Comparative Effects of Alkaloid and Saponin Fractions of *Rauwolfia vomitoria* on Social Behaviour and Depression in a CD1 Mouse Model of Memory Impairment

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Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aqueous root bark extracts of *Rauwolfia vomitoria* (RV) has been reported to reduce anxiety among other uses. The study compared the effect of alkaloid and saponin fractions of *RV* on social behaviour and depression in scopolamine-induced memory-impaired CD1 mice. The alkaloid and saponin fractions were extracted from the root bark of the plant using standard methods. Forty-two (42) CD1 mice were grouped into six (n=7): Control mice were given placebo; three groups were given scopolamine (1.0 mg/kg, i.p. (explain) for 5 days to induce memory impairment), with 2 of them treated with the alkaloid (0.15 mg/kg, p.o.) and saponin (0.10 mg/kg, p.o.) fractions of *R. vomitoria* respectively. The other 2 groups were given either of the alkaloid fraction or saponin fraction of RV. Treatment lasted 21 days, after which mice were given the forced swim test and tail suspension test to assess depression and nesting test to assess social behaviour. Results showed that latency to immobility for the alkaloid fraction-treated group decreased significantly while the duration of immobility increased significantly (P<0.05) compared to control. This shows a strong...
positive depressive symptom. The saponin fraction-treated group however showed a significant increase in the latency to immobility while the duration of immobility decreased significantly compared to control (P<0.05). Thus, the saponin fraction of R. vomitoria decreased depression. In the test to assess social behaviour, the alkaloid group showed a significant decrease in nesting score which indicates social loss whereas the saponin group showed a significant increase (P<0.05) in nesting score compared to control. Therefore, the saponin constituent of Rauwolfia vomitoria has a high antidepressant advantage over the alkaloids fraction.

Keywords: Rauwolfia vomitoria; alkaloid; saponin; social behavior; depression.

1. INTRODUCTION

Social behaviour is behaviour among two or more organisms typically from the same species that is usually beneficial to one or more of the individuals [1]. Depression on the other hand is a mood disorder that affects the way one thinks, feels and behaves. It is the feeling of sadness that can last from few days to a few years. Long term consequences of depression include coronary heart disease, type 2 diabetes mellitus and osteoporosis [2]. Depression is on the rise and it is expected that by 2030, depression will lead in global burden of diseases [3].

World Health Organisation (WHO) worldwide statistics in 2010 showed that in 2002, 91 million people suffer from behavioural disorders associated with the use of alcohol while 154 million people suffer depression [4]. According to WHO, there are 322 million people living with depression in the world and this is a major risk factor for suicide. The Society of Family Physicians of Nigeria (SOFPON) raised the alarm that 7 million Nigerians are living with depression and called for well-structured primary health centres that would help detect and treat depression early before the onset of suicide attempt [5]. In African societies and some areas in Japan and the United States of America, herbs have been used to treat mental and other disorders [6,7,8,9] probably because there are readily available and affordable by the poor. One of the herbs employed in mental health related issues is Rauwolfia vomitoria (RV). RV is a plant that belongs to the family Apocynaceae [10]. It is commonly called serpent wood and swizzle stick. In Nigeria, it is called Asofeyeje by the Yorubas, Akanta by the Igbos, Wada by the Hausas and Mboneba by the Efiks [11,12,13]. RV has been reported to be effective in the treatment of mental disorders, nervous disorders and insomnia [14]. It is also used against snake bite, as an anti-parasitic agent [10] and as an anti-psychotic and anti-hypertensive agent [15]. The plant has also been reported to have anticancer effect [16], anti-inflammatory effect [17], anti-pyretic effect [18] and to restore mental activities to normal [19]. Root bark extracts from R. vomitoria have also been reported to have many beneficial effects on some neurobehavioural parameters [20,21,22]. The therapeutic effects of Rauwolfia vomitoria are due to its phytochemical constituents. Sharma [23] reported that five crystalline alkaloids (ajmaline, ajmalicine, serpentine, serpentinine and yohimbine) and other constituents such as phytosterol, oleic and unsaturated alcohols can be isolated from the root of RV. A major alkaloid present in the plant is reserpine and reserpine is the major constituent of antihypertensive drugs [24] and has been used for the management of schizophrenia and psychiatric disorders [25]. Saponin had also been identified in the root of RV [26].

