoria* were collected from the University of Calabar farm, Calabar, and was identified and authenticated by a botanist in the Department of Botany, University of Calabar. The roots and the leaves were washed in water and the root-bark was defoliated, dried under a shed. The dried root-bark and leaves were blended into powdered form using a blender and kept in glass containers with plastic cover. The extraction method involved cold ethanolic extraction, where a known weight of the blended sample was soaked in ethanol for 24 hours and then the extract was filtered and evaporated to dryness at room temperature to obtain the crude extract.

2.2 *Experimental protocol*

The rats were divided into 6 groups labelled A, B, C, D, E, F with groups A and B were the normal control and olive oil control, groups C, D, E, and F as the experimental, each consisting of 5 rats. Group A animals, the normal control received 0.5ml/kg of normal saline while group B animals, the olive oil control received 0.5ml/kg for 7 days respectively. Olive oil was used as a vehicle in dissolving the extracts. Groups C and D received 200mg/kg and 300mg/kg of ethanolic root bark extract of *Rauwolfia vomitoria*, Groups E and F received 200mg/kg and 300mg/kg of ethanolic leaf extract of *Rauwolfia vomitoria* orally with the aid of orogastric tube.

2.3 Termination of the experiment

The animals were then sacrificed using chloroform inhalation method. The brain was extracted by opening up the skull; the cerebellum was excised and preserved using 10% formol saline. Routine histological process was carried out. The cerebellar sections were stained using Bielchowsky's (1904; 1909) methods for axons and neurofibrils,

3. Results

The cerebellar cortex of the normal and olive oil control rats shows normal cyto-architecture defined by a golden brown background as no neuron picked up the silver stain (Plate A & Plate 1B). The histological section of the cerebellar cortex of the experimental groups showed degenerated axons and neurofibrils in the Molecular layer (ML), Purkinje cell layer (PCL) and Granular layer (GL). The degeneration was more intense in groups C and D which received 200mg/kg and 300mg/kg of ethanolic root bark extract of Rauwolfia Vomitoria (Plate 1c, Plate 2c) when compared to the control and groups E and F which received 200mg/kg and 300mg/kg of ethanolic leaf extract (Plate 1d, Plate 2d).

4. Discussion

Silver impregnation histological techniques yield excellent visualization of degenerating neurons and their processes in animal models of neurological diseases. These methods also provide a particularly valuable complement to current immunocytochemical techniques for recognition of axon injury in the setting of brain or spinal cord trauma, ischemia, or neurodegenerative diseases (Tatyana and Mark, 2002). In this study, the treated groups showed a dose-dependent degree of silver impregnation of the cell bodies and axons. The sections of the cerebellar cortex of the treated group C, D, E and F showed various degrees of neurodegenerative changes highlighted by varying degrees of the silver stain impregnation which was more in groups that received ethanolic root-bark than in the groups that received leaf extract. The groups C and D which were given 200mg/kg and 300mg/kg of RV root-bark extract respectively, showed degenerated axons (A), and neuronal cell bodies (P) in the three layers in a dose-dependent manner. The histological sections of the groups E and F rats which received 200mg/kg and 300mg/kg of the leaf extract showed lightly impregnated neurons. The neuron cell bodies (especially Purkinje cell bodies) picked up the silver stain because of the degenerated neurofibrils in their perikaryon.

A higher degree of neurodegeneration was found in groups C and D which received the root-bark extract. This may have been due to the higher content of indole alkaloids (especially reserpine) in the root-bark than the leaf of Rauwolfia vomitoria. Ritter and Dinh (1988) reported that sections of the brain and spinal cord stained with the Carlsen-de Olmos cupric silver method revealed degeneration in cell bodies, axons and terminals. According to Switzer (2000) after a neuron is dead it disintegrates and it is the debris that pick up the silver stain. Silver stains are particularly well suited to localizing sites of injury, and determining the extent and time-course of degeneration (Jeanene and Karl, 1999). According to Cavanagh (1984) chemically induced neurodegeneration is usually characterised by different patterns of neuronal cell death, gliosis, swollen or destroyed axons, or destruction of the myelin sheath. NMDA mediated glutamatergic neurotransmission is related to various pathologies, including neurodegenerative diseases, ischemia, epilepsy and schizophrenia (Meldrum, 1994; Ozawa et al., 1998). Findings from the work of Megahed, et al (2006) showed that the administration of phenytoin alone affected the pyriform neurons; however, combination of therapeutic doses of acetaminophen and phenytoin caused extension of the neuronal degeneration to more than one layer of the cerebellar cortex.

