Effect Of Aqueous Extract Of Rauwolfia Vomitoria Root Bark On The Cytoarchitecture Of The Cerebellum And Neurobehaviour Of Adult Male Wistar Rats

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Citation

Abstract
Rauwolfia vomitoria is a natural medicinal herb which has been used over the years for the treatment of hypertension and mental disorders. The effect of aqueous extract of R.vomitoria root bark on the cyto-architecture of the cerebellum and neurobehaviour of adult male Wistar rats was studied. Fifteen adult male Wistar rats weighing between 150g and 170g were used for this study. They were divided into three groups, A, B and C of five rats each. Groups A and B were the experimental groups, while group C was the control. Oral doses of 600mg/kg and 500mg/kg body weight of the extract was administered to the rats in groups A and B respectively for seven days, while the control received distilled water. On the eight day, neurobehaviour test using the 'open field' was carried out and the animals were subsequently sacrificed. There were reductions in body weight in the experimental groups compared to the control. The neurobehaviour test showed reduced locomotion and exploratory activities in the experimental groups compared to the control and, histological result showed distortions of the cerebellar cells and layers of the experimental groups compared to the control. The extract affects the cerebellar cyto-architecture and neurobehaviour. Thus the use of the drug should be limited to management of a diseased condition.

INTRODUCTION
Medicinal plants are re-emerging health aid which has been observed in developing countries probably fuelled by the rising costs of orthodox drugs in the maintenance of personal health and well being . One of such medicinal plants is Rauwolfia vomitoria whose discovery is accredited to the 16th century German physician, Leonhart Rauwolf . It is also called African Serpent wood, African-snake root or swizzle stick. In Yoruba it is called asofeyeje, ira in Igbo, and wadda in Hausa, while it is either called mmoneba or utoenyin in Efik and Ibibio languages respectively .

The alkaloids of R. vomitoria are used mainly as anti-hypertensive agent and sedatives. Uses of its root, root bark and bark of stem are extensive, particularly for their aphrodisiac, antipsoric, abortive and insecticidal properties. They also include; anti-helminthic, apercent, dysenteric, astringent, bechic, cardiotoxic, diaphoretic, emetic, emminagogic, expectorant, haemostatic, hypotensive, vulnerary and febrifugic. It adverse effects include; decreased heart rate and blood pressure, which is due to dilatation of blood vessels. It also causes low sex drive, increase appetite, weight loss, swellings, stomach upset, hallucinations, poor co-ordination, dizziness, impairment of physical abilities and psychotic depression .

Reserpine is the most abundant and most active alkaloid in R. vomitoria whose action dominates the other alkaloids. It is an indole alkaloid known to irreversibly bind to storage vesicles of neurotransmitters in synapses. In low doses, it acts as an antidepressant while high doses cause monoamine depletion and depression. Reserpine also has peripheral action in many parts of the body, resulting in a preponderance of cholinergic part of nervous system; the gastrointestinal tract and smooth muscle vesicles. It is an anti-adrenergic drug which depletes stores of serotonin and nor-epinephrine in the brain reduced the accumulation of H – nor-epinephrine to varying extents in different regions of the brain .

Animals show different behaviour when placed in an environment different from theirs. But if these same animals are for example drugged, they will show behavioural
changes even in a known environment. To test the neurobehaviour of experimental animals, standard methods have been provided, but the most widely used is the ‘open field test’. Thus this research is designed to investigate the effect of aqueous extract of Rauwolfia vomitoria root bark on the histology of the cerebellum and neurobehaviour pattern of adult male Wistar rats.

MATERIALS AND METHODS
Fifteen adult male Wistar rats were bred in the animal house of the Department of Human Anatomy, University of Calabar. They were fed with normal rat chow and water was provided ad libitum throughout the duration of the experiment. The rats were weighed using the beam balance and the weights were ascertained to be between 150g to 170g before the commencement of the experiment.

PREPARATION OF HERB EXTRACT
The roots of R. vomitoria were dug out after identifying the tree. It was cleaned and the bark of the root was separated. They were sun-dried, blended into powder using an electric blender and extracted with ethanol using Soxhlet extractor and the extract weighed.

