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Co-Treatment of Rauwolfia Vomitoria and Gongronema Latifolium Affects Cerebellar Cell Population in Mice

Moses B Ekong, Aniekan Imo Peter

Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria.

***Corresponding Author:** Moses B Ekong, Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria.*

ABSTRACT

Rauwolfia vomitoria (RV) and Gongronema latifolium (GL) are medicinal plants. While RV is reported to possess adverse neural effects, GL has shown the potential to address these effects. This study therefore investigated the effects of co-treatment of RV and GL on the neurobehavior and histology of the cerebellar cortex of female mice. Twenty female albino mice were divided into 4 groups (n=6) administered 0.4 ml of 20% Tween, 150 mg/kg RV, 200 mg/kg GL and a combination of 150 mg/kg RV and 200 mg/kg GL (RV+GL) orally for seven days. On day 8 the animals were sacrificed and their brains excised and routinely processed using haematoxylin and eosin method. There were no differences in body and brain weights. Cerebellar cortical cyto-architectures were not affected, but cellular population was significantly ($p<0.05$) higher in the RV+GL, but not the RV and GL groups compared with the control group. In conclusion, RV+GL combination stimulated cerebellar cortical cellular increase which may affect the normal functions of the cerebellum.

Keywords: *Rauwolfia vomitoria, Gongronema latifolium, Cerebellum, body weight, brain weight;*

INTRODUCTION

The use of herbs for treatment of disease is popular in developing countries for diverse reasons (Vaughn, 2006; Nwangwu et al., 2009). These herbs which were the main stay of these societies have come handy presently due to the rising cost of orthodox drugs necessary for the management of disease conditions (Hoareau and DaSilva, 1999). Due to this increased use of herbal medication, so much research is ongoing into the pharmacological expunge activities, as well as their active constituents that influence biological processes and reverse disease state (Ugochukwu et al., 2003).

One such important medicinal plant is RV which belongs to the family *Apocynaceae* (Burkill, 1994). RV, commonly known as African serpent wood, and African snake root or swizzle stick is also called asofeyeje in Yoruba, ira in Igbo, wadda in Hausa, and eto mmoneba or utoenyin respectively in Efik and Ibibio local Nigerian languages (Mecha et al., 1980; Ehiagbonare, 2004).Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols, and reducing sugars (Akpanabiatu, 2006), with the active alkaloids including; rauwolfine, reserpine, rescinnamine, serpentine, ajmaline serpentinine, steroid-serposterol and saponin (Gill 1992).

Locally, RV is used in the management of ailments such as; mental disorder, hypertension, dysentery, jaundice, cerebral cramps and gastrointestinal disorders (Perry and Metzger 1980; Kutalek and Prinz, 2007). Research reports have showed that the RV has antioxidant, antipyretic, antiglycemic, anticonvulsant, analgesic, antipsychotic, and sedative properties among others (Amole et al., 2006; Akpanabiatu et al., 2009; Amole et al., 2009; Eluwa et al, 2009; Bisong et al, 2010 Bisong et al, 2011; Bisong et al, 2012). Adverse effects associated with this plant includes psychotic depression, poor co-ordination, dizziness, impairment of physical abilities, weight loss, hallucination and decreased heart rate and blood pressure (Vaughn, 2006).

Due to these adverse effects of RV, Ekong et al (2014), Ekong et al (2015), Ekong et al (2016), reported that the combination with an equally active plant, GL may ameliorate these adverse reports while still maintaining its usefulness. However, this combination effect on major decision centres of the brain is limited, and thus warranted this combination research with GL. GL belongs to the family, *Asclepiadaceae*, with common names as amaranth globe or bush buck, and is also known as utazi in Igbo, utasi in Efik and Ibibio, and arokeke in Yoruba languages of Nigeria (Edet et al., 2009). Phytochemical analysis of *G. latifolium* showed the presence of polyphenols, saponins, tannins, alkaloids, flavonoids, anthraquinones, cyanogenetic glycoside, glycides and hydroxymethyl anthra quinones (Alobi et al., 2012; Odo et al., 2013). The plant is used traditionally for the management of diseases such as diabetes and high blood pressure (Ugochukwu et al., 2003), for the control of body weight in lactating women and in promoting their fertility as well (Schneider et al., 2003). GL is also useful nutritionally as spice and edible vegetable for maintaining blood glucose level (Morebise et al., 2002; Nwangwu et al., 2009). Report have shown that *G. latifolium* has anti-oxidant, antimicrobial, analgesic, anti malarial, anti diabetic and anti-ulcer properties (Akuodo et al., 2010; Nwinyi et al., 2008; Atawodi, 2005; Edet et al., 2009; Odo et al, 2013). As both *R. vomitoria* and *G. latifolium* have closely related properties, this study investigated their combine effects on the histomorphology of the cerebellum of female mice.

