

Rauvolfia vomitoria Afzel. disrupts dentate gyrus cells

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Herbal remedy for neurological problems may have adverse effects, and could prove detrimental if not regulated properly. *Rauvolfia vomitoria* (RV) is a herb commonly associated with psychiatry management because of its antipsychotic and sedative properties. Here, we studied the effects of the root bark extract of *R. vomitoria* on the dentate gyrus of adult Wistar rats. Twenty four adult Wistar rats (220 g average) were divided into four groups (n=6); control (placebo), 200, 300 and 400 mg/kg RV root bark extract, respectively for 7 days. The animals were sacrificed 24 h after last administration, and the brains were processed for histology and immunoreactivity. Results showed hypertrophy and atrophy of granule cells in all 200, 300 and 400 mg/kg RV groups, respectively. There was increased neuron specific enolase and glial fibrillary acidic protein expressions in the 200 and 300 mg/kg RV groups, while these proteins expression were decreased in the 400 mg/kg RV group. These results suggest that RV cause dentate gyrus cell injury in a dose-dependent pattern, and may lead to degeneration and disruption of functions.

Keywords: Immunoreactivity, The poison devil's-pepper

Herbal remedy for neurological problems often results in two crucial problems viz. determination of dosage with regard to individual idiosyncrasy, and their non-recourse to the inherent adverse properties of these herbs. Though some of the herbs appear not to pose any threat to the subjects apparently, acute and chronic use of them may eventually lead to more devastating consequences. One such herb readily used in psychiatry conditions is *Rauvolfia vomitoria*, Afzel. known to be a sedative and an antipsychotic¹⁻³.

Rauvolfia vomitoria (RV), commonly called, The poison devil's-pepper from the family *Apocynaceae*, is a shrub whose root-bark and leaves are widely used as psychiatric drugs. Active constituents of this plant, which is mostly ascribed to its alkaloids and polyphenols compounds include; ajmalicine ajmaline, alstonine, reserpine, rescinnamine, serpentine, serpentinine and yohimbine among others⁴⁻⁷. There are beneficial, as well as adverse reports associated with RV, including cellular destruction of some brain areas, depression and Parkinsonism.^{1,2,5,8-17}

Sedatives have been reported to affect cognitive abilities^{18,19}. RV being a sedative may affect some of

these cognitive functions^{1,3,12}, although Ekong *et al.*¹¹ reported that 200 mg/kg of RV did not affect spatial learning and memory in 7-day treatment. However, depletion of dopaminergic stores by reserpine, one of RV constituents, is known to occur²⁰, which may still pose a problem to the functions of the dentate gyrus in the long run, as this brain area functions requires dopamine²¹. Thus, in this present study, we investigated the influence of the ethanol root bark extract of the medicinally important herb, *Rauvolfia vomitoria* Afzel. (RV) in the dentate gyrus cells to ascertain their viability.

Materials and Methods

Roots of *R. vomitoria* were harvested from a local farm in Ekpene Obo, Esit-Eket Local Government Area of Akwa Ibom State, Nigeria. The RV plant was identified and authenticated by the Curator of the plant herbarium unit of the Department of Botany and Ecological Studies of the University. The roots were washed to remove impurities and the bark was separated from the cambium, air-dried for one week and then pulverized into powder using kitchen blender. The RV root bark powder was extracted using 80% ethanol.

Eighteen adult Wistar rats weighing 220 g on the average were divided into four groups A-D (n=6) of

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control, 200, 300 and 400 mg/kg of root bark extract of *R. vomitoria*. The rats were cared following the guidelines for the care and use of experimental animals, and were maintained at the room temperature (27-30°C) and 12 h light and dark cycles at the animal facility of the Faculty of Basic Medical Sciences of the University. The rats were allowed free access to normal rat chow (Vital Feed Company Limited, Nigeria) and clean water *ad libitum*.

Briefly, a known weight of the blended sample was soaked in 80% ethanol for 24 h, the extract was filtered and concentrated using a rotary evaporator, and then dried in a Plus 11 Gallenkamp oven at 45-50°C. The dry extracts obtained were stored in a refrigerator at 4 °C until used. Distilled water was used as the vehicle to dissolve the extracts. Two grams of each extracts was dissolved in 30 mL of distilled water and the actual dosages were calculated based on the body weights of each rat.

