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Original Work

Effects of co-treatment of *Rauwolfia vomitoria* and *Gongronema latifolium* on neurobehaviour and the neurohistology of the cerebral cortex in mice

Moses Bassey EkongΨ†, Ubong Udo Ekpene‡, Fyneway Enang Thompson†, Aniekan Imo Peter†, Nsikan-Abasi Bassey Udoh†, Gabriel Joseph Ekandem†

†Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria
‡Department of Surgery, University of Uyo Teaching Hospital, Uyo, Nigeria

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ABSTRACT: *Rauwolfia vomitoria* and *Gongronema latifolium* are medicinal plants with antioxidant, antidiabetic and analgesic properties among others. *R. vomitoria* is reported to possess adverse neural effects, which *G. latifolium* has shown the potential to address. This study therefore investigated the effects of co-treatment of *R. vomitoria* and *G. latifolium* on the neurobehaviour and histology of the cerebral cortex of female mice. Twenty female Wistar mice were divided into 4 groups (A, B, C and D). Group A designated as the control received 0.4 mL of 20 % Tween, while groups B, C and D received oral doses of 150 mg/kg of *R. vomitoria* (RV), 200 mg/kg of *G. latifolium* (GL) and a combination of 150 mg/kg of *R. vomitoria* and 200 mg/kg of *G. latifolium* (RV+GL), respectively for seven days. Light and dark field behaviour test was carried out on day 8 and the animals were immediately sacrificed. Their brains were excised and routinely processed by haematoxylin and eosin method. There was no difference in body and brain weights, and the behavioural parameters. Cellular cyto-architecture showed higher glial population with no apparent histopathology. The cellular population was higher ($p<0.0001$) in the RV and RV+GL groups, while the GL group was less ($p<0.0001$) populated all compared to the control.In conclusion, the reported treatment regimes, RV administered singly and in combination with GL may not affect some neurobehavioural activities, but may result in cellular increase in the cerebral cortex.

KEY WORDS: ***Rauwolfia vomitoria; Gongronema latifolium; Cerebral cortex; Mice***

INTRODUCTION

Medicinal plants are health aids that have been in use for a long time in developing countries[1]. The use of these herbs for treatment of diseases is popular in developing countries for historical and cultural reasons[2,3], and is more economical because of the rising cost of orthodox drugs in the maintenance of personal health and well-being[1]. Herbal medicine has allowed for research into pharmacological activities of plants and their metabolites that influence biological processes and reverse disease states[4]. Unfortunately, a number of these herbs have shown adverse effects.

One such important medicinal plant is *Rauwolfia vomitoria* that belongs to the family *Apocynaceae*[5]. *R. vomitoria* has common names such as African serpent wood, and African snakeroot or swizzle stick. In local Nigerian languages, it is called *asofeyeje* in Yoruba, *ira* in Igbo, *wadda* in Hausa, and *eto mmoneba* or *utoenyin* in Efik and Ibibio, respectively[6]. Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols, and reducing sugars[7]. The active alkaloids of *R. vomitoria* include rauwolfine, reserpine, rescinnamine, serpentine, ajmaline serpentinine, steroid-serposterol and saponin[8].

Traditionally, *R. vomitoria* is used to manage ailments such as mental disorder, hypertension,

Ψ**Correspondence at:** Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria; Phone: +2348030868505; Email: mbe_flashpoint@yahoo.com

dysentery, jaundice, cerebral cramps and gastrointestinal disorders[9]. Research reports showed that the plant has antioxidant, antipyretic, antiglycemic, anticonvulsant, analgesic, antipsychotic, and sedative properties among others[10-13]. 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[3].

Due to these reported adverse effects of *R. vomitoria*, the present study considered the combined effects with another plant, *Gongronema latifolium*. *G.latifolium* belongs to the family, *Asclepiadaceae*, with common names as amaranth globe or bush buck. It is also known as *utazi* in Igbo, *utasi* in Efik and Ibibio, and *arokeke* in Yoruba languages of Nigeria[14]. Phytochemical analysis of *G. latifolium* showed the presence of polyphenols, saponins, tannins, alkaloids, flavonoids, anthraquinones, cyanogenetic glycoside, glycides, and hydroxymethyl anthraquinone[15]. The plant is used traditionally for the management of diseases such as diabetes and high blood pressure[4], for the control of body weight in lactating women and in promoting their fertility as well[16]. The plant is also useful nutritionally as spice and edible vegetable for maintaining blood glucose level[2]. Reports have shown that *G. latifolium* has anti-oxidant, antimicrobial, analgesic, antimalarial, antidiabetic, anti-ulcer properties[14,15]. As both *R. vomitoria* and *G. latifolium* have closely related properties, this study investigated their combined effects on neurobehavioural activities and the histomorphology of the cerebral cortex of female mice.