Materials and Methods

Twenty, three months old female albino mice weighing 18 - 23 g were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo, Uyo Nigeria. The animals were house in plastic cages with wire gauze roof and saw particles as bedding. The room temperature was between 26 - 29 ^{o}C, and 12:12 hours light and dark cycle was maintained throughout the duration of the experiment. The animals were handled according to International Guidelines for the regulation of laboratory animals, and were allowed to acclimatize for fourteen days before commencement of the experiment. They were fed with normal commercial pellet (Vital Feed Ground Cereal Ltd, Jos, Nigeria) and clean water *ad libitum* throughout the duration of the experiment.

Fresh leaves and roots of GL and RV respectively, were harvested from local farms in Ika and Esit Eket Local Government Areas respectively, of Akwa Ibom State in Nigeria. The plants were washed of dirt and air dried for one week, and were pulverized using a manually operated blender. 200 g of the leave powder of GL and 200 g of root powder of RV were extracted in 70 - 95 % as described by Ugochukwu et al. (2003). The extracts were concentrated using rotary evaporator and the concentrate was dried in a Plus 11 Gallenkamp oven at 45-50 ^{o}C. The dry extracts were refrigerated at 4 ^{o}C until use. Two grams each of the extracts were re-suspended in 20 mL of 20% Tween solution and the appropriate doses were calculated.

Experimental Protocol

The mice were divided into four groups of five mice each; the control that received 0.4 ml of 20 % Tween, and groups 150 mg/kg RV (RV), 200 mg/kg GL (GL) and a combination of 150 mg/kg RV and 200 mg/kg GL (RV+GL). The treatment which was for seven days was by oral gavages (Table 1). The body weights of the animals were taken prior and everyday till the end of the experiment.

On the 8th day, the animals were sacrificed after anaesthetizing with chloroform. The brains were collected by dissecting the skull, weighed and preserved in 10 % buffered formalin. They were further routinely processed for histological study using the haematoxylin and eosin staining method. Sections were viewed under the light microscope and photomicrographs were obtained using the microscope camera linked to a computer. Cellular population was determined by the Image J™ software.

Statistical Analysis

One way analysis of variance was used to compare the means for all group's activities, thereafter student Newman-Keul post-hoc test was carried out to find the level of significance at $p<0.05$. All the results were expressed as mean ± standard error of mean.

Table1. *Schedule of treatments of animals in control and treatment groups*

Group (n=5)	Treatment	Duration of treatment (days)
Control	0.4 mL of 20 % Tween	7
RV	150 mg/kg of *R. vomitoria*	7
GL	200 mg/kg of *G. latifolium*	7
RV+GL	150 mg/kg of *R. vomitoria* and 200 mg/kg of *G. latifolium*	7

RESULTS

Body Weight

There was a significant ($p < 0.05$) lower daily body weight observed in the group treated with 200 mg/kg per body weight of GL extract on days 6 and 7, compared with the control and the other test groups (Fig. 1). At the end of the experiment, the RV, Gl and RV+GL groups showed body weight increase; 1.22 %, 0.11 %, and 2.89 %, respectively, compared with the control group with body weight loss (0.27 %). There was no difference in the final day body weights, as well as the brain weights of the test groups compared with the control (Table 2). No difference existed in the brain-body weight ratio, which was 0.01 in all the experimental groups.

Histomorphological Observation

The histological section of the cerebellar cortex of the mice in the control group showed three layers; from the superficial to the deep surface were molecular layer, Purkinje layer and the granular layer, with the medulla being deep to it. The molecular layer had sparsely distributed cells. The Purkinje layer was a single layer of large Purkinje cells. The granular layer consisted of a large density of small granular cells interspaced with Glomeruli Island. Deep to the granular layer was the inner medulla having few sparse cells (Figure 2).

In the RV group, the cerebellar cortex showed slight dense cell population, although they appear unaffected compared with the control group (Figure 3). In the GL group, the cerebellar cortex showed less dense cell population compared with the control group (Figure 4). In the RV+GL group, the cerebellar cortex showed a dense population of cells, with slight neuronal size reduction compared with the control group (Figure 5).

The cell population estimation of the cerebellar cortex sectional area of 5733.81 μm^2 was significantly ($p<0.05$) higher in the RV+GL group compared with the control, RV and GL groups. The cerebellar cortical cell population of the RV group was also significantly ($p<0.05$) higher than the GL group. No difference was observed between the control and the RV and GL groups (Table 3).