5. Conclusion

Result observed in this study indicates that the ethanolic root bark of Rawolfia vomitoria was more neurotoxic than the leaf extract and may cause meuronal degeneration in the cerebellum of the Wistar rats

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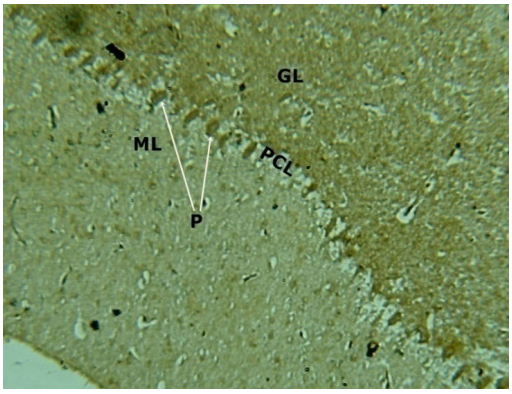

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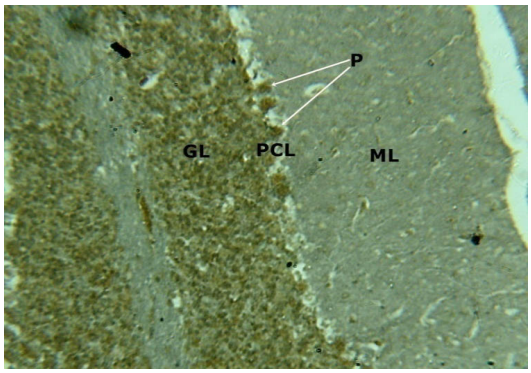

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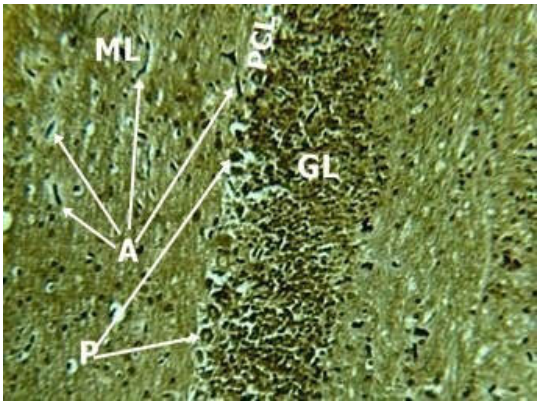

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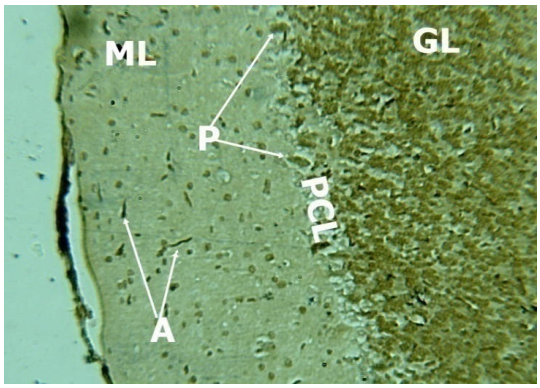


D

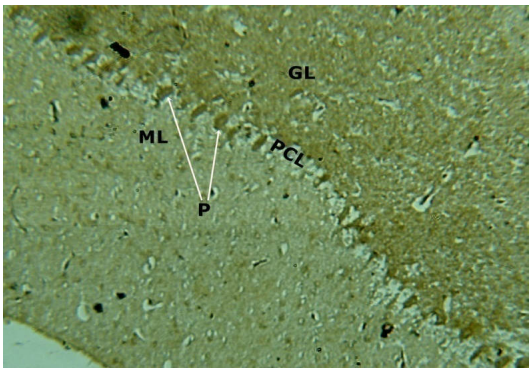
Plate 1: Photomicrographs of cerebellum of normal control, olive oil control and treated groups that received 200mg/kg root bark and leaf extracts of RV (Bielschowsky's method of silver impregnation; Mag. X 100 for all plates).