METHODOLOGY

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. Ethical approval was obtained from the Ethics Committee of the University of Uyo, and the animals were handled according to international guidelines as laid down by the National Institute of Health (NIH) of the United States of America for the regulation of laboratory animals. They were housed 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 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 *G. latifolium* and *R. vomitoria*, 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 leaf powder of *G. latifolium* and 200 g of root powder of *R. vomitoria* were extracted respectively in 70 - 95 % alcohol as described by Ugochukwu et al[4]. The extracts were concentrated using rotary evaporator and the concentrates were dried in a Plus 11 Gallenkamp oven at 45-50 ºC. 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: A, B, C and D, of five mice each. Group A was the control and received 0.4 mL of 20 % Tween, while groups B, C and D were the treatment groups and received respectively 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*. 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.

Table 1: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
Group B	150 mg/kg of *R. vomitoria*	7
Group C	200 mg/kg of *G. latifolium*	7
Group D	150 mg/kg of *R. vomitoria* and 200 mg/kg of *G. latifolium*	7

Neurobehaviour test

Light and dark neurobehaviour test was carried out on the eighth day. Briefly, the apparatus used for the light and dark field test was constructed of white plywood. The maze was a rectangular box with open roof of 45 x 27 cm. It was divided into a small (18 x 27 cm) and large (27 x 27 cm), chambers by a flat board, with an opening (7.5 x

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7.5 cm) at the floor level linking the two chambers. The small chamber was painted black, with the large chamber painted white. The floor was covered by Plexiglas and the large chamber was divided into nine squares (9 x 9 cm) by blue lines, while the floor of the small chamber was divided into six squares (9 x 9 cm) by white lines[17](Costall*et al*., 1993). The maze was located in a 1.8 x 4.6 cm test room lit by a 60-Watt red lamp for background lighting.

The mice were carried to the test room in home cages an hour before the test, and were handled by the base of their tails at all times. Each mouse was placed in the proximal right-hand corner of the large chamber and allowed to explore the apparatus for five minutes. After the five-minute test, the mouse was returned to its home cage and the maze was cleaned with 70% ethyl alcohol and allowed to dry before the introduction of the next mouse. Behaviour was scored manually, and each trial was recorded for subsequent analysis using a video camera positioned above the apparatus. The counting was also done manually. The parameters scored included; line crossing (ambulatory activities), rearing, grooming, transition frequency, duration spent in the light and dark chambers respectively, defecation and urination.

Tissue processing

Immediately after the neurobehaviour test 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 by 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 ImageJ™ software.

Statistical analysis

One way analysis of variance (ANOVA) was used to compare the means for all groups' 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.

RESULT

Anthropometry study

There was a significant ($p<0.05$) lower daily body weight observed in the group treated with 200 mg/kg per body weight of *G. latifolium* extract on days 6 and 7, compared with the control group, and the other treatment groups administered 150 mg/kg extract of *R. vomitoria* and a combination of 150 mg/kg extract of *R. vomitoria* and 200 mg/kg extract of *G. latifolium* per body weight respectively (**Figure 1**).

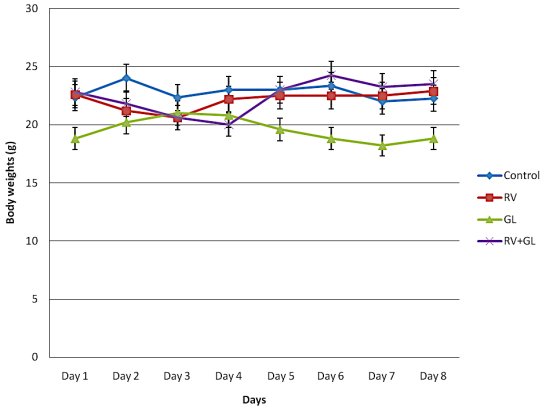

Figure 1: Daily body weight measure of the mice in all groups

At the end of the experiment, the groups treated with *R. vomitoria* alone, *G. latifolium* alone and the combined *R. vomitoria* and *G. latifolium* showed body weight increase (1.22 %, 0.11 %, and 2.89 %, respectively) compared with the control group which had body weight loss (0.27 %). There was no difference in the final day body weights, as well as the brain weights of the groups treated with *R. vomitoria* alone, *G. latifolium* alone and the combined *R. vomitoria* and *G. latifolium* compared with the control (**Table 2**). No difference existed in the brain-body weight ratio, which was 0.01 in all the experimental groups.