Saponin is an important phytochemical of many medicinal plants and its role as natural cure for depression had been supported [27]. There are serious limitations in terms of safety, efficacy, tolerability and therapeutic success for current antidepressants [27]. In spite of the use of RV as an antipsychotic agent and in the treatment of mental disorders [14], it remains uncertain which of the phytochemicals is most effective. Considering the potential of saponin in the treatment of depression and the less side effects associated with herbal therapy compared with orthodox drugs, this study sought to compare the effects of alkaloid and saponin fractions of root bark extract of Rauwolfia vomitoria on depression and social behaviour in scopolamine-induced memory-impaired CD1 mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty-two male CD1 mice weighing 15-27 g were obtained from the animal house of Department of Physiology, University of Calabar. The study was approved by the ethics committee on animal
experimentation of the Faculty of Basic Medical sciences, University of Calabar. The mice were acclimatized for seven (7) days, given rat feed and water ad libitum and exposed to 12/12 hours light/dark cycle. They were kept in properly ventilated transparent plastic cages in the animal house of Department of Physiology.

2.2 Plant Material

After identification of Rauwolfia vomitoria plant by a botanist in the Biological Science Garden of the University of Calabar, country name the plant was harvested and the roots washed using water so as to remove debris and the root bark was peeled off. The root bark was sundried and blended to fine powder.

2.3 Extraction and Isolation of Saponins

Saponins were isolated according to the method of Majinda [28]. The root bark of Rauwolfia vomitoria was sundried and ground into fine powder and oven-dried at 40°C to a consistent weight of 390.93 g. The powdered material was defatted with n-hexane and the dried marc extracted with MeOH in a Soxhlet for 72 hours. The extract was then concentrated under reduced pressure and partitioned successfully using n-hexane (for further removal of fats), ethyl acetate and n-BuOH. The n-BuOH soluble fraction and the aqueous part containing the major saponin triterpene fractions were pooled and concentrated. Saponin compounds were detected on thin layer chromatography (TLC) after spraying with 10% (v/v) H$_2$SO$_4$ in the Lieberman-Buchard reagent and triterpene saponins produce blue violet spots on heating. Weight of saponin extract was 5.7 g (1.46%).

2.4 Extraction and Isolation of Alkaloids

The plant material was dried to a constant weight in an oven set to 40°C and was reduced to a moderately coarse powder by grinding and sieving. This was to facilitate maximum effective contact of the solvent with the ruptured alkaloid bearing tissues and cells. The coarse powder was first defatted with n-hexane in order to remove oils and fats in a soxhlet apparatus. The n-hexane fraction was shaken with a dilute mineral (HCl) which was tested for the presence of alkaloids. The dried marc was subsequently subjected to exhaustive extractions with 80% methanol to obtain crude extracts. The crude methanol extract was washed with n-hexane in a separation flask in order to remove any remaining oil or fat. The methanol layer was subsequently reduced in volume (concentrated) by mildly evaporating the solution. The weight of alkaloid extract was 8.16 g giving a percentage yield of 2.09%.

2.5 Experimental Design

The forty-two CD1 male mice were randomly assigned into six groups of seven rats each thus:

Group 1 (Control group): Received normal feed and water

Group 2 (Alkaloid-treated group): Received alkaloid (0.15/kg)

Group 3 (Saponin-treated group): Received saponin (0.10/kg)

Group 4 (Scopolamine-treated group): Received scopolamine (1mg/kg) to induce memory impairment.

Group 5 (Sco+Alk group): Received scopolamine and alkaloid

Group 6 (Sco+Sap): Received scopolamine and saponin

All groups had access to feed and water throughout the duration (21 days) of the experiment.

