**ANTIPSYCHOTIC EFFECT OF THE LEAVES OF STACHYTARPHETA CAYENNENSIS (L.C. RICHE) VAHL. VERBENACEAE IN MICE.**

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**ABSTRACT**
The antipsychotic effect of the extracts of the leaves of *Stachytarpheta cayennensis* was examined following ethnomedicinal claims for its use in the management of mental illness in Nigeria, Ghana and other tropical parts of the globe. The apomorphine and amphetamine-induced stereotyped behavior models were used in this study to see if the extract or its fractions would abolish the stereotypy in mice. The method of Siqueira et al (1998) was employed in this study and the Kruskal Wallis non-parametric ANOVA followed by Mann-Whitney U-test was employed for statistical analysis. The results showed that only the methanolic extract of the leaves of *Stachytarpheta cayennensis* but none of the fractions thereof prevented stereotyped behavior induced by amphetamine. The extract did not abolish stereotypy induced by apomorphine. The inhibition of amphetamine stereotypy also occurred at only one dose (20 mg/kg, i.p.). The inferences from this study include the fact that for the antipsychotic effect of the leaves of *Stachytarpheta cayennensis*, fractionation would lead to loss of activity. More importantly the methanolic extract of the leaves of *Stachytarpheta cayennensis* possesses antipsychotic effect and this justifies its ethnomedicinal use in the management of mental illness in some regions of the world.

**INTRODUCTION**
The use of herbs ramifies all health conditions including psychiatry or management of mental challenges. There are specialist herbalists in areas of mental health in most primary societies, just as we have in maternal/child health, orthopaedics and virtually all health conditions [1]. Some plants have been scientifically verified as possessing antipsychotic properties e.g. *Rauwolfia vomitoria* or *Rauwolfia serpentina*[1], [2], [3]. The antipsychotic agents are distinguished from both antidepressants and anxiolytics by animal experiments. The primary site of action of antipsychotics is the dopaminergic synapse [4]. Depression of dopaminergic transmission by antipsychotics is caused either by emptying the dopamine stores, as obtained with the action of reserpine, or by blocking the dopamine receptors. In addition to this main action antipsychotic agents reduce, to varying degrees, noradrenergic and serotonergic transmission. Histamine and acetylcholine receptors may also be blocked by some of these antipsychotics [5], [6]. The striatum, which has an inhibitory effect on the initiation and execution of motor activities, makes the function of dopamine clear as an inhibitory transmitter [4]. Dopamine possibly depresses perception too. Dopamine inhibits the activity of neurons in the striatum, consequently striatal inhibition of sensory and motor function. Lowering the dopaminergic influence on the striatum (and perhaps son certain limbic and cortical structures) increases the activity and excitability of these structures, resulting in psychomotor depression and apathy [5].

The antipsychotic effect of the extracts of the leaves of *Stachytarpheta cayennensis* was examined following ethnomedicinal claims for its use in the management of mental illness in Nigeria, Ghana and other tropical parts of the globe [2], [7]. *Stachytarpheta cayennensis* leaves have been scientifically documented as a sedative, anthelmintic, antiulcer, antacid, analgesic, anti-inflammatory, anti-diarrhoeal, vasodilator and a lavacidal agent [8], [9], [10], [11], [12], [13], [14]. The ethnomedicinal use of the leaves of *Stachytarpheta cayennensis* in the management of mental illness was therefore scientifically verified in this report.

**RESULTS**
SCCR, SCBT, SCAQ and SCEA (10-75 mg/kg, i.p.) were administered to mice to observe the effect of the extract on amphetamine and apomorphine-induced stereotyped behaviour in the test animals. Only SCCR (20 mg/kg, i.p.) abolished stereotyped behaviour induced by amphetamine in mice. The extract and all the fractions at all doses did not abolish stereotypy induced by apomorphine (data not included). The result was subjected to two-way analysis of variance and a relationship was established between dose of extract and time of observation of stereotypy (Table 1.).

