COMPARATIVE ANALYSIS OF ADVERSE EFFECTS POSED BY THE ADMINISTRATION OF ETHANOLIC ROOT BARK AND LEAF EXTRACTS OF RAUWOLFIA VOMITORIA (APOCYNACEAE) ON NEUROBEHAVIOUR OF ADULT WISTAR RATS

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ABSTRACT

Background: Rauwolfia vomitoria is widely used in traditional medicine for diverse purposes. People believe that the plant has no adverse impact on humans since it is a natural product. Objective: The purpose of this study was to comparatively analyze the extent of adverse impact posed by either the administration of ethanolic root bark and leaf extracts of Rauwolfia vomitoria on neurobehavioural parameters of adult Wistar rats. Methods: 30 adult rats were used for this investigation and were randomly divided into six groups (n=5). Several neurobehavioural parameters were assessed and determined using open field maze method. Results: From this investigation it is observed that the root-bark and leaf extracts impair locomotory and exploratory behavior in a dose-dependent manner, the root-bark extract was found to cause more impairment than the leaf extract. It was also observed that the root-bark extract caused more reduction in anxiety than the leaf extract. This adverse impact may be due to the presence of a known sedative agent, reserpine, in the root-bark than the leaf and care /precaution must be taken during the consumption of these plant extracts.

KEYWORDS: Rauwolfia vomitoria, Neurobehaviour, adverse impact, reserpine.
1. INTRODUCTION

*Rauwolfia vomitoria* is a shrub or a small tree of about 8cm and is extensively grown in Congo, Africa for commercial purposes due to its medicinal value. The branches are whorled and the nodes enlarged and lumpy. Older parts of the plant contain no latex and the leaves are in threes, elliptic – acuminate to bradly lanceolate. The flowers are minute, sweet-scented; branches of inflorescences are distinctly puberulous with hardly any free corolla lobes. Fruits are fleshy and red in colour [1]. *Rauwolfia vomitoria* belongs to a genus called Rauwolfia. This generic name is in commemoration of a 16th century German Physician, Leonhart Rauwolf, who travelled widely to collect medicinal plants [2]. Rauwolfia is a genus of evergreen trees and shrubs in the dogbane family, Apocynaceae and contains about 85 species which can mainly be found in the tropics.

Some of the species of Rauwolfia include *Rauwolfia cattria* (South African quinine tree), *Rauwolfia canescens*, *Rauwolfia micrantha*, *Rauwolfia sachetiae* (French Polynesia), *Rauwolfia sandwicensis*, *Rauwolfia serpentine* (snakeroot), *Rauwolfia tetraphylla* and *Rauwolfia vomitoria* (poison devil’s pepper). The specific epithet “vomitoria” refers to the purgative and emetic properties of the bark.

*Rauwolfia vomitoria* occurs naturally in gallery forests but is mostly found in forest regrowth where fallow periods are prolonged. It is associated with palms, *Tremagouineensis* and Combretum species. It is a hermaphroditic species and its fruits are dispersed by birds. It is native in Cameroon, Democratic Republic of Congo, Ghana, Liberia, Nigeria, Senegal, Sudan, and Uganda. It is currently considered endangered species. Seedling production is best achieved through vegetative propagation using total immerses method in 50 ppm indole butyric acid where 80% in rooting stem cuttings was achieved [2]. It is commonly known as serpent wood, Swizzle stick in English, Asofeyeyeji in Yoruba, Wadda in Hausa, Eto mmmomba and Eto untoenyin in Efik. *Rauwolfia vomitoria* is one of the herbs used in treating mental disorders. It is used for treating hypertension, stroke, insomnia and convulsion including psychotopic disorders [1]. *Rauwolfia vomitoria* is used traditionally against snake bites, fever and nervous disorders. In Ghana and Nigeria, it is used as emetic and purgative; children are treated with this plant for cerebral cramps, jaundice and gastrointestinal disorders. Aqueous solution of the bark of *Rauwolfia vomitoria* can be used against such parasites as lice and scabies [3]. In Mali, the roots of *Rauwolfia vomitoria* are used to treat hemorrhoids and hepatomegaly. It is also used in Mali as sedative for mentally ill persons,
good for treating tetanus and epilepsy. In the Congo Basin, Rauwolfia species together with traditional ash salt is used against diarrhea and with red palm oil against elephantiasis of the legs. It is used as abortifacient because it contracts the uterus after administration\(^{[4]}\).