EXPERIMENTAL PROTOCOL
The animals were weighed before the commencement of the administrations and at the end of administrations. Groups A and B were administered 600mg/kg and 500mg/kg per body weight of Rauwolfia vomitoria respectively for seven days by oral gavage, while the control group received distilled water. The rats were fed with normal rat chow and clean water ad libitum. On the eighth day, neurobehaviour test using the ‘open field’ was carried out. The neurobehaviour of the animals were determined for total locomotor activities, exploration and anxiety.

STATISTICAL ANALYSIS
One-way analysis of variance (ANOVA) was used to compare the group’s mean for the open field activities, for treatment or administration and their interactions. Thereafter the post-hoc test using student-Newman-Keul method was carried out to find the level of significance at p<0.05. All the results are expressed as mean±standard error of mean.

RESULTS
ANTHROPOLOGICAL STUDY
There was an increase in weight of rats in the control group while there were decreased weights in the experimental groups. Food and water intake was also reduced and the rats were inactive in the experimental groups.

There was a significant (p<0.01) weight loss observed in the two groups of animals administered 600mg/kg and 500mg/kg per body weight extract of R. vomitoria compared to the control which gained weight.

Figure 1
Figure 1: The percentage weight change in groups A, B and the control (group C)

Figure 2
Figure 2: Line crossing in the open field between groups A, B and the control (group C)

NEUROBEHAVIOUR OBSERVATIONS
LINE CROSSING
The line crosses of the control (group C) was significantly (p<0.001) higher than groups A and B. Both experimental groups almost had zero line crosses during the course of the experiments. This result is seen in Fig. 2.

Figure 2
Figure 2: Line crossing in the open field between groups A, B and the control (group C)

CENTRAL SQUARE FREQUENCY AND
DURATION
There were no central square frequency and duration in the experimental groups compared to the control.

STRETCH ATTENDS
There were no stretch attends in the experimental groups compared to the control.

REARING
The rearing of the control was significantly (p<0.001) higher than the experimental groups. This result is seen in Fig. 3.

Figure 3
Figure 3: Frequency of rearing in the open field between groups A, B and the control (group C)

Both doses of extract of R. vomitoria caused significant (p<0.01, 0.001) decrease in the groups administered with the extract of R. vomitoria. This is shown in Fig. 4.

Figure 4
Figure 4: Frequency of grooming in the open field between groups A, B and the control (group C)

The defecation of the control was significantly (p < 0.01, 0.001) higher than the experimental groups. However, the number of urine puddles was not significantly different between the groups and so was not shown.

HISTOLOGICAL OBSERVATION
Photomicrograph of the control showed the cerebellar cortex made up of three layers: the outer molecular layer containing stellate and basket cells, the middle Purkinje layer with a single layer of large Purkinje cells, and the inner granular layer contain numerous granule and Golgi cells (Plate 1).

Photomicrograph from group A animals which received 600mg/kg of extract of R. vomitoria showed slight reduction in cellular sizes in the molecular and Purkinje layers. Distortions were observed in Purkinje layer. The granular layer also showed distortions (Plate 2).

In group B, which received 500mg/kg of extract of R. vomitoria, the molecular and Purkinje layer appeared normal compared to the control though there was slight Purkinje cell size reduction. There were distortions in the granular cell layer (Plate 3).
**Figure 6**
Plate 1: The control showed the cerebellar cortex made up of three layers: the outer molecular layer, the middle Purkinje layer with a single layer of large Purkinje cells, and the inner granular layer contain numerous granule and Golgi cells. Mag x400. H&E

**Figure 7**
Plate 2: Group A which received 600mg/kg of extract of R. vomitoria showed slight reduction in cellular sizes in the molecular and Purkinje layers. Distortions were observed in Purkinje layer. The granular layer also showed distortions. Mag x400. H&E

**Figure 8**
Plate 3: Group B which received 500mg/kg of extract of R. vomitoria, the molecular and Purkinje layer appeared normal compared to the control though there was slight Purkinje cell size reduction. There were distortions in the granular cell layer. Mag x400. H&E

**DISCUSSION**

**MORPHOLOGICAL CHANGES**

The reduction in weights observed in the experimental groups and their lack of activity may be due to the effect of the extract of R. vomitoria. These groups were administered 600mg/kg and 500mg/kg of R. vomitoria root bark extract. R. vomitoria contains reserpine as its major alkaloid. Reserpine impairs mucosal quality by increasing cholinergic activity in gastric tissue which reduces nutrients absorption and loss of appetite. It also causes drowsy, hypnotic and sedative tendencies. Thus the low food and water intake and inactivity of the rats seen in this study may be as a result of gastric intolerance caused by reserpine as well as drowsy, hypnotic and sedative tendencies and these may have been the reason for the weight loss.