The control group (A) was administered distilled water (3 mL/kg), while groups B, C and D were administered by oral gavages doses of 200 mg/kg (0.66 mL), 30 mg/kg (0.99 mL) and 400 mg/kg (1.32 mL) body weights of the extracts of RV, respectively, for seven days. On day 8, the animals were anaesthetized with ketamine hydrochloride (Rotex Medical, Germany; 10 mg/kg, i.p.) and sacrificed. The thorax of each animal was dissected and transcardially perfused with 1M phosphate buffered saline followed by 10% buffered formalin. The skull was later excised, whole brains removed and post-fixed in 10% buffered formalin for 48 hours. The tissues were routinely processed for paraffin wax embedding. About 10 µm thick paraffin sections were then routinely processed for histomorphology with haematoxylin and eosin stains (H&E) and immunolabelled for neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP).

Briefly, serial paraffin sections on slides were brought to water and antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven (100 W) for 5 min, followed by protein block using 3% hydrogen peroxide for 10 min. Sections were thereafter preincubated in 2% normal goat serum for 30 min, and incubated in monoclonal mouse anti-enolase-2 (Novocastra, Leica Biosystems, 22C9, 1:100) for neuron specific enolase and mouse monoclonal anti GFAP (Novocastra, Leica Biosystems, NCL-L-GFAP-GA5, 1:100) for GFAP. These were followed by one hour incubation in goat anti-mouse secondary antibody (1:100) for one hour.

Detection of reaction was by means of the avidin-biotin complex with diaminobenzidine as the chromagen. Sections were then counterstained with Harris haematoxylin, dehydrated, cleared and cover slipped with distyrene plasticizer xylene (DPX). Processed slides were viewed under the light microscope and photomicrographs obtained using a computer assisted digital microscope's camera.

Cellular density was determined manually by means of ImageJ® software. 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. One way analysis of variance was used to analyse obtained data, followed with post-hoc Tukey's test. Data are presented as Mean ± Standard Error of Mean, and data with probability level $P < 0.05$ was regarded as significant.

Results

Morphology/Body weight

At the beginning of the experiments, all the animals were apparently healthy and agile, with normal faecal boli. However, in the course of the experiment all the animals administered doses of RV appear generally weak, dull and drowsy compared with the control group. There was also decreased food and water intake in these test groups.

The initial body weights of all the test groups; 200 mg/kg RV extract (221.67±9.68 g), 300 mg/kg RV extract (213.00±9.00 g), and 400 mg/kg RV extract (227.83±5.52 g) were not different from the control group (218.00±4.88 g). However, their final body weights were significantly ($P < 0.05$) lower; 200 mg/kg RV extract (166.33±10.75 g), 300 mg/kg RV extract (152.83±6.54 g), and 400 mg/kg RV extract (146.67±7.39 g) compared with the control group (218.00±4.03 g). There was loss of body weight in all the test groups, with the body weight loss being dose dependent; 200 mg/kg RV extract (55.34 g, 25.01%), 300 mg/kg RV extract (66.33 g, 31.14%), and 400 mg/kg RV extract (75.00 g, 32.93%) (Fig. 1).

Histomorphology study

The dentate gyrus of the control group showed three layers, from the outside inwards; molecular, granular, and polymorphic. The molecular layer showed sparsely distributed cells, while the granular layer had a dense population of granule cells that project to Cornu Ammonis 3 (CA3) of the hippocampus. There were

numerous small size cells in the subgranular zone of granular layer. The polymorphic layer also showed sparse cell density (Fig. 2A).

The section of the dentate gyrus of the 200 mg/kg RV extract group showed slight hypertrophy and karyorrhexis of the nuclei in some of the granule cells. There were few small size cells in the subgranular zone, with these being more numerous in the polymorphic layer compared with the control group (Fig. 2B). The section of the dentate gyrus of the 300 mg/kg RV extract group showed hypertrophy and karyorrhexis of the nuclei in some of the granule cells, though the subgranular zone showed few small size cells extending into the granular and polymorphic layers compared with the control group (Fig. 2C). The section of the dentate gyrus of the 400 mg/kg RV extract group showed atrophy of most of the granule cells compared with the control group (Fig. 2D).