2.6 Induction of Memory Impairment

Memory impairment was induced by intraperitoneal administration of 1.0mg/kg body weight of scopolamine (Sigma-Aldrich Ltd, Canada).

2.7 Behavioural Assay

Nesting behaviour test: The social behaviour of the mice was assessed using nesting behaviour test as described by Bender et al. [29] and Deacon [30], and used by Bisong et al. [21]. Mice were housed individually and tested in their home cages. All enrichment objects in the home cages of the mice were removed one hour before giving the mice nesting materials. About 3 g of nesting material was supplied to each mouse in its home cage and allowed form 24 hours after which the nests were assessed according to Deacon’s rating scale (Table 1) [30]. This was based on what was seen. The cage was carefully brought out for observation so as not to cause panic on the mouse which can lead to destruction of the nest so built.
Table 1. Deacon’s rating scale for assessing social behavior

<table>
<thead>
<tr>
<th>Rating</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nestlet not noticeably touched (90% or more intact)</td>
</tr>
<tr>
<td>2</td>
<td>Nestlet partially torn (50-90% intact)</td>
</tr>
<tr>
<td>3</td>
<td>Nestlet mostly shredded, often no identifiable nest site, 50-90% shredded, also, less than 50% remains intact, but less than 90% is within a quarter of the cage floor (i.e., not gathered into a nest site but spread throughout cage)</td>
</tr>
<tr>
<td>4</td>
<td>An identifiable, but flat nest, more than 90% of the nestlet is torn, the nest is uneven, material is gathered into a nest within a quarter f the cage floor, but the nest is flat with walls higher than mouse body height for less than 50% of its circumference</td>
</tr>
<tr>
<td>5</td>
<td>A (near) perfect nest, more than 90% of the nestlet is torn, nest is fairly even, the nest is a crater, with walls higher than the mouse body for more than 50% of its circumference</td>
</tr>
</tbody>
</table>

Deacon, 2006

**Tail suspension test:** Tail suspension test according to Steru et al. [31] is a mouse behaviour test useful in the screening of potential antidepressant drugs and assessing of other manipulations that are expected to affect depression-related behaviours.

A 12 cm paper tape was cut. The middle was made unstick by pasting it on any surface while the end at least 2cm was sticky. A stopper was put through the tail of the animals and one sticky was used to wrap round the tail of the animals while the other end was attached to the tail suspension apparatus. The animal was allowed for five minutes. A video camera was used to video the experiment since four animals were checked at once. The behaviour scored include: Latency to immobility, duration of immobility and defecation.

**Forced swim test:** The forced swim test is a rodent behavioural test used for evaluation of antidepressant drugs [32]. Water was used in this test and a transparent container with thermometer was used to check the water and be sure it was at least 30°C. The mice were introduced into the water individually and allowed for 4 minutes. A stopwatch was used to check:

- Latency time (time taken for the mice to move their hind paws to the time they first stop)
- Mobility (Total time the mice used their hind paws to move before the expiration of the swim).
- Immobility (Total duration of inactivity).
- Defecation

After the test, the animals were dried using a towel before being returned to their cages.

2.8 Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Computer software, Statistical Package for Social Sciences (SPSS) (version 17, Microsoft Company, USA) and Microsoft Excel were used for the analysis. Differences among groups were analysed using one-way analysis of variance followed by post hoc multiple comparison (least square difference procedure). p<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

*Rauwolfia vomitoria* (RV) has been employed in the treatment of mental and nervous disorders owing to its phytochemical composition [14,23]. This study compared the effects of alkaloid and saponin fractions of root bark extract of *Rauwolfia vomitoria* on depression and social behaviour in scopolamine-induced memory-impaired CD1 mice.