2. MATERIALS AND METHODS

Breeding of Animals
Thirty (30) adult Wistar rats weighing 160g – 250g were obtained from the Department of Biochemistry, University of Calabar. They were housed in the animal house of the Department of Anatomy under standard conditions. The animals were fed with standard diet and allowed access to drinking water \textit{ad libitum}. They were randomly divided into 6 groups (n=5).

Preparation of Extracts
The root-bark and leaves of \textit{Rauwolfia vomitoria} were obtained from the University of Calabar farm, Calabar. They were identified and authenticated by a botanist in the Department of Botany, University of Calabar. The roots and the leaves were washed in water and the root-bark was defoliated and dried. The dried root-bark and leaves were blended into powdered form using a Binatone kitchen blender. The blended sample was soaked in ethanol for 24 hours and the extract was filtered and evaporated to obtain the crude extract.

Experimental protocol
The animals were randomly divided into 6 groups of 5 animals each labelled A, B, C, D, E, F. Groups A and B were the normal control and olive oil control respectively. Groups C, D, E, and F served as the experimental. Group A animals received 0.5ml/200g of normal saline while group B animals received 0.5ml/200g for 7 days respectively. The ethanolic extracts of Rauwolfia vomitoria root-bark and leaf were administered orally to the animals with the aid of orogastric tube. Open field maze was used for neurobehavioural assessment. The following behaviours were assessed: Line Crossing, Center Square Entries, Center Square Duration, Rearing, Stretch Attend Postures, Grooming, Freezing, Walling frequency, Walling duration.

3. Statistical analysis
Statistical analysis was performed using analysis of variance (ANOVA) and post-hoc test. The experimental groups responsible for the differences were determined using post-hoc test. All values were expressed as mean±SEM of mean. Values were statistically significant at P<0.05.
4. RESULTS
The stretch attend posture (SAP) in the open field maze was significantly reduced (P<0.05) for the experimental groups compared to the control groups (fig. 1). Within the treated groups, stretch attend posture was lower in the groups C and D (1.00±0.71; 0.00±0.00) that received 200mg/kg and 300mg/kg of root-bark extract compared to groups E and F (2.75±1.49; 0.75±0.48) which received 200mg/kg and 300mg/kg of ethanolic leaf extracts of *Rauwolfia vomitoria*. The frequency of walling was significantly reduced in the experimental group rats (P<0.05) compared to olive oil and normal control group rats (fig 2). There was also a significant reduction in group C and D (7.75±2.10; 2.00±1.68) which received 200mg/kg and 300mg/kg of *Rauwolfia vomitoria* root-bark extracts respectively compared to groups E and F (16.25±2.98; 10.50±1.32) which received 200mg/kg and 300mg/kg of leaf extract respectively.

The frequency of line crossing in open field maze (OFM) was significantly reduced in all the experimental groups compared to normal control and olive oil control groups at P<0.05 (fig. 3). There was also reduction in line crossing frequency in the experimental group C and D (17.25±6.61 and 2.50±0.87) which received 200mg/kg and 300mg root-bark extract compared to group E and F (44.25±5.04 and 20.50±8.96) which received 200mg/kg and 300/kg leaf extract.

Freezing duration in the experimental groups C, D, E and F showed a statistically significant increase (P<0.05) when compared to the normal and olive oil control groups (P<0.05). There was no statistically significant difference in this parameter among groups that received different doses of ethanolic root-bark extract and those which received leaf extract (figure 4). The grooming frequency was significantly reduced in all the experimental groups and the olive oil control group compared to the normal control group (P<0.05). The experimental groups C, D, E and F showed a significant reduction in grooming frequency compared to the olive oil control group (figure 5). However, there was a significant reduction (P<0.05) in the grooming frequency in the experimental group D (0.75±0.48) rats which received 300mg/kg of root-bark extract compared to group F (2.75±1.60) which received 300mg/kg of leaf extract.