Table 2: 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'; NS-No significant difference at $p<0.05$ compared to the control group; RV- *R. vomitoria*; GL-*G. latifolium*

Neurobehaviour study

There was no difference in line crossing, transition frequency, grooming, duration in light and dark

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chambers, defecation and urination in the groups treated with *R. vomitoria* alone, *G. latifolium* alone and the combined *R. vomitoria* and *G. latifolium* compared with the control group (**Table 3**).

Table 3: Light dark field behavioural test of the mice in all groups

Groups (n=5)	Line crossing F=0.86 p=0.491	Transition F=1.04 P=0.411	Grooming F=1.67 P=0.226	Rearing F=0.82 P=0.506	Duration in light (min) F=0.96 P=0.442	Duration in Dark (min) F=1.00 P=0.426	Defecation F=0.45 P=0.723
A (Control)	63.67 ±1.76	15.67 ±2.33	3.67 ±0.33	36.00 ±1.00	3.36 ±0.07	2.24 ±0.07	2.33 ±0.67
B (RV)	68.50 ±4.17^{NS}	19.00 ±1.15^{NS}	4.00 ±0.58^{NS}	31.00 ±1.08^{NS}	2.08 ±0.32^{NS}	2.52 ±0.32^{NS}	1.00 ±0.41^{NS}
C (GL)	73.20 ±7.45^{NS}	17.20 ±2.87^{NS}	7.20 ±1.83^{NS}	33.80 ±2.63^{NS}	1.61 ±0.34^{NS}	3.00 ±0.34^{NS}	1.60 ±0.68^{NS}
D (RV+GL)	76.00 ±3.08^{NS}	21.00 ±0.91^{NS}	5.75 ±0.85^{NS}	35.25 ±2.95^{NS}	1.75 ±0.19^{NS}	2.85 ±0.19^{NS}	2.00 ±1.22^{NS}

Data is presented as 'mean±standard error'; NS-No significant difference at $p<0.05$ compared to the control group; RV- *R. vomitoria*; GL-*G. latifolium*

Histomorphological study

The histological section of the cerebral cortex of the mice of the control group showed six cortical layers; from the superficial to the deep surface were marginal zone, cortical plate, sub-plate, intermediate zone, sub-ventricular zone and the ventricular zone. The marginal zone consisted mostly of nerve fibres with sparsely populated neurons and glia. The cortical plate showed numerous pyramidal neurons and glia. The sub-plate showed less number of pyramidal and more granular neurons, as well as glia. The intermediate, sub-ventricular and the ventricular zones were indistinguishable, with the layers showing numerous neurons and glia (**Figure 2**).

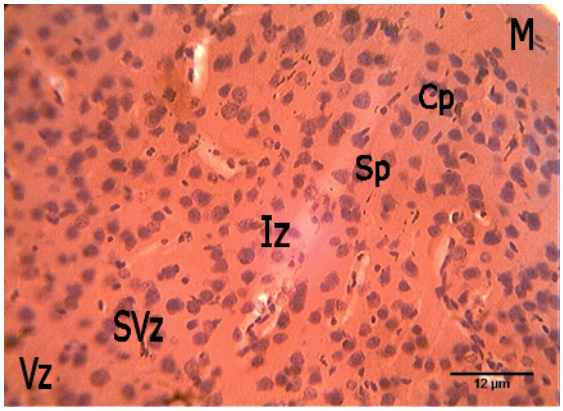

Figure 2: The section of the cerebral cortex of the control group showed six cortical layers. The layers from superficial to the deep were; M= marginal zone, Cp= cortical plate, Sp= sub-plate, Iz= intermediate zone, SVz= sub-ventricular zone and the Vz= ventricular zone. H & E. Mag. x400

In the group treated with *R. vomitoria* alone, the histological section of the cerebral cortex showed more glial density, with the neurons appearing unaffected compared with the control group (**Figure 3**). In the group treated with *G. latifolium* alone, the histological section of the cerebral cortex showed a lesser glial density, but the pyramidal and granular neurons appeared slightly reduced in size in all the cortical layers compared with the control group (**Figure 4**). In the group treated with a combination of *R. vomitoria* and *G. latifolium*, the histological section of the cerebral cortex showed a higher cellular population density, with slight neuronal size reduction compared with the control group (**Figure 5**).