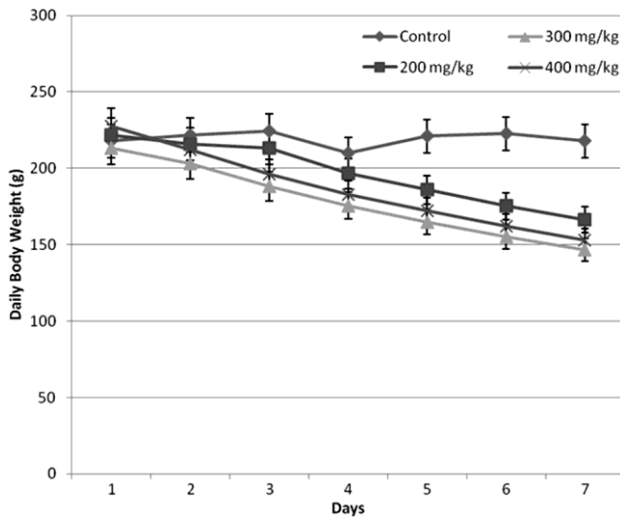


Fig. 1 — Daily body weight change of experimental groups

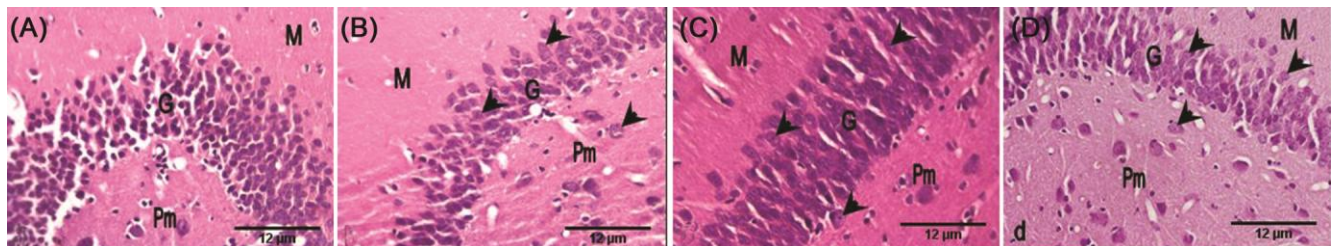


Fig. 2 — The photomicrographs of the dentate gyrus of the control group and test groups. (H&E, X200). (A) The control showed three layers; molecular (M), granular (G), and polymorphic (Pm). The molecular showed sparsely distributed cells, while the granular layer had a dense population of granule cells. There were numerous small size cells in the subgranular zone of granular layer. The polymorphic layer also showed sparse cell density; (B) The 200 mg/kg *Rauvolfia vomitoria* (RV) extract group showed slight hypertrophy and karyorrhexis of the nuclei in some of the granule cells. There were few small size cells in the subgranular zone, which were numerous in the polymorphic layer; (C) The 300 mg/kg RV extract group showed hypertrophy and karyorrhexis of the nuclei in some of the granule cells, though the subgranular zone showed few small size cells extending into the granular and polymorphic layers; and (D) 400 mg/kg RV extract group showed atrophy of some of the granule cells.

Cellular population were significantly ($P < 0.05$) lower in the all the test groups; 200 mg/kg (166 ± 7.90), 300 mg/kg (134 ± 8.84), 400 mg/kg (103 ± 5.99) compared with the control group (270 ± 10.41). These differences were also significant ($P < 0.05$) among the test groups (Fig. 3).

Immunolabelling study

There was prominent neuron specific enolase (NSE) expression in all the layers of the dentate gyrus of the control group (Fig. 4A), while the NSE expression was enhanced in the dentate gyrus of the 200 mg/kg RV group compared with the control group (Fig. 4B). There was also enhanced NSE expression in the dentate gyrus of the 300 mg/kg RV

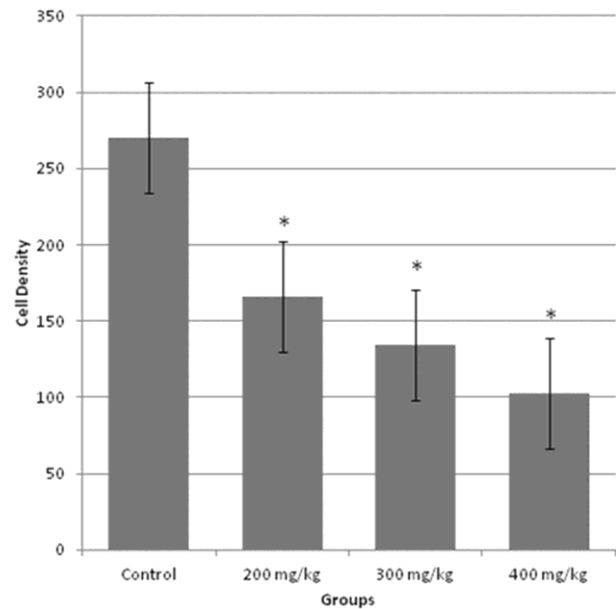


Fig. 3 — The dentate gyrus cell density. [* Significantly different from the control group at $P < 0.05$]

