Rauwolfia vomitoria Root Bark Extract Affects the Cervical Ventral Horn Cells of the Spinal Cord

Moses Bassey Ekong*, Ngozi Jane Muonagolu, Utibeabasi Bassey Akpan
Department of Anatomy, University of Uyo, Uyo, Nigeria

ABSTRACT

Background: Rauwolfia vomitoria is a tropical shrub of Apocynaceae family, and a widely used nutraceutical containing bioactive alkaloids such as reserpine and yohimbine with reported antipsychotic, sedative and Parkinson-like effects. The pathway by which these become evident is through the spinal cord, a major reflex centre and conduction pathway of the brain and the rest of the body. This study therefore investigated the role of R. vomitoria on the microstructure of the cervical ventral horn cells of Wistar rats.

Methods: Twenty-four adult male Wistar rats with average weight 221 g were divided into four groups (n=6); control, 200 mg/kg, 300 mg/kg and 400 mg/kg body weight of R. vomitoria root bark extract administered orally for seven days. On the eighth day, the animals were anesthetized with ketamine hydrochloride (10 mg/kg; i.p); and perfusion-fixed sacrificed. The cervical vertebrae were exposed and cracked-open and the spinal segment C3-C5 were excised and post-fixed in 10 % buffered formalin for 48 hours. The tissues were then routinely processed for histological study using haematoxylin and eosin staining method.

Results: The results showed general weakness, dull and drowsy behaviour, with hypertrophy of the ventral horn neurons in the 200 mg/kg, 300 mg/kg and 400 mg/kg R. vomitoria groups. There was hyperplasia 300 mg/kg and karyorrhectic appearance of some of the ventral horn neurons with no difference (p > 0.05) in the spinal cervical ventral horn cell population between the test groups and the control.

Conclusion: R. vomitoria caused some behavioural changes and alterations in the cellular integrity of the C3-C5 spinal ventral horn neurons.

Keywords: Rauwolfia Vomitoria; Spinal Cord; Neurons; Glial Cells; Wistar Rats

Introduction

Over the years, nutraceutical extracts have been reported to contain therapeutic agents, and have been extensively explored for their potentials in the treatment of a wide range of health conditions.[1] One of such herbs widely used for its antipsychotic effects is Rauwolfia vomitoria. [2] This herb also known either as African serpent wood, African-snake root or swizzle stick is also known in local Nigerian languages; asofeyeje in Yoruba, ira in Igbo, wadda in Hausa, and mmoneba or utenyi in Efik and Ibibio languages, respectively.[3] R. vomitoria extracts are rich in β-carboline and indole alkaloids, many of which are contained in its stem, leaf and root. R. vomitoria is also rich in saponins and flavonoids.[4,5] Of the several alkaloid constituents, reserpine and yohimbine are the best known in terms of mechanism of action.[6-8] The R. vomitoria is an antihypertensive and sedative agent,[3,9,10] and is used for the treatment of hypertension, schizophrenia, paranoia and cancer.[2,11,12] It acts as an antidepressant in low doses, while high doses cause monoamine depletions and depression.[13] Other uses of this plant include treatment of a variety of ailments such as fever, general weakness, gastrointestinal diseases, liver diseases, psychosis, pain and cancers.[14,15] However, despite these various health benefits, some side effects such as drowsiness, nasal congestion, paradoxical anxiety, depression and Parkinson-like symptoms have been reported.[6,12,16] Histological alteration in different brain areas have been reported,[3,17-22] Also, overdose may cause respiratory depression, slowed heartbeat, hypotension, confusion, tremors, convulsions and gastro-intestinal distress.[16] One pathway by which these tremors and convulsion become evident is through the spinal cord.[23,24]

The spinal cord is a major reflex centre and conduction pathway of the body and the brain, and also forms a direct pathway by which motor function is influenced.[25,26] Sedation is reported as one of the actions of R. vomitoria on the nervous system, and how this may influence the spinal cord is not known. The reported plethora of side-effects and the likely effect on the motor neurons of spinal cord necessitated this study of R. vomitoria root bark extract on the ventral horn cells of the cervical spinal segment of Wistar rats.

