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Neurohistological Study of Ethanolic Root Bark and Leaf Extracts Of Rauwolfia Vomitoria on Reactive Astrocytes in the Cerebral Cortex of Adult Wistar Rats

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Abstract: Astrocytes are neuroprotective glial cells that protect and nourish neurons of the central nervous systems. *Rauwolfia vomitoria* is a common medicinal plant used for the treatment of various diseases such as insanity, insomnia, hypertension, malaria etc. The comparative examination of reactive astrocytes in the cerebral cortex of adult albino wistar rats following the administration of crude ethanolic root bark and leaf extract of *Rauwolfia vomitoria* was studied using 25 mature Wistar rats of both sexes. The animals were divided into 5 groups, labeled A, B, C, D, and E. Group A was the control, while groups B, C, D, and E were the experimental. Oral doses of 200 mg/kg and 300 mg/kg body weight of the root bark extract were administered to groups B and C animals, while groups D and E animals received 200 mg/kg and 300 mg/kg body weight of the leaf extract respectively for seven days. On the 8th day, the rats were sacrificed; their brains were surgically extracted, and routinely processed for neurohistological study of Astrocytes using Hortegas lithium carbonate method for reactive astrocytes. Results showed hyperplasia of reactive astrocytes in the root bark groups, while there was hypertrophy of reactive astrocytes in the leaf extract groups when compared to the controls. Thus, the plant may have some adverse effects on neurons within the cerebral cortex.

Keywords: Astrocytes; Hyperplasia; Hypertrophy; Neurons; *Rauwolfia vomitoria*; Wistar rats.

1. Introduction

Medical plants are known to play major roles in health care delivery notwithstanding the advances in modern medicine [1]. These plants are distributed worldwide and are more abundant in tropical regions [2, 3]. Interests in the investigation of higher plants as sources for new lead structures and for development of phototherapeutic agents with proven efficacy, safety and quality have been demonstrated by pharmaceutical companies [4, 5].

Herbal medicine has become a way of life in Nigeria and other West African Countries, due to their been readily available and cheap. It could also be partly due to the identification of some indigenous medicinal plant that is more effective but less toxic than most pharmaceutical agents [6].

Rauwolfia vomitoria is one of the commonly used medicinal plants in Nigeria; it is used for the treatments of various ailments such as snakebites, insect bites, hypertension, mental illness, diabetes and insomnia [7]. It is commonly called serpent wood or Swizzle stick in English, Asofeyeje in Yoruba, Wadda in Hausa, Ira in Igbo, Eto mmongeba in Efik, Eto utoenyin in Ibibio and Mmongeba-ebot in Annang languages respectively [8]. The roots and

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leaves are the parts of the plant that are mostly used by local traditional medicine practitioners. Investigations into its phytochemical analysis have revealed more than 50 active indole alkaloids, each possessing remarkable pharmacological activities, with Reserpine being the most active alkaloids found predominantly in the roots than in the leaves [9, 10]. Reserpine is however known to have peripheral action in many parts of the body, especially depleting stores of cathecholomines to varying extents in different regions of the brain [11, 12].

In Nigeria, traditional medicine practitioners prescribe and administer herbal extracts to patients without proper dose regiment, adequate knowledge about their indications and safety has led to this present study. Therefore, this work focused on investigating the likely neurohistological effects of the crude ethanolic root bark and leaf extracts of *Rauwolfia vomitoria* (*Apocynaceae*) on reactive astrocytes in the cerebral cortex of adult albino Wistar rats.

2. Materials and Methods

Twenty-five adult Wistar rats of both sexes were bred in the animal house of the Department of Human Anatomy, University of Calabar Nigeria. They were fed with normal rat chow, and water was provided *ad libitum* throughout the duration of the experiment. The rats were kept under standard room temperature of 25–27°C. The animals were divided into five groups designated as A, B, C, D, and E, each consisting of five rats. The group A animals were the control, and groups B, C, D, and E were the experimental animals. The use of experimental animals was in accordance with the internationally accepted principles for laboratory animal use and cares as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.1. Preparation of the Herbal Extract

The roots and leaves of *Rauwolfia vomitoria* plant were collected from University of Calabar farm and were identified and authenticated by the Botanist at the botanical garden of the University of Calabar, Nigeria. The roots and the leaves were washed with water to remove the impurities. The roots bark were defoliated, dried in Carbolite moisture extraction drying oven (Grant Instruments, Cambridge, England) at 40°C–50°C as well as the leaves for 3 hours. The dried root barks and leaves were blended into powdered form 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