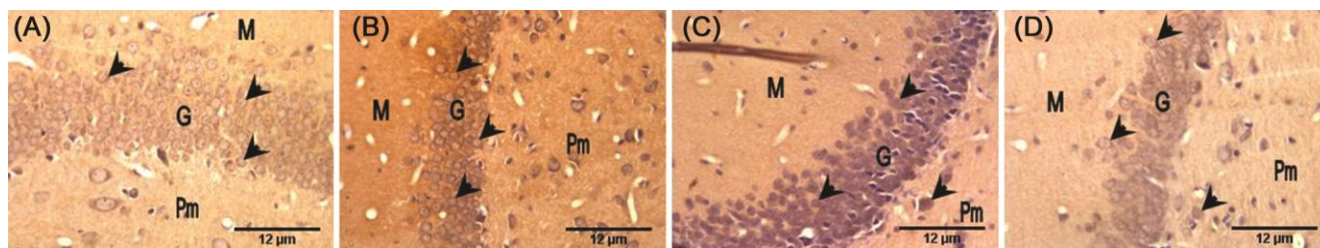


Fig. 4 — The photomicrographs of the dentate gyrus of the control group and test groups (NSE, X200). Control group (A) showed prominent neuron specific enolase (NSE) expression in all the layers of the dentate gyrus; There was enhanced NSE expression in the dentate gyrus of the (B) 200 and (C) 300 mg/kg RV group; and (D) 400 mg/kg RV group showed less prominent NSE expression in the dentate gyrus.

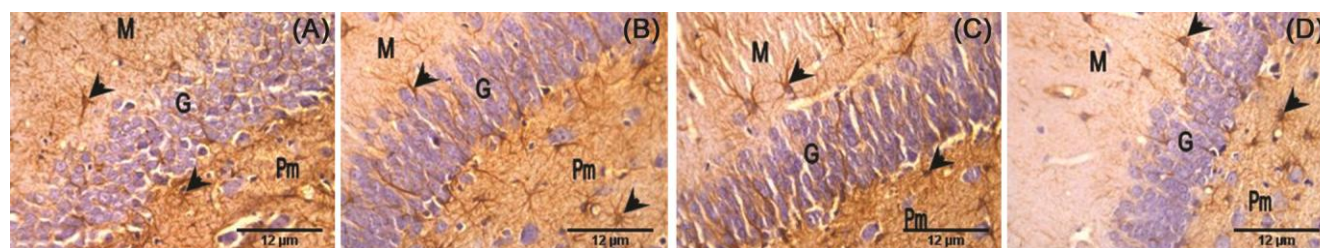


Fig. 5 — The photomicrographs of the dentate gyrus of the control group and test groups (GFAP, X200). Control group (A) showed glial fibrillary acidic protein (GFAP) expression in the dentate gyrus of the control group. The soma and processes were more prominent in all the three layers; There was marked increase GFAP expression in the dentate gyrus of the (B) 200 and (C) 300 mg/kg RV group; whereas 400 mg/kg RV group (D) showed decrease GFAP expression in the dentate gyrus.

group compared with the control group (Fig. 4C), while there was less prominent NSE expression in the dentate gyrus of the 400 mg/kg RV group compared with the control group (Fig. 4D).

Glial fibrillary acidic protein (GFAP) expression in the dentate gyrus of the control group rats was very prominent. This GFAP expression was much in the soma and processes in all the three layers (Fig. 5A). There was marked increase GFAP expression in the dentate gyrus of the 200 mg/kg RV group compared with the control group rats (Fig. 5B). There was also marked increase GFAP expression in the dentate gyrus of the 300 mg/kg RV group compared with the control group rats (Fig. 5C), while GFAP expression in the dentate gyrus of the 400 mg/kg RV group was decreased compared with the control group rats (Fig. 5D).