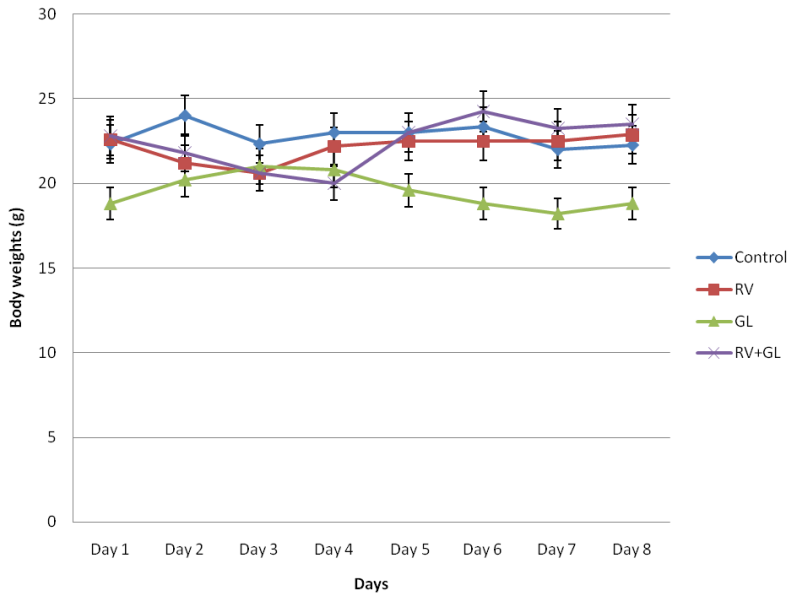

Figure1. *Daily body weight measure of the mice in all groups*

Table2. *Body and brain weights of the mice in all the groups*

Groups (n=5)	Body weight (g) F=3.39,P=0.054	Brain weight (g) F=0.67, P=0.578
Control	22.27±0.38	0.47±0.03
B (150 mg/kg of RV)	22.88±0.91^{NS}	0.40±0.04^{NS}
C (200 mg/kg of GL)	18.82±1.61^{NS}	0.46±0.02^{NS}
D (150 mg/kg of RV & 200 mg/kg of GL)	23.48±0.85^{NS}	0.43±0.05^{NS}

Data is presented as mean ± standard error of mean

NS - No significantly different at p<0.05 compared to the control group, RV- R. vomitoria, GL-G. latifolium

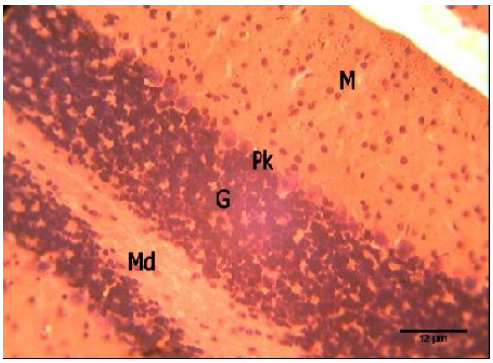

Figure2. *The section of the cerebellum of the control group showed its three cortical and medulla layers.*

The layers from superficial to the deep were; M= molecular layer, Pk= Purkinje and Gr=granular plate. H & E. Mag. x400

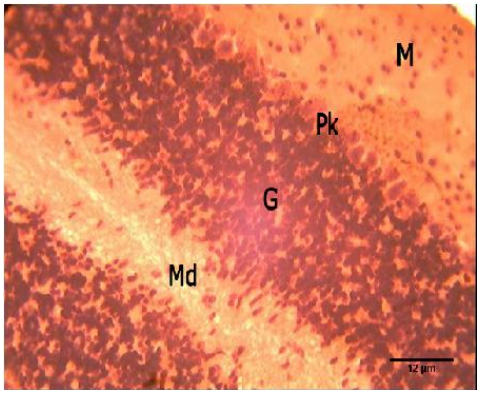

Figure3. *The histological section of the cerebellum of mice that received 150 mg/kg of root bark extract of R. vomitoria, showed. H & E. Mag. x400.*

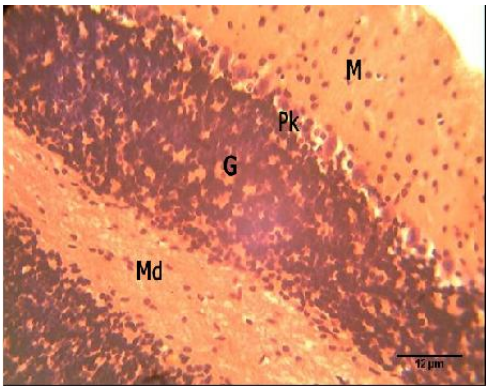

Figure4. *In this section of the cerebellum of mice that received 200mg/kg of leaf extract of G. latifolium, showed. H & E. Mag. x400.*

