

# *Rauvolfia vomitoria* and *Gongronema latifolium* stimulate cortical cell proliferations

## ABSTRACT

**Introduction:** *Rauvolfia vomitoria* (RV) and *Gongronema latifolium* (GL) are herbs with closely related and diverse medicinal properties. The combination of both plants is reported to have the potentials for brain functions and structure protection. **Aim:** This study therefore investigated the interaction of these herbs on the histomorphology of the cerebral cortex of mice. **Materials and Methods:** 24 male Wistar mice of body weight 15-26 g were divided into 4 groups. The mice were administered respectively, 0.5 mL of Tween 20, 150 mg/kg of *R. vomitoria*, 200 mg/kg of *G. latifolium*, and a combination of 150 mg/kg of *R. vomitoria* and 200 mg/kg of *G. latifolium* (RV+GL), orally, and daily for seven days. On day 8, the animals were sacrificed and their brains preserved, and the cerebral cortices were excised for routine histology. Cellular densities were quantified using ImageJ™. **Results:** All the groups gained body weight, which was however lower in the test groups compared with the control group. No difference was observed in whole brain weight in all the experimental groups, while histomorphological studies of the cerebral cortex showed higher cellular density and smaller cellular sizes in the RV, GL and RV+GL groups. The RV+GL group also showed slightly larger cells in the cortical plate compared with the control group. The mean cellular population of sections of the cerebral cortex were also higher in test groups RV and GL, but not the RV+GL group. **Conclusion:** This study showed that *R. vomitoria* root bark and *G. latifolium* leaf extracts either singly or in combination may stimulate cellular proliferation at the given dose, which may serve a protective or deleterious role.

**Key words:** Brain, cerebral cortex, *Gongronema latifolium*, mice, *Rauvolfia vomitoria*

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## INTRODUCTION

The rising cost of modern and more efficacious orthodox drugs, its availability and safety have led to the patronage of alternative medication.<sup>[1]</sup> Most of these alternatives are herbs, and reports show that these herbs have wide ranges of useful medicinal properties.<sup>[2-4]</sup> Herbs such as *Rauvolfia vomitoria* (RV) and *Gongronema latifolium* (GL) have closely related, as well as diverse properties,<sup>[5-9]</sup> and these have led to their medicinal uses.

RV belongs to the family *Apocynaceae*, and it is commonly called serpent wood or swizzle stick. In Nigerian languages,

it is known as eto mmong eba eboto in Ibibio, akanta in Igbo, asofeyeje in Yoruba, and wada in Hausa. RV contains alkaloid which includes ajmaline, ajmalicine, reserpine, serpentine, serpentinine, and yohimbine among others.<sup>[3,10]</sup> This herb has been reported to improve immunity and restore hematologic parameters.<sup>[11]</sup> Analgesic, anticonvulsant, and antipsychotic effects have been reported,<sup>[5,12,13]</sup> while it was previously reported that RV restores brain mental activities.<sup>[14]</sup>

Side effects of RV in animal models have been reported: In the fetus, hepatotoxicity, cardiotoxicity, hypertrophy, and

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hyperplasia of osteoblasts and osteoclasts in the femur have been reported.<sup>[15-17]</sup> Depression and Parkinsonism,<sup>[10]</sup> degenerative changes in cerebellar and cerebral cortical cytoarchitectures, and neurobehaviors have also been reported in adults.<sup>[18-22]</sup>

RV causes sedation,<sup>[18,19,22]</sup> which if not controlled may result in death due to appetite loss. The effectiveness of RV in combination with other herbs such as GL has been reported with minimal side effects;<sup>[19-23]</sup> hence, this study is to investigate if the combination herbs could be beneficial to the cerebral cortical morphology.

GL belongs to the family *Asclepiadaceae*, and it is commonly called amaranth globe and bush buck. In Nigerian languages, it is known as utasi in Ibibio, utazi in Igbo, and arokeke in Yoruba.<sup>[8]</sup> The plant is used for both medicinal and nutritional purposes.<sup>[24,25]</sup> GL contains alkaloids, saponins, tannins, flavonoids, and glycosides.<sup>[25,26]</sup> Properties such as hypoglycemic, antibacterial, antioxidant, anti-inflammatory, antiulcer, analgesic, antidiabetic, and antipyretic activities have been reported.<sup>[4,7,9,25,27,28]</sup> GL is also reported to be a bittering agent, as well as having cardioprotective properties.<sup>[6,29]</sup>

Both plants show potentials for brain function improvement and protection<sup>[13,22,23]</sup> although a recent report showed no effect on spatial learning and memory, as well as some biomolecules and enzyme activities.<sup>[23]</sup> Therefore, this investigation was to investigate the combined properties of these herbs on the histology of the cerebral cortex of mice.