Discussion

This study was to assess the effect of RV on the dentate gyrus of adult Wistar rats. The groups administered different doses of RV showed general weakness, dull and drowsy behaviour, decreased food and water intake, and body weights loss. These behavioural and morphological changes are the sedative effects and visible signs of RV toxicity as previously reported^{1,3,10,12,22,23}. Body weight loss and

other observed effects have been attributed to the action of its constituents especially reserpine, implicated in sedation and appetite loss²³⁻²⁶. Appetite loss is reported to result in body weight loss,²⁶ which has also been reported. This result supports previous reported studies^{1,3,12}.

The results of the histomorphology of the present study showed hypertrophy of the granule cells with some of them having karyorrhectic nuclei in the 200 and 300 mg/kg RV groups. Hypertrophy is an adaptive change in tissues to cope with trauma, and may result from increase demand on the cells due to tissue injury²⁷. However, karyorrhexis indicates a pathological process²⁸, a prerequisite to cell death, which may have been the case in the present study. This result corroborates previous reports that RV causes brain cells injury^{1,2,12,29}.

There was atrophy of the granule cells in the 400 mg/kg RV group. Atrophy indicates cell death process, which occur when there is disruption of trophic signals to cells among other causes³⁰, resulting in loss of function²⁸. This result indicates that high dose of RV may initiate neuronal degeneration, and it supports previous studies of oral RV administration in brain tissues^{1,10,12}.

Cellular density appeared reduced in all the RV groups which may indicate cell death. Previous

reports in rat brain tissues have shown similar loss of cells^{10,12}. These are at variance with reports on mice brain tissues where there was hyperplasia^{1,10,31}. It is reported that different animal species react differently to exogenous stimuli³². These differences in brain tissue reaction may be due to animal species difference.

Immunolabelling results showed enhanced NSE expression in the dentate gyrus of the 200 and 300 mg/kg RV groups. Increased NSE expression indicates neuronal damage^{33,34}. NSE is a brain-specific glycolytic enzyme and cytosolic protein²² and serves as a marker of neuronal functional metabolic activity and synaptic connections³⁵, an increase in NSE may be due to increase neuronal metabolism leading to neuronal injury. Ekong *et al.*¹², have also reported similar effect on olfactory bulb cells.

There was decreased NSE expression in the dentate gyrus of the 400 mg/kg RV group, an indication of neuronal degeneration. RV constituent, reserpine is reported to cause dopaminergic cell death³⁰, which the dentate gyrus is replete with. This degeneration of neuron may lead to its reduced NSE expression which supports the report of Kirino *et al.*³¹ Decreased NSE also indicates that its role in neuronal glycolysis is also reduced leading to a concomitant reduction in neurotransmitter production and axonal transport³⁵, usually associated with degenerative processes.

There was marked increased GFAP expression in the olfactory bulb of the 200 and 300 mg/kg RV groups. Increased GFAP expression is indicative of the up-regulation of the GFAP protein in the astrocytes, which usually arise following CNS injury or trauma and also in the process of neurodegeneration³⁸. Increased GFAP expression is essential for the process of reactive astrogliosis and glial scar formation^{39,40}. Reserpine, a component of RV is reported to cause GFAP increase in astrocytes⁴¹, and is also reported to cause neuronal death³⁶. The increase expression of GFAP indicates that 200 and 300 mg/kg RV resulted in injury to the dentate gyrus, and has been previously reported in other brain areas^{12,29}.

There was decrease GFAP expression in the dentate gyrus of the 400 mg/kg RV group. Decreased GFAP expression may indicate a decrease GFAP either due to a decrease in the number of astrocytes, cytoskeletal destabilization or degradation and loss of GFAP antigenicity⁴²⁻⁴⁴. It is reported that high dose of reserpine acts as a depressant, and this may have equally played out in the 400 mg/kg RV group as depressed brain results in GFAP downregulation⁴⁵.

The dentate gyrus is an integral portion of the hippocampal formation⁴⁶, and acts as a pre-processor of information to the hippocampus proper by receiving excitatory neuron input from the entorhinal cortex and sending excitatory output to the hippocampal CA3 region via the mossy fibres⁴⁷. These processes may be altered with RV administration due to neuronal and glia injuries that may initiate cellular death processes.

Conclusion

The results of this study indicate that administration of ethanolic extract of root bark of the herb, *Rauwolfia vomitoria* (RV) stimulates dentate gyrus cells injury that may lead to degenerative changes in its cytoskeletal proteins, and these effects were dose-dependent.

Conflict of Interest

Authors declare no conflicts of interests.

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