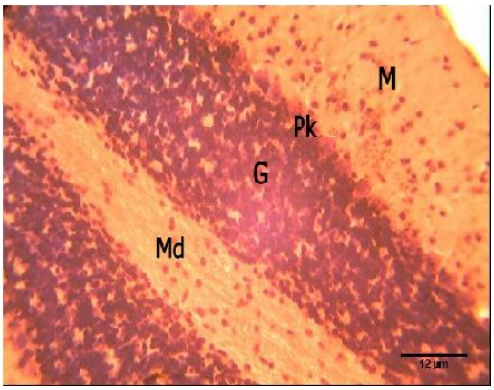

Figure5. *In this section of the cerebellum of mice that received a combination of 150 mg/kg of root bark extract of R. vomitoria and 200 mg/kg leaf extract of G. latifolium, showed. H & E. Mag. x400*

Table3. *Cellular population of the cerebellar cortex of mice in all groups*

Groups (n=5)	Cellular Population P<0.0001 F=13.28
Control	2442 ± 14.71
RV	2474 ± 23.44^{d}
GL	2403 ± 6.395^{b,d}
RV + GL	2534 ± 10.83**

Data is presented as mean ± standard error of mean

** - *Significant different at p<0.001 compared to Control group*

b - *Significant different at p<0.001 compared to RV group*

d - *Significant different at p<0.001 compared to RV+GL group*

RV - *R. vomitoriaGL* - *G. latifolium*

Discussion

This study investigated the effects of co-treatment of RV and GL on cerebellar neurohistology in female mice. In this study, there was no difference in body weight in the test groups compared with the control group, an indication that the administered extracts may not have effect on body weight. Body weight is an important index in the determination of well being (CMAJ, 1926), and in the present study, the animals may be said to be healthy. The present results are similar to Ekong et al (2014) and Ekong et al (2015b) who reported no difference in body weight. However, the present result is at variance with other studies that reported body weight loss in rats upon administration or GL and RV (Ezeonwu, 2013; Ekong et al, 2015a). This difference may be due to the specie, as well as the sex of the animals, as males and females have been reported to differ in their pharmacokinetics and pharmaco dynamics of drugs (Soldin and Mattison, 2009; Soldin et al, 2011).

There was no difference in the brain weights of the test groups compared with the control, as well as in the brain-body weight ratios, which indicate that the brain morphology and the organosomatic index were not affected, further supporting the body weight results. The present results are similar to previous reports (Ekong et al, 2014; Ekong et al, 2015b). These results also indicate that *R. vomitoria* and *G. latifolium* may be useful in controlling body weight gain. The result of this study corroborates a previous report that *G. latifolium* is used traditionally in the control of body weight gain in lactating women (Schneider et al. 2003).

Histomorphological and morphometrical study of the cerebella sections showed no apparent adverse effect of the extracts in all the test groups compared with the control group. However, there was increased cell population in the RV+GL group compared with the control and the RV and GL groups. It is reported that cellular population change affects cognitive abilities either positively or negatively (Seigers et al 2008; Wang et al, 2013), which the RV and GL combination may influence. But reports by Ekong et al. (2014) and Ekong et al. (2016) did not showed difference in cognition despite cellular increase. The present results is at variance with previous reports of RV and GL on the cerebral cortices and cerebella were adverse histomorphologies of these brain areas were observed (Eluwa et al. 2009; Ekong et al. 2017). Cerebellar cortical cellular increase in the RV+GL group may be due to gliosis, which usually result when the brain is traumatized by chemical agents (Roumier et al. 2008). However, neurogenesis may not be ruled out if the combination herbs act as an antidepressant, which have been reported to increase neurogenesis in the adult rodent brain (Sairanen et al. 2005; Acosta et al., 2010), and Ponti et al (2008) had reported that cerebellar neurogenesis is possible in rabbits (Ponti et al. 2006; Ponti et al. 2008), which may also occur in mice.

The cerebellum maintains balance of posture and co-ordinates the timing and force of muscle groups to produce fluid limb or body movements. Reports have also shown that the cerebellum is important for learning, and is also involved in certain cognitive functions (Doya 2000; Timmann and Daum 2007). The cerebellar changes as reported in this study may or may not affect the functions of the cerebellum.

In conclusion, dosages of RV and GL administered singly did not affect the female mice body weights and cerebellar cortical histomorphology and cell density. However, their combination stimulated cerebellar cortical cellular increase which may or may not affect the normal functions of the cerebellum.

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