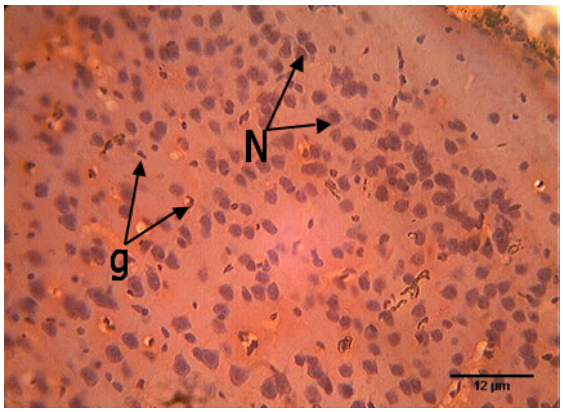

Figure 3: The histological section of the cerebral cortex of mice that received 150 mg/kg of root bark extract of R. vomitoria, showed a denser population of glia (g), with the pyramidal neurons (N) appearing unaffected compared with the control group. The layers from superficial to the deep were; M= marginal zone, Cp= cortical plate, Sp= sub-plate, Iz= intermediate zone, SVz= sub-ventricular zone and the Vz= ventricular zone. H & E. Mag. x400

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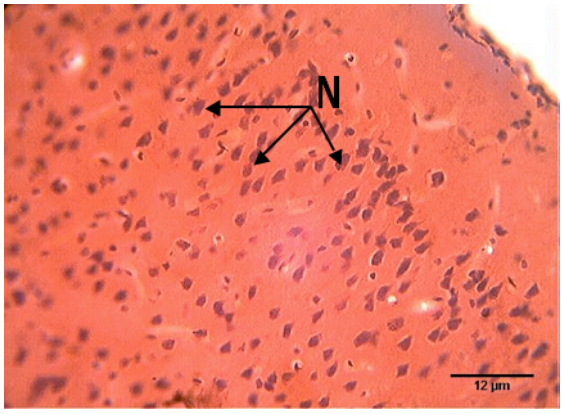

Figure 4: In this section of the cerebral cortex of mice that received 200mg/kg of leaf extract of G. latifolium, showed a less dense population of glia, but the pyramidal and granular neurons (N) appeared slight reduced in size in all the cortical layers compared with the control group. The layers from superficial to the deep were; M= marginal zone, Cp= cortical plate, Sp= sub-plate, Iz= intermediate zone, SVz= sub-ventricular zone and the Vz= ventricular zone. H & E. Mag. x400.

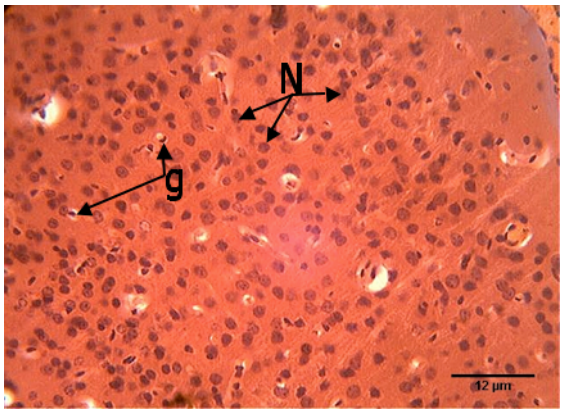

Figure 5:In this section of the cerebral cortex of mice that received a combination of 150 mg/kg of root bark extract of *R. vomitoria* and 200 mg/kg leaf extract of *G. latifolium*, showed a higher cellular population density, with slight neuronal size reduction compared with the control group. The layers from superficial to the deep were; M= marginal zone, Cp= cortical plate, Sp= sub-plate, Iz= intermediate zone, SVz= sub-ventricular zone and the Vz= ventricular zone. H & E. Mag. x400.

Stereological estimation of the cerebral cortical section area of 4673.76 µm^2 showed a significantly ($p<0.0001$) higher cellular population in the groups treated with *R. vomitoria* alone, and the combined *R. vomitoria* and *G. latifolium*, while the group treated with *G. latifolium* alone, the cellular population was significantly ($p<0.0001$) lower compared with the control group. The group treated with combination of *R. vomitoria* and *G. latifolium* had a significantly ($p<0.0001$) higher cellular population compared with the groups treated with *R. vomitoria* alone, and *G. latifolium*, while the group treated with *G. latifolium* alone had a significantly ($p<0.0001$) lower cellular population compared with the group treated with *R. vomitoria* alone (**Table 4**).