## MATERIALS AND METHODS

Thirty male Wistar mice of age 3 months and body weight 15–26 g obtained from the animal facility of the Faculty of Basic Medical Sciences of the University of Uyo, Nigeria, were handled in accordance with the international guidelines for the use and care of laboratory animals. The animals were provided with normal mice chow (Vital Feed, Jos, Nigeria) and water *ad libitum* throughout the duration of the experiment.

### Preparation of herbs

Fresh leaves and roots of GL and RV were, respectively, obtained from local farms in Ika and Esit Eket in Akwa Ibom State of Nigeria. Samples of the plants were authenticated by a botanist in the Department of Botany of the University. The root barks were separated from the cambium, and together with the leaves were cleaned of impurities. The parts of these plants were air-dried and crushed to fine powder and were macerated in 75–80% ethanol, using Soxhlet extractor. The extracts were concentrated using a rotary evaporator, and the concentrates were 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. Tween 20 was used as the

vehicle to dissolve the extracts. Two gram of each extract was dissolved in 30 mL of Tween 20, and the actual dosages were calculated.

### Experimental protocol

The mice were weighed and divided into four groups of six mice each. Animals in Group 1 (control) were administered 0.5 mL of Tween 20. Group 2 animals were administered 150 mg/kg of RV. Group 3 animals were administered 200 mg/kg of GL, whereas Group 4 animals were administered a combination of 150 mg/kg of RV and 200 mg/kg of GL. All the treatments were by oral gavages, carried out at 8 am every day for 7 days [Table 1].

Twenty-four hours after the last administrations, the animals were anesthetized using chloroform fumes and were humanely sacrificed. The brains were removed, weighed, and preserved in 10% buffered formalin for 7 days. Each cerebral cortex was excised, routinely processed and stained following the hematoxylin and eosin staining method. The nuclei of the cells were quantified using ImageJ™ software (version 1.37c, NIH, USA) with the aid of a computer operated digital camera linked to the microscope to determine the cellular population, and photomicrographs of the sections were obtained.

### Statistical analysis

One-way analysis of variance was used to compare the means for treatment and their interactions; thereafter, the *post hoc* test using Student–Newman–Keuls multiple comparisons test was carried out to find the level of significance at  $P < 0.05$ . All the results were expressed as mean  $\pm$  standard deviation.

## RESULTS

### Weight changes study

There was gradual increase in body weights in all the experimental groups, but from day 6 of the administration of the extracts, there was loss of body weights in the test groups which received 150 mg/kg of RV and 200 mg/kg of GL either alone or in combination (RV + GL), compared with the control group, which was increased during the same period [Figure 1]. The percentage increase in body

**Table 1: Schedule of administration of the extracts to the animals**

Groups (n=6)	Treatment (mg/kg)	Duration of treatment (days)
Group 1 (control)	20% Tween	7
Group 2	150 of RV	7
Group 3	200 of GL	7
Group 4	Combination of 150 of RV and 200 of GL	7

RV = *Rauvolfia vomitoria*; GL = *Gongronema latifolium*

**Table 2: Body weights and body weights change**

Groups (n=6) (mg/kg)	Day 1 body weight (g)	Day 8 body weight (g)	Body weight change (g)	Body weight change (%)
Group 1 (control)	15.75±0.57	20.25±5.17	4.5	28.57
Group 2 (150, RV)	26.00±0.71*	27.17±1.64* <sup>d</sup>	1.17	4.5
Group 3 (200, GL)	25.00±1.00* <sup>d</sup>	25.97±2.54* <sup>d</sup>	0.97	3.88
Group 4 (150, RV and 200, GL)	19.10±0.36* <sup>b</sup>	19.83±1.54 NS	0.63	3.28

Data is presented as mean±SD. \*Significant different at  $P < 0.05$  compared to Group 1; <sup>b</sup>Significant different at  $P < 0.05$  compared to Group 2; <sup>d</sup>Significant different at  $P < 0.05$  compared to Group 4; NS=No significant difference at  $P < 0.05$  compared to Group 1; RV=*Rauvolfia vomitoria*; GL=*Gongronema latifolium*; SD=Standard deviation

weights during the experimental period for the RV, GL, and RV + GL groups were 4.50%, 3.88%, and 3.28%, respectively, compared with the control group (28.57%) [Table 2].