**NEUROBEHAVIOUR TEST**

The open field apparatus is used to assess the emotionality of animals in a novel environment. It is also used to assess locomotor and exploratory behaviour. The frequency of line crossing strongly correlates with the distance covered and it assesses the horizontal locomotion of the animals. The frequency of line crossing in the groups of rats administered 600mg/kg and 500mg/kg of R. vomitoria was significantly lower than that in the control group. This implies that the horizontal locomotor activity was greatly reduced following administration of the extract.
There were no central square frequency and duration as well as stretch attends in the experimental groups compared to the control. Since there was a significant lack of line crossing in the experimental groups, it follows that there will be no central square frequency and duration. The lack of stretch attend follows the inactivity of the animals in the experimental groups. Reserpine cause drowsy, hypnotic and sedative tendencies, and these conditions usually result in lack of movement as seen in this study. Thus the animals never portrayed exploratory behaviour.

The frequency of rearing was also significantly lower in both test groups administered 600mg/kg and 500mg/kg of R. vomitoria when compared to control. Rearing is a form of vertical locomotor behaviour and it assesses the level of exploration in animals in the open field apparatus. This behaviour was significantly reduced, implying that the extract of R. vomitoria reduced exploratory behaviour in the open field.

Grooming is a displacement reaction. It also shows the level of excitability of the animal. In the study, the animals administered 600mg/kg and 500mg/kg of R. vomitoria extract showed grossly reduced frequency of grooming compared to their control. Thus the test animals were far less excitable compared to control.

The exploratory behaviours in rodents have also been used in the open field maze to assess excitability, emotionality and locomotor phenomena. Since all the above parameters/behaviours were reduced following oral administration of both high and low doses of R. vomitoria, it means the extract reduced excitability and locomotor as well as exploratory behaviour. The extract thus has a strong sedative property.

This is consistent with the fact that one of the main alkaloids found in the plant extract, reserpine, has been found to have some depressive effects on the nervous system. Reserpine causes depletion of the peripheral stores of catecholamines, which accounts for much of the beneficial antihypertensive effect employed over the years. However, depletion of central stores of neurotransmitter amines is responsible for the antipsychotic effects and consequently its adverse side effects such as sedation, depression inability to perform complex tasks and Pseudo-Parkinsonism.

The number of faecal boli was also significantly decreased in the test groups when compared to control. This however may not be very informative as there is still some controversy over whether the number of faecal boli and number of urine puddles can be used to accurately assess the level of anxiety in animals in the open field apparatus.

Locomotion activation results from brain activation which manifest as excitation of the central neurons and an increase in cerebral metabolism. Brown et al. reported that natural occurring locomotor activation (like searching, grooming and rearing) and inhibition (like rest and sleep) may differ in its underlying mechanisms from similar behaviours induced by drugs.

**HISTOLOGICAL CHANGES**

There were distortions observed in the experimental groups which received 600mg/kg and 500mg/kg of root bark extract of R. vomitoria. These may be due to reserpine, which is the most active alkaloid of R. vomitoria. Reserpine depletes stores of serotonin and nor-epinephrine in the brain and reduces the accumulation of H\(^+\) – nor-epinephrine to varying extents in different regions of the brain, and Cavanagh had earlier stated that chemically induced neurodegeneration is usually characterised by different patterns of neuronal cell death, gliosis, swollen or destroyed axons, or destruction of the myelin sheath. These effects are usually preceded by changes on biochemical targets, as seen in this study. This distortion may disturb the inhibitory circuit signaling within the cerebellum and causes exhibition of unsteady gait and involuntary postural and kinetic movements.

R. vomitoria, a medicinal herb very effective in the treatment of different psychotic disorders can cause different adverse effects in individuals. These include: reduction of body weight, mild distortions of the cerebellum and reduction in locomotion and exploratory behaviours. Thus the use of the drug should be limited to management of a diseased condition.

**References**

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