A: Cerebellar cortex of normal control rats showing a normal cerebellar architecture and normal neurons, molecular layer (ML), Purkinje cell layer (PCL), granular layer (GL), Purkinje cells (PC).

B: Cerebellar cortex of olive control showing a molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P)

C: Cerebellar cortex - 200mg/kg root-bark extract showing degenerated axon (A) in the molecular layer (ML); degenerated neurofibrils in Purkinje cells layer (P) and in granular layer.

D: Cerebellar cortex - 200mg/kg leaf extract showing few Degenerated axons (A) in the molecular (ML), Purkinje cells layer (P) and granular layer.



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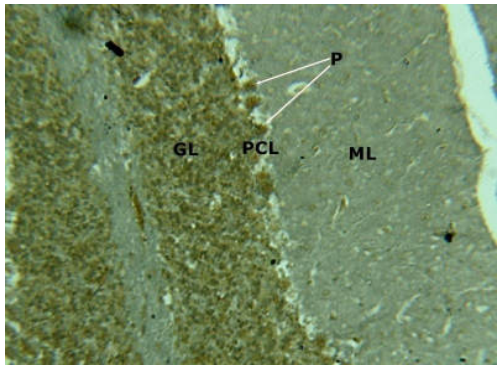

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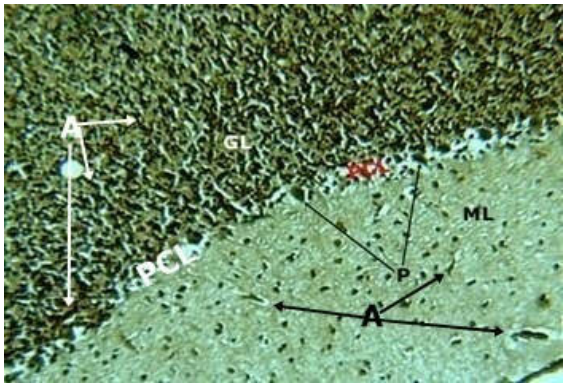

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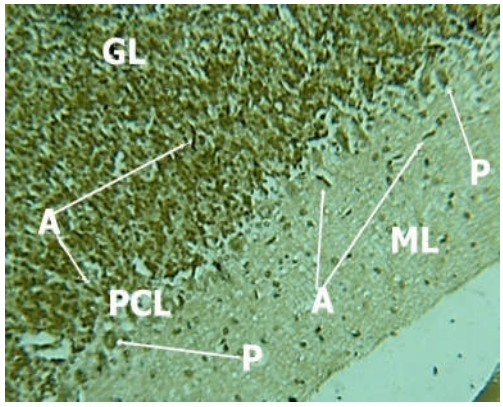

D

Plate 2: Photomicrographs of cerebellum of normal control, olive oil control and treated groups that received 200mg/kg root bark and leaf extracts of RV (Bielschowsky's method of silver impregnation; Mag. X 100 for all plates).

A: Cerebellar cortex of normal control rats showing a normal cerebellar cytoarchitecture and normal neurons, molecular layer (ML), Purkinje cell layer (PCL), granular layer (GL), Purkinje cells (PC).

B: Cerebellar cortex of olive control showing a molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P).

C: Cerebellar cortex - 300mg/kg of root bark showing degenerating axons and neurofibrils in the Molecular layer (ML) Purkinje cell layer (PCL) and Granular Layer (GL).

D: Cerebellar cortex - 300mg/kg leaf extract showing few Degenerated axons (A) in the molecular (ML), Purkinje cells layer (P) and granular layer.