Table 4:Cellular population of the cerebral cortex of mice in all groups

Groups (n=5)	Cellular population P<0.0001 F=10688
Control	3631±6.65
B (150 mg/kg of RV)	4024±8.44***,c,d
C (200 mg/kg of GL)	3159±13.42***,d
D (150 mg/kg of RV & 200 mg/kg of GL)	5473±8.82***

Data is presented as mean ± standard error of mean ***Significant difference at $p<0.001$ compared to Control group; c - Significant difference at $p<0.001$ compared to group C; d - Significant difference at $p<0.001$ compared to group D; RV-*R. vomitoria*; GL - *G. latifolium*

DISCUSSION

The most potent alkaloids of the root bark extract of *R. vomitoria*, reserpine has been found to have anti-depressant effect in low dose[18]. In this study, 150 mg/kg of *R. vomitoria* was used, which indicates that it contained less concentration of the active components thus, decreasing the depressive nature of the herb. Antidepressants have been reported to have different effects on body weight. They may be either neutral to weight gain and loss, or may cause either weight gain or loss[19,20]. Body weight is an important index in the determination of the well being of an individual[21]. In this study, no difference existed in the body and brain weights of the groups treated with either *R. vomitoria* alone or in combination with *G. latifolium* compared with the control group. The result may indicate that the size of the body and brain of the animals may not have been affected by the treatment regimes. A previous report with similar treatment resulted in reduced body weights of the male mice[22]. The difference may be due to the sex of the animals, because males and females differ in their pharmacokinetics and pharmacodynamics of drugs[23,24]. Another report showed that the same treatment may result in body weight loss in male rats[25]. The difference may be due to the species of the animal.

A lower body weight was however observed in the group treated with *G. latifolium* alone compared

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with the control group, and the other treatment groups. This result indicates that *G. latifolium* may cause body weight loss, which corroborates to a previous report that the plant is used traditionally in the control of body weight gain[16]. Other studies also corroborate the body weight lowering property of *G. latifolium*[25-27]. However, body weight loss was not observed in the group with *R. vomitoria* and *G. latifolium* combination, indicating a possible interacting effect of *R. vomitoria* on *G. latifolium*, thereby modulating its weight controlling property.

The light and dark field neurobehavioural test showed no difference in ambulatory activities and aversion to the light chamber compared with the control group, which is an indication that the herbal treatment regimes may not have affected the animal's behaviour. The light and dark exploration test provides a means of examining anxiety like-behaviour in rodents, as most mice naturally demonstrate a preference for the dark compartment[28]. The frequency of line crossing strongly correlates with the distance covered and it assesses the horizontal locomotion (ambulation) of the animals[29]. As no difference was observed in the present study, the treatment regimes may not affect anxiety, which is similar to a previous report where a standard antidepressant, selegiline produced the same effect[30]. The present study is in line with a previous report where the same treatment regimes did not affect the behaviour of the mice[22]. However, the present study is at variance with another report where lower treatment regimes of *R. vomitoria* administered intraperitoneally on CD1 mice decreased these anxiety related behaviours[11]. This difference may be due to the route of administration, as intraperitoneal route may provide an easy avenue to the systemic circulation[31].

Cerebral cortical cyto-architecture showed high glial and general cellular population density in both groups treated with either *R. vomitoria* alone or in combination with *G. latifolium* compared with the control group. The cellular population was lower in the group treated with *G. latifolium* alone compared with the control and other treatment groups. The apparent high glia population density is indicative of trauma from the treatment regimes, which was not observed in the group treated with *G. latifolium* alone. The high general cellular population density may be due to either gliosis and/or neurogenesis. Gliosis usually result when the brain is traumatized by chemical agents and/or infections[32], and the plants may have done that.

Antidepressants and antioxidants have been reported to stimulate neurogenesis in adult rodent brains[33,34]. Both plants used in the present study showed antioxidant properties[14,15], while *R. vomitoria* may act as antidepressant in low concentration[18]. Hence, it may also be possible that the general cellular population increase may have resulted from neurogenesis.

The cerebral cortex is the part of the brain primarily responsible for cognitive abilities[35]. It is reported that cellular population change affects cognitive abilities either positively or negatively[36,37]. Wang *et al*[37] reported that *Ginkgobiloba* extract promotes proliferation of endogenous neural stem cells, which might be a reason it improves memory loss and cognitive impairments in patients with senile dementia[38,39]. Thus, it is possible that the increased cellular population observed in the present study may lead to improved cognitive abilities.

CONCLUSION

In conclusion, the treatment regimes, *R. vomitoria* administered either alone or in combination with *G. latifolium* may cause cerebral cortical cells proliferation, but may not affect anxiety-like behavioural activities and body weight of mice.

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