There was a decrease in central square crossing frequency in the experimental groups compared to the control groups (P<0.05). The rats in groups C and D (0.00±0.00 and 0.00±0.00) which received 200mg/kg and 300mg/kg of ethanolic root-bark extract showed
lack of exploratory behaviour as they did not enter the centre square. The rats in groups E and F (0.50±0.50 and 0.25±0.25) which were given 200mg/kg and 300mg/kg of ethanolic leaf extract showed a higher central square frequency compared to those which received root-bark extract (figure 6).

![Graph showing comparison of frequency of stretch attend posture (SAP) during the open field maze test in the control and tests groups. Values are mean ± SEM. *p<0.05 vs NC (normal control) a = p<0.05 vs olive oil control.]

![Graph showing comparison of frequency of walling during the open field maze test in the control and tests groups. Values are mean ± SEM. *p<0.05 vs NC a = p<0.05 vs olive oil control b = p<0.05 vs test 1 c = p<0.05 vs test 2.]
Fig. 3 Comparison of frequency of line crossing during the open field maze test in the control and tests groups. Values are mean ± SEM.

*p<0.05 vs NC
a = p<0.05 vs olive oil control
b = p<0.05 vs test 1
c = p<0.05 vs test 2
d = p<0.05 vs test 3

Fig. 4 Comparison of freezing duration during the open field maze test in the control and tests groups. Values are mean ± SEM.

*p<0.05 vs NC
a = p<0.05 vs olive oil control
Fig. 5 Comparison of grooming frequency during the open field maze test in the control and tests groups. Values are mean ± SEM.
*p<0.05 vs NC
a = p<0.05 vs olive oil control
b = p<0.05 vs test 1
c = p<0.05 vs test 2

Fig. 6 Comparison of frequency of centre square entry (CSE) during the open field maze test in the control and tests groups. Values are mean ± SEM.
*p<0.05 vs normal control
a = p<0.05 vs olive oil control
5. DISCUSSION

Neurobehaviour tests were carried out to evaluate the effects of *Rauwolfia vomitoria* (RV) on anxiety, locomotory and exploratory behaviours using open field maze. The data gotten were analyzed using ANOVA. Data gotten from this study have shown that there was no statistically significant difference between the group A rats which received distilled water and group B which received olive oil in any of the parameter measured.

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety. In this study, the open field maze was used to test the effects of *Rauwolfia vomitoria* on the exploratory and locomotory behaviour, as well as fear and anxiety of adult Wistar rats. The parameters used included stretch attend posture, walling, line crossing frequency, rearing, freezing duration, grooming frequency and center square crossing.

The stretch attend posture (SAP) is a sign of hesitation and risk assessment which indicates that the animal is hesitant to move from its present location to a new position. A high frequency of these postures indicates a higher level of anxiety. In this study, SAP was significantly reduced (P<0.05) in the test groups C, D, E, and F as compared to the control groups A and B. This indicates a reduced anxiety due to the anxiolytic, anti-psychotic and depressive properties of the administered root-bark and leaf extracts due to the reserpine contents. Groups C and D which were given 200mg/300mg of RV root-bark extract respectively, showed a much more lower SAP (1.00±0.71 and 0.00±0.00 respectively) than the groups E and F (2.71±1.49and 0.75±0.48) which 200mg/kg and 300mg/kg of ethanolic extract of *Rauwolfia vomitoria* leaf, showing that the root-bark is more anti-psychotic than the leaf. There was also a dose-dependent reduction in SAP between groups C and D and between groups E and F, due to the varying reserpine contents in each extract and dose.