There was no difference in the whole brain weight of all the test groups compared with the control group. The mean cellular population in sections of the cerebral cortex were significantly ( $P < 0.05$ ) higher in the RV and GL groups, but not the RV + GL group compared with the control. However, the cellular population of the RV + GL was significantly ( $P < 0.05$ ) lower than the RV and GL groups [Table 3].

### Histomorphological study

The histological section of the cerebral cortex of the mice of the control group showed six cortical layers; from the superficial inwards were marginal zone, cortical plate, subcortical plate, intermediate, subventricular, and the ventricular zones. The marginal zone consisted mostly of fibers with sparse cell density. The cortical plate showed much cell density. The subcortical plate had less cellular density. The intermediate, subventricular, and the ventricular zones were not distinguishable, with the layers showing numerous cells [Figure 2].

In the group administered RV alone, the histological section of the cerebral cortex showed more cellular density, and smaller cellular sizes compared with the control group. Every other area of the section appeared like the control [Figure 3]. In the group administered GL alone, the histological section of the cerebral cortex also showed much cellular density and smaller cellular sizes in all the cortical layers compared with the control group. Every other area of the section appeared like the control [Figure 4]. In the group administered the combination of RV and GL (RV + GL), the histological section of the cerebral cortex showed much cellular density as well, but smaller cellular sizes in the intermediate zones through to the ventricular zones. However, the cortical cells were slightly larger compared with the control group [Figure 5].

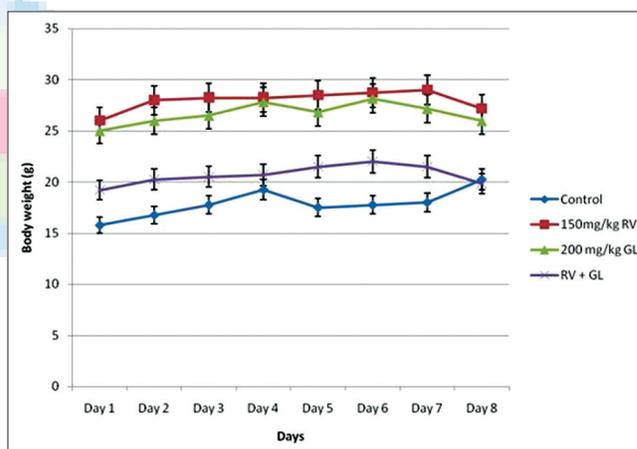
### DISCUSSION

Most alternative medicine practitioners use herbs without taking into consideration the potent adverse effects that

**Table 3: Whole brain weight and cellular population of the cerebral cortex of the mice**

Groups (n=6) (mg/kg)	Whole brain weight (g)	Mean cellular population
Group 1 (control)	0.40±0.00 NS	1725±35.36
Group 2 (150, RV)	0.48±0.02 NS	1941±40.90*
Group 3 (200, GL)	0.42±0.02 NS	1875±57.01* <sup>b</sup>
Group 4 (150, RV and 200, GL)	0.40±0.06 NS	1779±59.13 <sup>b</sup>

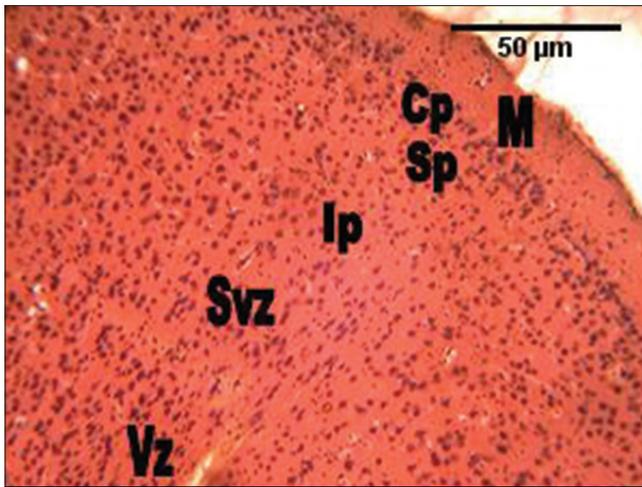
Data is presented as mean±SD. \*Significant different at  $P < 0.05$  compared to Group 1; <sup>b</sup>Significant different at  $P < 0.05$  compared to Group 4. RV=*Rauvolfia vomitoria*; GL=*Gongronema latifolium*; SD=Standard deviation; NS=No significant