Depression and social behaviour were studied using the tail suspension test (TST) and the forced swim test (FST). These tests are based on the principle that when faced with a life threatening situation, rodent will continue to make frantic effort to escape and therefore will continue to scamper for safety. In the TST, latency of immobility was significantly decreased in all treated groups except the saponin-treated group compared with control. It was however significantly increased in the saponin-treated group compared with control (Fig. 1). On the other hand, duration of immobility was significantly increased in all treated groups except the saponin-treated group compared with control (Fig. 1). On the other hand, duration of immobility was significantly increased in all treated groups except the saponin-treated group compared with control. It was however not significantly different between control and saponin-treated groups (Fig. 2). These results are similar to those obtained in
the forced swim test (Figs. 4 and 5). The latency and duration of immobility are parameters used to assess depressive state. The present results indicate that alkaloid fraction of RV causes depression owing to the fact that mice that received alkaloid fraction had significantly decreased latency of immobility and significantly increased duration of immobility in both the tail suspension and forced swim tests. The saponin fraction of RV on the other hand showed anti-depressive effect as the mice that received this fraction had significantly increased latency of immobility in both the TST and FST. Although the duration of immobility was not significantly different between control and saponin-treated mice in the TST, however, in the FST, it was significantly decreased in the saponin-treated mice compared with control. Latency of immobility and duration of immobility were significantly increased and decreased respectively in the saponin-treated mice compared with the alkaloid-treated mice in both the TST and the FST. The present results indicate that alkaloid fraction of *Rauvolfia vomitoria* has a positive depressive effect whereas the saponin fraction has anti-depressive effect.

The significantly decreased latency of immobility and significantly increased duration of immobility observed in the scopolamine-treated group during the tail suspension test and forced swim test is an indication that memory impairment causes depression. The depressive effect of the alkaloid can be further seen in the Sco+Alk-treated group where latency of immobility and duration of immobility were significantly decreased and increased respectively in both tests compared with the scopolamine-treated group. This indicates that the alkaloid fraction on its own has a positive depressive effect and this worsens the depression associated with memory impairment. In the Sco+Sap-treated group, latency of immobility and duration of immobility were significantly increased and decreased respectively during the TST and FST compared with the scopolamine-treated and Sco+Alk-treated groups. This results further indicate the anti-depressive effect of the saponin fraction of RV. Saponin had been previously reported to exhibit anti-depressive effect in mice [33].

The frequency of defecation is also used as a measure of depression. The greater the depressive state, the higher the frequency of defecation and vice versa. Our result for frequency of defecation further shows the anti-depressive effect of the saponin fraction of RV root bark and the depressive effect of the alkaloid fraction. The frequency of defecation was significantly increased in the Alkaloid- and Scopolamine-treated groups compared with control (Fig. 6) implying that memory impairment and the alkaloid fraction caused depression. On the other hand, the saponin fraction of the root bark of the plant showed anti-depressive effect normally and in a memory impaired state as seen in Fig. 6 where frequency of defecation was significantly decreased in the saponin-treated group compared with control and also significantly decreased in the Sco+Sap-treated group compared with control and Scopolamine-treated groups.

In general, alkaloid fraction of the root bark of *Rauvolfia vomitoria* has a positive depressive effect in normal and memory-impaired mice whereas the saponin fraction has anti-depressive effect in normal and memory-impaired mice.

Nesting score significantly decreased in the Alkaloid-treated, Scopolamine-treated and Sco+Alk-treated groups compared with Control (Fig. 3). These results indicate that the alkaloid fraction of RV on its own causes social loss (impairment in social relationship). Also, memory impairment impairs social relationship to a larger extent as seen in the Scopolamine-treated group which had a significantly decreased nesting score compared with control, Alkaloid-treated and Saponin-treated groups. The Sco+Alk-treated group had a zero nesting score indicating that the alkaloid fraction in a memory impaired state causes complete social loss. The effect of the alkaloid fraction may be due to the effect of reserpine being the main alkaloid in RV root bark and which has been previously reported to decrease social behaviour in CD-1 mice [21]. On the other hand, the saponin fraction of RV had no significant effect on social behaviour in normal mice as nesting score was not significantly different between the Control and Saponin-treated group. However, the saponin fraction was able to improve social behaviour in memory-impaired mice. This is seen in our results where nesting score was significantly increased in the Sco+Sap-treated group compared with Scopolamine- and Sco+Alk-treated groups. In general, our results show that the alkaloid fraction of RV root bark on its own suppresses social behaviour in mice and further worsens the situation in a memory-impaired state whereas the saponin fraction on its own has the potential to improve social behaviour in normal mice and actually improves social behaviour in memory-impaired mice.
Fig. 1. Comparison of latency to immobility in the tail suspension test in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root (age) bark.