Materials and Methods:

Twenty-four (24) adult albino Wistar rats of average weight 221 g were used for this study. Ethical approval was obtained from the Faculty of Basic Medical Sciences
Ethical Committee, and the experiment was carried out at the Animal House Facility of the Faculty of Basic Medical Sciences of University of Uyo, Nigeria. The animals were allowed free access to rat chow (Vital Feed Company Limited, Nigeria) and clean water ad libitum.

**Preparation of *R. vomitoria* Roots:** Roots of *R. vomitoria* were identified and harvested in a local farm at Ekpene Obo in Esit-Eket Local Government Area of Akwa-Ibom State, Nigeria. The roots were washed and the bark was separated from the cambium, air-dried for one week and then pulverized into powder using kitchen blender. The *R. vomitoria* root bark powder was extracted using 80% ethanol. Dry extracts were obtained using a rotary evaporator and Plus 11 Gallenkamp oven at 45-50 ºC and stored in a refrigerator at 4 ºC until use.

**Experimental protocol:** Two grams of the *R. vomitoria* root bark extract was dissolved in 30 ml of distilled water and the actual dosages were calculated based on the body weights of each rat. The animals were divided into four groups (n = 6) of control, 200 mg, 300 mg and 400 mg of *R. vomitoria* per kilogram body weight (kg b.w.), which also served as the dosage regimens. The control group received 2 ml/kg of distilled water, and all administrations were done orally for seven days. The animals were monitored for behavioural changes throughout the duration of the experiment. On day 8, the animals were anaesthetised with ketamine hydrochloride (Rotex Medical, Germany; 50 mg/kg, i.p) and sacrificed. The thorax of the animals was dissected and transcardially perfused with 1M phosphate buffered saline followed by 10 % buffered formalin. The cervical vertebrae were exposed and cracked open, and the spinal segment C3-C5 were excised and post-fixed in 10 % buffered formalin for 48 hours. The tissues were then routinely processed for histomorphology with haematoxylin and eosin stains (H&E) through paraffinized 10 micrometre thin sections. The sections were quantified by means of ImageJ® software (version 1.37c). Briefly, images of the whole dentate gyrus was obtained for each section and randomly mapped with the ImageJ® gridlines. Counting of cell nuclei was done manually taking into consideration the nuclei on the upper and right borders of the mapped areas. Data were analyzed using one way analysis of variance by means of Graphpad prism (version 5) and post-hoc Tukey test used to compare group difference. Statistically significance was determined at probability level p ≤ 0.05, and data represented as mean ± standard error of mean.

**Results**

**Behavioural appraisal:** At the beginning of the experiments, all the animals were apparently healthy and agile, with solid faecal boli. However, in the course of the experiment all the animals administered doses of *R. vomitoria* appeared generally weak, dull and drowsy compared with the control group. The faecal boli appeared semi solid, while there was also decreased food and water intake in these test groups.

**Histological Observations:** The ventral horn of the control group showed distinctive appearance of meshwork of cell neurons and glial processes. Glial cells appeared more numerous than neurons, with no obvious histopathology observed (Figure 1 and 2a). The ventral horn of the 200 mg/kg *R. vomitoria* group showed slight hypertrophy especially of the neurons. The glial cells also appear less distinct compared with the control (Figure 2b). The ventral horn of the 300 mg/kg *R. vomitoria* group showed hypertrophy especially of the neurons and hyperplasia of the glia cells with pyknotic appearance of the nuclei (Figure 2c). The ventral horn of the 400 mg/kg *R. vomitoria* group showed hypertrophy of cell neurons with karyorrhectic appearance of some of the nuclei (Figure 2d). There was no difference (p > 0.05) in cell population between the test groups and the control. Also, there was no difference (p > 0.05) in cell population among the test groups (Figure 3).