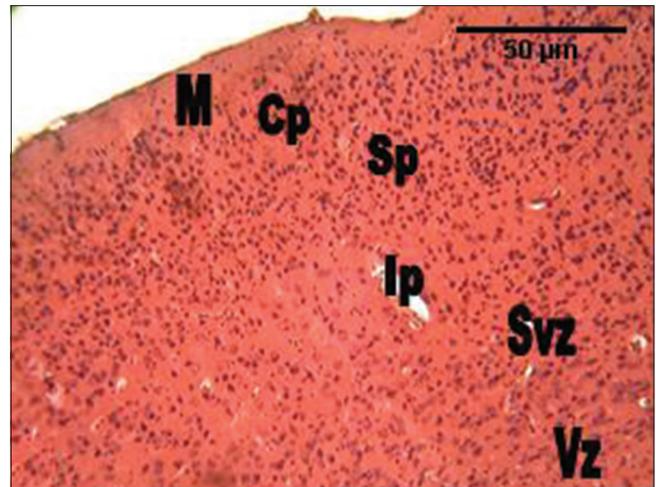
**Figure 1: Daily body weights of the mice**

may result from such. RV has been reported to have adverse effects added to its useful properties.<sup>[17-19]</sup> As such, the combination with other equally useful herbs may modulate the adverse effects while still maintaining its useful abilities;<sup>[19]</sup> thus, the use of RV in combination with GL in this study.

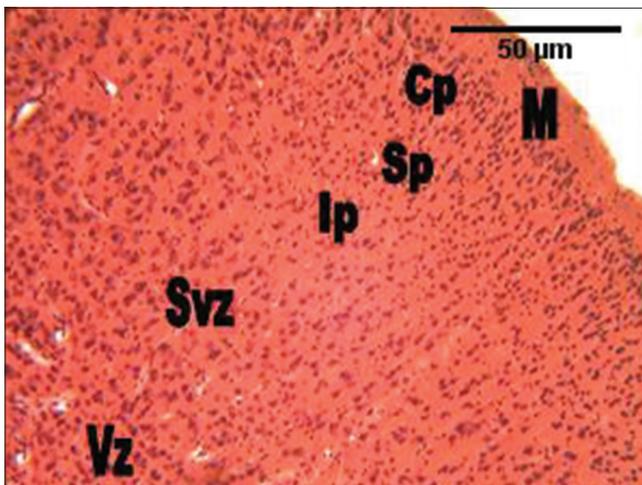
All the groups gained body weight in the course of the experiment. However, there was a loss in body weights in the test group animals from day 6 of the experiment. The gain in body weight was lower in the test groups compared with the control group. There are reports that individual administration of RV and GL result in body weight loss in rats.<sup>[18,19,22,30,31]</sup> However, no difference in body weights was reported when mice were administered same.<sup>[20,22]</sup> The



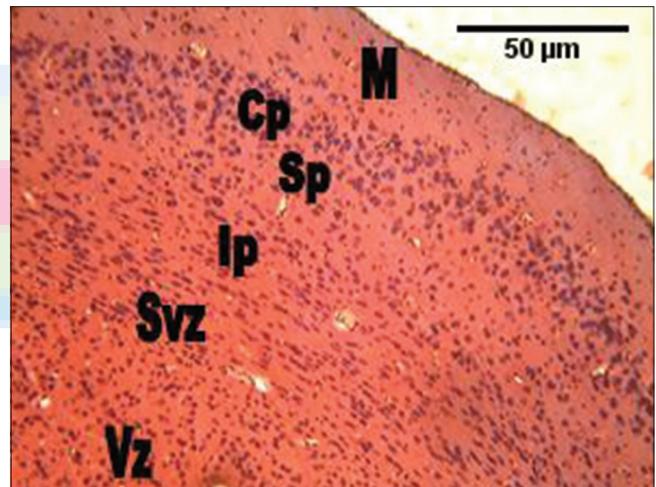
**Figure 2:** The photomicrograph of the cerebral cortex of the control group showing the marginal zone containing mostly processes, with sparse cell density. The cortical plate shows much cell density. The subcortical plate has less cellular density. The intermediate, subventricular and the ventricular zones are not distinguishable, with the layers showing numerous cellular densities. M = Marginal zone; Cp = Cortical plate; Sp = Subcortical plate; Ip = Intermediate; Svz = Sub-ventricular; and the Vz = Ventricular zones (H and E, ×100)



