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 221g 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 eight day, the animals were anaesthetized 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 *utoenyin* 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 flavinoids.[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 depletion 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 °C and stored in a refrigerator at 4 °C 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 anaesthetized 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. Statistical significance was determined at probability level $p \leq 0.05$, and data represented as mean $\pm$ 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

Fig. 1: Photomicrograph of the section of the spinal cord segment C3-C5. Central canal (CC); Dorsal horn (DH), Ventral horn (MH); Ventral Funiculus (VF); H&E, $\times 40$.

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; 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, $\times 400$

Figure 3: Ventral Horn Cell Population of the Spinal Cord Segment C3-C5 Data presented as Mean $\pm$ Standard Error of Mean; Data is not significantly different from the control at $p \leq 0.05$; $p = 0.1419$; 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]. 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.

Acknowledgment

We acknowledge with thanks Dr Monday Akpanabiatu who made available the roots of the *R. vomitoria*, and the Animal House Facility personnel who took care of the animals.

References

1. Valnet J. The practice of aromatherapy: A classic compendium of plant medicines and their healing properties. Original ed. Rochester, NY: Healing Arts Press. 1982.
2. Obembe A, Sokombab EN, Sijuwolaa OA, et al. Antipsychotic effects and tolerance of crude *Rauwolfia vomitoria* in Nigerian psychiatric inpatients. *Phytother Res* 1994;8:218-223.
3. Eluwa MA, Idumesaro NB, Ekong MB, Akpantah AO, Ekanem TB. Effects of aqueous extract of *Rauwolfia vomitoria* root bark on the cytoarchitecture of the cerebellum and neurobehaviour of adult male Wistar rats. *IJAM* 2008; 6(2):3081.
4. Bemis DL, Capodice JL, Gorroochurn P, Katz AE, Buttyan R. Anti-prostate cancer activity of a β -carboline alkaloid enriched extract from *Rauwolfia vomitoria*. *International J Oncol* 2006;29:1065.
5. Duez P, Chamart S, Vanhaelen M, Vanhaelen-Fastré R, Hanocq M, Molle L. Comparison between high-performance thin-layer chromatography-densitometry and high-performance liquid chromatography for the determination of ajmaline, reserpine and rescinnamine in *Rauwolfia vomitoria* root bark. *J Chromatography A* 1986;356:334.
6. Barcelos RCS, Benvegnü DM, Bouffleur N, et al. Short term dietary fish oil supplementation improves motor deficiencies related to reserpine-induced parkinsonism in rats. *Lipids* 2010;46(2):143-149
7. Henry J, Scherman D. Radioligands of the vesicular monoamine transporter and their use as markers of monoamine storage vesicles. *Biochem Pharmacol* 1989;38(15):2395-2404.
8. Hai-Bo L, Yong P, Lu-qi H, Jun X, Pei-Gen X. Mechanism of selective inhibition of yohimbine and its derivatives in adrenoceptor α_2 subtypes. *J Chem* 2013;(2013):783058
9. Oyedeji L (2007). *Drugless Healing Secret*. 3rd ed. Ibadan: Panse Press Ltd.
10. Ekong MB, Peter AI, Ekpene UU, et al. *Gongronema latifolium* modulates *Rauwolfia vomitoria* induced behavior, biochemicals, and histomorphology of the cerebral cortex. *Int J Morphol* 2015;33:77.
11. Shamon SD, Perez MI. Blood pressure lowering efficacy of reserpine for primary hypertension. *Cochrane Database Syst Rev* 2009; CD007655.
12. Leão AHFF, Aldair JS, José RS. Molecular, neurochemical, and behavioral hallmarks of reserpine as a model for parkinson's disease, new perspectives to a long-standing model. *Brain Pathol* 2015;25:377-390.