Reserpine has been used over the years as an antipsychotic agent due to its sedative properties as a result of depletion of catecholamines. Reserpine irreversibly blocks the vesicular monoamine transporter (VMAT) which normally transports free norepinephrine, serotonin and dopamine from the cytoplasm of the presynaptic nerve terminal into storage vesicles for subsequent release into the synaptic cleft. Unprotected neurotransmitters are metabolized in the cytoplasm and consequently never reach the synapse. These neurotransmitters (catecholamines) are responsible for the high anxiety in animals during ‘fight or flight’ situations. Therefore, by interfering with the sympathetic nervous system, reserpine reduces anxiety in the animals as seen in this work. LaBuda and Fuchs (2002),

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however, reported that the interference of reserpine with normal catecholamine activity by depleting norepinephrine and dopamine from sympathetic nerve terminals blocked the anxiolytic action of ethanol.

The mechanism of reserpine's toxic effects is similar to the mechanism of its pharmacologic effects. Reserpine inhibits normal sympathetic activity in both the CNS and peripheral nervous system by binding to catecholamine storage vesicles. This prevents the normal storage of catecholamines and serotonin in the nerve cell, with the result being catecholamine depletion. Reserpine has also been said to inhibit catecholamine synthesis by blocking the uptake of dopamine into the storage vesicle.\cite{9,10}

This work is in line with the work of Eluwa et al (2009)\cite{11} who reported a lack of SAP as a result of the inactivity of the rats in the experimental which received different doses of aqueous RV root-bark extract, and this was said to be as a result of the sedative properties of RV. According to Molewijk et al (1995)\cite{12}, the stretched attend posture is sensitive to a number of anxiolytic agents and thus mirrors a crucial element in the temperamental dimension of harm avoidance; namely, anxiety, worry and apprehension, especially in anticipation of future problems.\cite{13}

Grooming behaviour is a displacement response and is expected to be displayed in a novel environment (Espejo, 1997). It is a sign of anxiety and apprehension. In this study, grooming frequency was significantly reduced in the treated groups C, D, E and F, compared to the control groups, indicating a decrease in anxiety. The root-bark extract groups exhibited a lower grooming frequency than the leaf extract groups. This reduction in anxiety is due to the anxiolytic effects of the reserpine content in RV, especially in the root-bark where its content is much higher. The binding of reserpine to the storage vesicles causes catecholamines to leak into the synapse so that they are not available for release when the pre-synaptic neuron is stimulated.\cite{10} This reduces the animals risk assessment behaviour, making them calm effects even in a novel environment, or in ‘fight or flight’ situations. This is in line with the findings made by Eluwa et al (2009)\cite{11} who recorded reduced frequency of grooming in animal groups administered with 500mg/kg and 600mg/kg of RV root-bark extract compared to the control. Odo et al (2007)\cite{15} also reported a reduction in the frequency of grooming rats that were treated with artesunate than in the control (P<0.01).
Walling is a measure of exploration. In this study, walling was significantly reduced (P<0.05) in RV treated groups C, D, E and F compared to control groups A and B. Walling was significantly reduced in experimental group C (7.75±2.10) and D (2.00±1.68) rats which received 200mg/kg and 300mg/kg of root-bark extract compared to the groups E and F (16.25±2.98 and 10.50±1.32) which received 200mg/kg and 300mg/kg RV leaf extract (P<0.05). This is in line with findings made by Odo et al., (2007)\[15\] who reported that there was a decrease in locomotor activity (line crossing) and exploratory (rearing and walling) activities in comparison with the control (P<0.05) in groups of rats administered with artemisinin.

Line crossing frequency and the frequency of rearing are usually used both as measures of locomotory activity, as well as measures of exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and/or a lower level of anxiety.\[5\] In this study there was significantly reduced frequency of line crossing in the treated groups C, D, E, F compared to the control groups A and B indicating a reduction in exploratory and locomotory, as well as increased anxiety behaviours due to the effects of the leaf back and leaf extracts. The group C and D which received 200mg/kg and 300mg/kg RV root-bark extract respectively showed a more reduced line crossing frequency than the group E and F which received 200mg/kg 300mg/kg RV leaf extract respectively. This shows that the root-bark extract affects exploratory and locomotory activities more than the leaf extract.