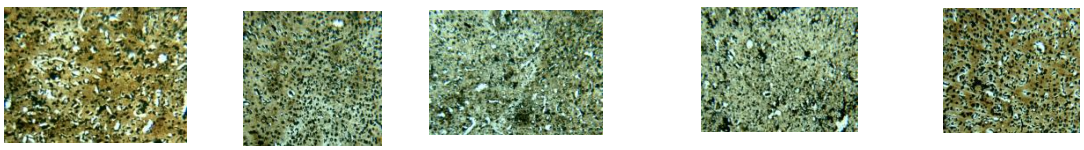
The twenty-five adult wistar rats received oral doses of 200 mg/kg and 300 mg/kg per body weight of ethanolic root bark and 200 mg/kg and 300 mg/kg per body weight of leaf extracts of *Rauwolfia vomitoria* were administered to rats in groups B, C, D, and E, respectively for seven days with the aid of an orogastric tube.

2.3. Termination of Experiment

On the eight day, the rats were sacrificed by chloroform inhalation method. The brains were extracted and preserved using 10% formaldehyde. Following complete fixation of the whole brain, the cerebral cortex was excised. Routine histological tissue processing was carried out, and the brain sections were subjected to special staining method using Hortegas lithium carbonate method for reactive astrocytes [13].

3. Results

Neurohistological study of astrocytes in cerebral cortex using Hortegas lithium carbonate method showed normal non- reactive protoplasmic astrocytes stained black in the control groups A (Plate 1A). Sections of cerebral cortex of group B animals that received 200mg/kg of the rootbark extract of Rauwolfia vomitoria showed proleferation of reactive astrocytes than in group D animals that received 200mg/kg leaf extract when compared to the controls (Plate 1B & D). Cerebral cortex section of group C that received 300mg/kg rootbark extract of Rauwolfia vomitoria showed hyperplasia of reactive astrocytes while in group E animals that received 300mg/kg leaf extract of Rauwolfia vomitoria showed hypertrophy of reactive astrocytes (Plate 1 C & E).



A **B** **C** **D** **E**

Plate 1: Photomicrographs of the cerebral cortex of controls and treated groups who received 200mg/kg and 300mg/kg root bark and leaf extracts of Rauwolfia vomitoria (Hortegas lithium carbonate method. Mag. x100 for all plates).

Group A: Cerebral cortex controls showing normal astrocytes stained black. Group B: 200mg/kg of root bark extract showing hyperplasia of reactive astrocytes. Group D: 200mg/kg of leaf extract showing hypoplasia of reactive astrocytes. Group C: 300mg/kg of root bark extract showing hyperplasia of reactive astrocytes. Group E: 300mg/kg of leaf extract showing hypertrophy of reactive astrocytes.

4. Discussion

Astrocytes are major class of glial cells which regulate neurotransmitter systems, synaptic processing, ion homeostasis, antioxidant defenses and energy metabolism [14]. They are Stellate cells of two types: protoplasmic, which occur in the gray matter, and fibrous, which occur in the white matter. Furthermore, they provide support for nerve fiber tracts and also participate in the exchange of fluids, gases, and metabolites among nervous tissue, blood, and cerebrospinal fluid [15]. Following injury or trauma to the CNS, astrocytes function in scar formation by proliferating cell processes, forming an area of gliosis around the neurons and are termed reactive astrocytes. Special stains containing heavy metals are used to detect astrocytes such as the Hortegas lithium carbonate method for demonstrating reactive astrocytes.

This study revealed the presence of reactive astrocytes in the experimental groups than in the controls resulting in hyperplasia and hypertrophy of reactive astrocytes in the cerebral cortex sections that received root bark and leaf extracts. The appearance of reactive astrocytes in the cerebral cortex sections may be due to injury to the neurons since astrocytes help in the healing and recovery process of injured neurons of various nervous system pathology [16]. Astrocytes regulate ionic concentration, tight junctions and also serve as an intermediary station for converging nutrients to injured neurons [17].

The appearance of reactive astrocytes may be caused by the activities of the ethanolic extracts of *Rauwolfia vomitoria*, especially the root bark extract, indicating early signs of neuronal injury and cell loss, whereby the functional capability of neurons and even the astrocytes itself may be compromised. The appearance of reactive astrocytes was also reported by Eluwa, *et al.* [18] on fetal cerebral cortex of wistar rats after the administration of oral doses of ethanolic root bark and leaf extracts of *Rauwolfia vomitoria*. This is also in line with the study by Ekanem, *et al.* [19] who reported that combination therapy of antimalarial drugs, mefloquine and artequin, induces reactive astrocytes formation in the hippocampus of Wistar rats.

We therefore conclude that care must be taken in the use and administration of these extracts of the plant especially by local traditional medicine men as the plant may possibly have adverse effects on neurons within the cerebral cortex that can impair the function of the brain as a whole.

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