13. Lopez-Munoz F, Bhatar V, Alamo C, Cuenca E. Historical approach to reserpine discovery and its introduction in psychiatry. *Actas Esp Psiquiatr* 2004;32(6):387-395.
14. Bisong SA, Brown R, Osim EE. Comparative effects of *Rauwolfia vomitoria* and chlorpromazine on locomotor behaviour and anxiety in mice. *J Ethnopharmacol* 2010;132:334-339.
15. Campbell-Tofte JI, Molgaard P, Josefsen K. Randomized and double-blinded pilot clinical study of the safety and anti-diabetic efficacy of the *Rauwolfia-Citrus* tea, as used in Nigerian traditional medicine. *J Ethnopharmacol* 2011;133:402-411.
16. Okpako DT (1991). *Principles of Pharmacology: A Tropical Approach*. Cambridge University Press, New York.
17. Ekong MB, Ekpene UU, Nwakanma AA, Peter AI, Etuknw BT. *Rauwolfia vomitoria* and *Gongronema latifolium* stimulate cortical cell proliferations. *Ann Bioanthropol* 2016;4:41-46.
18. Ekong MB, Ekpene UU, Thompson FE, Peter AI, Udoh NB, Ekandem GJ. Effects of co-treatment of *Rauwolfia vomitoria* and *Gongronema latifolium* on neurobehaviour and the neurohistology of the cerebral cortex in mice. *Internet J Med Update* 2015;10(1):3-10.
19. Ekong MB, Peter AI, Edagha IA, Ekpene UU, Friday DA. *Rauwolfia vomitoria* inhibits olfaction and modifies olfactory bulb cells. *Brain Res Bullet* 2016;124:206.
20. Ekong MB, Ekpene UU, Nwakanma AA, Bello EF. The combination of the extracts of *rauwolfia vomitoria* and *gongronema latifolium* show protective effects on the cerebellum. *Synergy* 2017;5:29-34.
21. Ekong MB, Nwakanma AA. *Rauwolfia vomitoria* and *gongronema latifolium* extracts influences cerebellar cortex. *ADCN* 2017;1(3):1-6.
22. Ekong MB, Peter AI. Co-treatment of *rauwolfia vomitoria* and *gongronema latifolium* affects cerebellar cell population in mice. *J Animal Husbandry Dairy Sci* 2017;1(1):1-7.
23. Meythaler JM, Tuel SM, Cross LL. Spinal cord seizures: a possible cause of isolated myoclonic activity in traumatic spinal cord injury: case report. *Paraplegia* 1991;29:557-560.
24. Hashimoto T, Sato H, Shindo M, Hayashi R, Ikeda S. Peripheral mechanisms in tremor after traumatic neck injury. *J Neurol Neurosurg Psychiatry* 2002;73(5):585-587.
25. Rothwell J. Reflex pathways in the spinal cord. In: *Control of Human Voluntary Movement*. Springer, Dordrecht. 1994
26. West KB, Applegate E. *Today's Medical Assistant: Clinical and Administrative Procedures*. 3rd ed. Elsevier Health Sciences. 2015
27. Kusumoto FM. *Cardiovascular Pathophysiology*, Hayes Barton Press. 2004
28. Kroemer G, Galluzzi L, Vandenabeele P. Classification of cell death: recommendations of the nomenclature committee on cell death. *Cell Death Differ* 2009;16(1):3-11.
29. Porth C. *Essentials of Pathophysiology: Concepts of Altered Health States*. Lippincott Williams & Wilkins. 2011
30. Kaminsky N, Bihari O, Kanner S, Barzilai A. Connecting malfunctioning glial cells and brain degenerative disorders. *Genomics Proteomics Bioinform* 2016;14(3):155-165.
31. Eluwa MA, Ekanem TB, Udoh PB, et al.. Teratogenic effects of crude ethanolic root bark and leaf extracts of *rauwolfia vomitoria* (apocynaceae) on the femur of albino wistar rat fetuses. *J Histol* 2013;(2013):363857.

***Corresponding author:**

Dr. Moses B. Ekong, Department of Anatomy, University of Uyo, Uyo, Nigeria

Phone: +2348030868505

Email: mbe_flashpoint@yahoo.com

Financial or other Competing Interests: None.