**Figure 3:** The photomicrograph of the cerebral cortex of the group administered *Rauvolfia vomitoria* (RV) alone showing more cellular density, and smaller cellular sizes compared with the control group. M = Marginal zone; Cp = Cortical plate; Sp = Subcortical plate; Ip = Intermediate; Svz = Sub-ventricular; and the Vz = Ventricular zones (H and E, ×100)



**Figure 4:** The photomicrograph of the cerebral cortex of the group administered *Gongronema latifolium* alone showing much cellular density and smaller cellular sizes in all the cortical layers compared with the control group. M = Marginal zone; Cp = Cortical plate; Sp = Subcortical plate; Ip = Intermediate; Svz = Subventricular; and the Vz = Ventricular zones (H and E, ×100)



**Figure 5:** The photomicrograph of the cerebral cortex of the group administered the combination of *Rauvolfia vomitoria* and *Gongronema latifolium* (*Rauvolfia vomitoria* + *Gongronema latifolium*) showing much cellular density, but smaller cellular sizes in the intermediate zones through to the ventricular zones. However, the cortical cells were slightly larger compared with the control group. M = Marginal zone; Cp = Cortical plate; Sp = Subcortical plate; Ip = Intermediate; Svz = Sub-ventricular; and the Vz = Ventricular zones (H and E, ×100)

present study may be due to the administered dose and the specie of the animal and is at variance with previous reports.

GL was previously reported as a traditional tool in the control of body weight gain by lactating women.<sup>[32]</sup> While groups administered either RV or GL alone or their combination did show body weight gain, it appeared controlled compared with the control group. These results further buttress that RV and GL may be useful in body weight gain control.

Morphometric studies showed no difference in whole brain weight in all the experimental groups, an indication that the size of the brains of the animals was not affected by the administered substances which are in line with previous studies.<sup>[20,21]</sup> Histomorphological and morphometrical study of the cerebral cortex showed higher cellular density, and smaller cellular sizes in the RV and GL groups though the RV + GL group cellular density was however not significant, but showed much cellular, but smaller cellular sizes in the intermediate zones through to the ventricular zones, and

the cortical cells were slightly larger compared with the control group.

The histomorphology result of this study indicates that the extracts either singly or in combination have the potential to stimulate cellular proliferation in the cerebral cortex. The reported cellular proliferation may be due to either gliosis and/or neurogenesis. Gliosis usually result when the brain is traumatized by chemical agents and/or infections,<sup>[33,34]</sup> as in this case, the administered substances. It is reported that reserpine, which is the most abundant and most active alkaloid in RV have both depressive and antidepressant effects on the nervous system.<sup>[35,36]</sup> Antidepressants have been reported to increase neurogenesis in the adult rodent hippocampus.<sup>[37-39]</sup> It is possible that neurogenesis may be stimulated as well in the cerebral cortex in this study, as previous studies have also shown that neurogenesis occurs in the cerebellum of rabbits.<sup>[40,41]</sup>

The cerebral cortex is the outer layer of mammalian cerebrum and plays a major role in memory, attention, perception, awareness, thought, language, and consciousness.<sup>[42]</sup> The morphological changes in the cerebral cortex as reported in this study may be due to plasticity usually associated with morphological changes in neuronal distribution.<sup>[43]</sup> While this morphological changes may be beneficial for normal function and for compensatory recovery,<sup>[44]</sup> it may also be deleterious,<sup>[45]</sup> resulting in the inhibition of the processing abilities of the cortex.

## CONCLUSION

This study showed that RV root bark and GL leaf extracts either singly or in combination may stimulate cellular proliferation at the given dose, which may serve either a protective or deleterious role.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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