*-*significant at $p<0.05$ vs control; a-* at $p<0.05$ vs alk; b-* at $p<0.05$ vs sap; c-* at $p<0.05$ vs scop; d-* at $p<0.05$ vs scop+alk

Fig. 2. Comparison of duration of immobility in the tail suspension test in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root bark.

*-*significant at $p<0.05$ vs control; a-* at $p<0.05$ vs alk; b-* at $p<0.05$ vs sap; c-* at $p<0.05$ vs scop; d-* at $p<0.05$ vs scop+alk
Fig. 3. Comparison of nesting score in the nest building task in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root bark

*-*significant at p<0.05 vs control; a- at p< 0.05 vs alk; b- at p< 0.05 vs sap; c- at p< 0.05 vs scop; d- at p< 0.05 vs scop+alk

Fig. 4. Comparison of latency to immobility in the forced swim test in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root bark

*-*significant at p<0.05 vs control; a- at p< 0.05 vs alk; b- at p< 0.05 vs sap; c- at p< 0.05 vs scop; d- at p< 0.05 vs scop+alk
Fig. 5. Comparison of duration of immobility in the forced swim test in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root bark

*significant at p<0.05 vs control; a- at p< 0.05 vs alk; b- at p< 0.05 vs sap; c- at p< 0.05 vs scop; d- at p< 0.05 vs scop+alk

Fig. 6. Comparison of frequency of defecation in the forced swim test in a scopolamine model of memory-impaired mice treated with alkaloid and saponin fractions of *Rauwolfia vomitoria* root bark

*significant at p<0.05 vs control; a- at p< 0.05 vs alk; b- at p< 0.05 vs sap; c- at p< 0.05 vs scop; d- at p< 0.05 vs scop+alk
Alkaloids such as reserpine which forms a major proportion of the Rauwolfia alkaloids increase removal of monoamine neurotransmitters from neurons, decreasing the size of the neurotransmitter pools, and thereby decreasing the amplitude of neurotransmitter release [34]. Reserpine irreversibly blocks the neuronal H+-coupled vesicular monoamine transporters (VMAT2) thus inhibiting uptake and reduces stores of the monoamine neurotransmitters norepinephrine, dopamine, serotonin and histamine in the synaptic vesicles of neurons [35] thereby causing depression.

This result implies that the saponin fraction of RV can be employed in the treatment of depression considering the fact that social loss precipitates depression [21]. This results agrees with the previous report of Wang et al. [36] that Tribulus terrestris saponins antagonized chronic mild stress and produced anti-depressive effect in rats. Similarly, this research agrees with the research of Abbas et al. [27] who in a preclinical report supported the role of saponins as natural cure for depression. The saponins restore monoaminergic tone and enhance neurotrophic factors as well as exhibited adaptogenic effects via normalising hypothalamus-pituitary-adrenal axis, corticosterone levels and oxidative stress [27], a possible mechanism by which it reduces depression.

4. CONCLUSION

Alkalo id fraction of Rauwolfia vomitoria root (age) bark extract caused suppression of social behaviour and depression in scopolamine model of memory impairment in mice whereas the saponin fraction of the root bark of Rauwolfia vomitoria improved social behaviour and exhibited anti-depressive effect in scopolamine model of memory impairment in mice. The employment of saponin isolated from the root bark of Rauwolfia vomitoria in the formulation of antidepressants is therefore strongly encouraged.

ETHICAL APPROVAL

Ethical standards laid down in 1964 declaration of Helsinki [37] were strictly adhered to in handling the animals and during the experiments. Approval was given for the research from the Animal Ethics committee of the Faculty of Basic Medical Science, University of Calabar Nigeria with approval No. 16PHY20817.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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