**Discussion**

This study investigated the effect of *R. vomitoria* on the ventral horn cells of the spinal cervical segment of Wistar rats. The groups administered different doses of *R. vomitoria* showed general weakness, dull and drowsy behaviour, and body weights loss. These behavioural and morphological changes are the visible signs of *R. vomitoria* toxicity as previously reported.[3,17-22] Histologically, there was slight hypertrophy of the neurons with less distinct glial cells in the spinal cervical ventral horn, and no difference...
Figure 2: Photomicrographs of Sections of the Ventral Horn of Spinal Segment C3-C5. a. The ventral horn of control group shows distinctive appearance of meshwork of neurons and their processes, as well as glial cells; b. The ventral horn of the 200 mg/kg R. vomitoria group shows slight hypertrophy of neurons and less distinct glial cells; c. The ventral horn of the 300 mg/kg R. vomitoria group shows hypertrophy and hyperplasia of cell neurons with pyknotic (Pk) appearance of some nuclei; d. The ventral horn of the 400 mg/kg RV group shows hypertrophy of neurons with karyorrhectic (Kx) appearance of some of the nuclei. Neurons (N); neuronal processes (Np); glial cells (G); H&E, ×400

Figure 3: Ventral Horn Cell Population of the Spinal Cord Segment C3-C5. Data presented as Mean ± Standard Error of Mean; Data is not significantly different from the control at p ≤ 0.05; p = 0.1419; F-ratio = 2.031 RV = Rauwolfia vomitoria
(p > 0.05) in cell population in the 200 mg/kg R. vomitoria group compared with the control. Hypertrophy, which is the enlargement of cells and its organelles usually arise as compensation reaction to trauma of the tissue.[27] R. vomitoria have been reported to cause such trauma in different body tissues,[3,17-22] indicating that it may also affect spinal cervical ventral horn cells as in the present study. The non-prominent glia cells may be due to cell division changes, which may have inhibited their staining ability. This is because R. vomitoria stimulates gliosis. [10,17-22]

In the 300 mg/kg R. vomitoria group, there was hypertrophy of the neurons and hyperplasia of the glia cells with pyknotic appearance of some of the nuclei in the spinal cervical ventral horn, and no difference (p > 0.05) in cell population compared with the control. This result is similar to that of the 200 mg/kg R. vomitoria group and more probably due to the higher dosage. This result is similar to that of the 200 mg/kg R. vomitoria group and more probably due to the higher dosage. These additional features signify changes due to trauma which has progressed to the early cell death process. This is because pyknosis signify an irreversible condensation of chromatin in the nucleus of a necrotic cell.[28]

In the 400 mg/kg R. vomitoria group, there was hypertrophy of neurons with karyorrhectic appearance of some of the nuclei in the spinal cervical ventral horn cells, and no difference (p > 0.05) in cell population compared with the control. This result is similar to that of the 200 and 300 mg/kg R. vomitoria groups and more probably due to the higher dosage. These additional features signify changes which has progressed to the cell death process as karyorrhexis signifies an irreversible condensation of chromatin in the nucleus of a necrotic cell.[28]

The histopathological changes of the cervical ventral horn cells of the test groups could also be due to increased tissue demand, hormonal dysfunction, chronic inflammatory response or compensation for damage.[29] The This may hinder the role of these cells in supporting motor functions. [30] R. vomitoria has been reported to cause loss of cellular integrity of the cerebral cortex, cerebellum and olfactory bulb in animal models and, hypertrophy and hyperplasia of osteoblasts, which supports the present results. [3,10,17-22,31]

**Conclusion**

R. vomitoria alter behavioural and cellular architecture of the spinal ventral horn C3-C5 segments and these changes were dose dependent.

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*Corresponding author:
Dr. Moses B. Ekong, Department of Anatomy, University of Uyo, Uyo, Nigeria
Phone: +2348030868505
Email: mbe_flashpoint@yahoo.com

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