This may be as a result of the reserpine content of RV especially in the root-bark which suppresses the central nervous system thereby sedating the animals. This is in agreement with the findings of Eluwa et al (2009)\[11\] where 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 implying that the horizontal locomotor activity was greatly reduced following administration of the extract.

Freezing Duration (FD) is a measure of anxiety. In this study, the experimental groups C, D, E and F showed a statistically significant increase in FD compared to the normal and olive oil control groups (P<0.05). This may not have been due to increase in anxiety, but could have been as a result of lethargy and sedation caused by the extracts given. The main adverse effects described with the therapeutic administration of reserpine include lethargy and sedation, psychiatric depression; hypotension.\[16,17\] Reserpine suppresses the central nervous system and it is known to possesses antihypertensive effect as a result of the fact that it
causes the depletion of the peripheral stores of catecholamines.\textsuperscript{18} It also causes the depletion of central stores of neurotransmitter amines which is responsible for the antipsychotic effects and consequently its adverse side effects such as sedation, depression and inability to perform complex tasks and Pseudo-Parkinsonism.\textsuperscript{19,20}

This is in line with another study\textsuperscript{15} where rats administered with artesunate showed marked increase in the frequency and duration of freezing than in the control rats (P<0.01).

The number of central square entries (CSE) and the duration of time spent in the central square are measures of exploratory behaviour and anxiety. A high frequency/duration of these behaviours indicates high exploratory behaviour and low anxiety levels.\textsuperscript{5,20} In this study, the CSE was much higher in the control groups A and B than in the treated groups C, D, E and F showing that \textit{Rauwolfia vomitoria} causes reduction in exploratory behaviour in the treated groups. However, groups C and D which received 200mg/kg and 300mg/kg root-bark extract showed no CSE. This may have been due to lethargy which is one of the side effects of reserpine.\textsuperscript{16,17} This shows that the root-bark extract affects exploratory and locomotory behaviour more than the leaf extract due to a higher reserpine content in the root-bark. Reserpine is one of the major alkaloids found in RV.\textsuperscript{7} Reserpine has drowsy, hypnotic and sedative tendencies\textsuperscript{21} and these conditions usually result in lack of movement as seen in this study. The higher content of this alkaloid in the root-bark as compared to the leaf of this plant may have been responsible for the lower exploratory and locomotory behaviour found in the groups which received the root-bark extract compared to those which received the leaf.

Another alkaloid of importance found in RV is alstonine. Alstonine possesses anti-psychotic and anxiolytic, properties\textsuperscript{7} and unlike reserpine, does not cause motor deficits\textsuperscript{22} Therefore, it may not have been responsible for the impairment of motor behaviour found in this study. However, its anxiolytic properties may be implicated in the low anxiety level found in some of the measures of the open field test.

This is in line with the work of Bisong et al (2012)\textsuperscript{23} who found decreased locomotor behaviour (p < 0.05) and reduced vacuous chewing movement in mice treated with RV when compared to the Reserpine-treated group of mice and chlorpromazine groups. The results of this study is at variance with the findings of Ekong et al (2009)\textsuperscript{24} where higher doses of amodiaquine was found to have caused higher locomotory and exploratory activities and reduced anxiety due to its ability to cause agitation, aggressiveness, confusion, personality
changes and psychotic symptoms as earlier reported by Tester-Dalderup (1984).[25] This may be due to the fact that amodiaquine has psychotic effects while Rauwolfia vomitoria has anti-psychotic effects.

6. CONCLUSION
Neurobehavioural studies showed that the root-bark and leaf extracts impair locomotory and exploratory behavior in a dose-dependent manner, the root-bark extract was found to cause more impairment than the leaf extract. It was also observed that the root-bark extract caused more reduction in anxiety than the leaf extract. This may have been due to the higher content of a known sedative agent, reserpine, in the root-bark than the leaf.

6. REFERENCES