**DISCUSSION**
The effect of SCCR was abolition of amphetamine-induced stereotyped behaviour in mice.

This may suggest that SCCR contains dopamine modulating compound(s) since it is known that amphetamine induced stereotyped behaviour is mediated by hyperactivity of dopaminergic mechanisms of the nigrostriatal and mesolimbic pathways [15]. The role of cerebral dopaminergic systems in the amphetamine-induced stereotypy has been reported [16]. Amphetamine indirectly causes the release of norepinephrine,
dopamine, and serotonin from nerve terminals. The drug causes
the release of norepinephrine more potently than it causes the
release of dopamine; it is least effective as a serotonin releasing
agent. Amphetamine enters nerve terminals either by inward
transport through monoaminergic transporters or by lipophilic
diffusion; the latter process is much less efficient, however [17].
At plasmalemmal monoaminergic transporters, amphetamine
indirectly increases the efflux of monoamines that they are
released into the synaptic cleft. Evidence also suggests that
amphetamine directly decreases the inward transport of
monooamines at monoaminergic transporters; note that the drug
may also directly inhibit monoamine reuptake to some degree [18].
At the serotonin, dopamine, and norepinephrine transporters,
recent reports have concluded that the inward and outward
transport mechanisms are independently modulated; the facilitated
exchange diffusion model commonly used to describe amphetamine-mediated monoamine efflux is therefore unlikely to be
accurate [19]. Amphetamine’s vesicle-depleting effect
represents the rate-limiting step in the monoamine efflux induced
by the drug. Amphetamine may redistribute monoamines such as
dopamine from vesicles to the cytoplasm through two possible
mechanisms. First, the inhibitory effect of the drug on vesicular
monoamine transporter-2 (VMAT-2) may inhibit monoamine
uptake into vesicles. Second, amphetamine may decrease VMAT-2
activity by reducing the transmembrane pH difference that drives
amine uptake. Both mechanisms are likely to be involved in the
amphetamine-induced stereotyped process [18]. Amphetamine-
mediated release of monoamines may also be dependent on
mechanisms not involving plasmalemmals transporters. For
example, the influx of calcium ions through P-type voltage activated
calcium channels has been shown to partially media
teamphetamine-induced dopamine release in some cases; the PKC-
mediated potentiation of P-type channels via phosphorylation is
a likely cause of this effect. The degree of involvement of P-type
channels in amphetamine-induced monoamine release in vivo is
unknown. Amphetamine has also been shown to exhibit agonist
activity at a rat trace amine receptor that is known to exist in
humans [17], [19]. In summary, amphetamine acts preponderantly
pre-synaptically while apomorphine acts postsynaptically.

The rationale for engaging the two inducers of stereotypy is to
establish whether the test compound acts pre- or post synaptically.
SCCR inhibited only amphetamine-induced stereotyped behaviour
in mice at a particular dose in the range of the sedative doses i.e
20mg/kg, i.p. Other doses lower or higher than this value did not
prevent stereotyped behaviour induced by amphetamine. There
was no abolition of stereotypy due to apomorphine. The results
show that SCCR is effective in inhibiting excessive psychomotor
activity. Future study will be extended to include the effect of the
extract and fractions on amphetamine induced gnawing. This study
also established that SCBT, SCAQ and SCEA did not induce
abolition of amphetamine induced stereotypy. This study also
showed that the antidopaminergic principle in the leaves of
S. cayennensis is contained only in the methanol: water extract or
the parent extract. Further fractionation of the extract or attempt at
isolation or purification of the extract may result in loss of this
important antipsychotic activity. It is interesting and equally
surprising that the active antipsychotic principle is not partitioned
into any of the phases (aqueous, butanol or ethylacetate). This
may suggest that the antipsychotic principle may be due to a
combination of more than one compound acting in synergy. This
observation is an additional point in favour of the use of the
extract of the leaves of the plant rather than fractions or isolates
or purified forms.

Table 1. Effect of extract (SCCR) on amphetamine induced stereotyped behaviour in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stereotypy at specified time in minutes (n=5); Observation made for 2 minutes at each time.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mins.</td>
</tr>
<tr>
<td>Normal Saline</td>
<td>3.0 ± 0</td>
</tr>
<tr>
<td>SCCR 10 mg/kg</td>
<td>2.40±0.55</td>
</tr>
<tr>
<td>SCCR 20 mg/kg</td>
<td>0.60±0.55*</td>
</tr>
<tr>
<td>SCCR 50 mg/kg</td>
<td>0.80±0.45*</td>
</tr>
<tr>
<td>SCCR 75 mg/kg</td>
<td>2.40±0.45</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. Number of mice per treatment group is 5

Administration of SCCR (20 mg/kg, i.p.) abolished stereotyped
behaviour induced by amphetamine in mice. The lower dose (SCCR
10 mg/kg, i.p.) and higher doses of SCCR 50-75 mg/kg, i.p) did not
abolish stereotypy in mice. Abolition of stereotypy is taken as
values less than 1.

*indicates significant difference from the normal saline control
(p< 0.05). Results are subjected to two-way analysis of variance
(2-way ANOVA) followed by LSD Post Hoc Test.

MATERIALS AND METHODS

Plant Materials

S. cayennensis leaves were collected from the wild on the campus
of Obafemi Awolowo University, Ile-Ife, Nigeria during the months
of August and September. The staff of the Department of Botany
(Professor O. Olorode and Dr. H. C. Illoh), Faculty of Science of
the Obafemi Awolowo University identified the plants as S.
cayennensis. Herbarium voucher (FHI-106491) for S. cayennensis
has been deposited with the National Herbarium, Forestry
Preparation of Plant Extracts for biological activities

The leaves of the plants were air-dried for 48 hours in the laboratory at a room temperature of about 27 ± 1°C. The dried leaves were pulverized and 500g of the powdered leaves of the plant was soaked in 3 litres of 1:1 methanol: water solution for 48 hours. The marc was re-extracted twice and the combined extracts were concentrated in vacuo to yield 88.52 g (17.7%) methanolic extracts. The extract obtained was concentrated to a waxy cake on dryness. The methanolic extract (SCCR) was successively partitioned into ethylacetate (SCEA), n-butanol (SCBT) and water (SCAQ). The fractions were concentrated to give ethylacetate fraction (19.20 g, 21.69%), butanol fraction (35.52 g, 40.13%), and aqueous fraction (28.82 g, 32.56%). The partitioning of the methanolic extract into butanol and ethylacetate was to locate the fraction with the active principle, particularly to see if the polar medium (butanol) or the non-polar medium (ethylacetate) contains the active pharmacological principle. 20ml stock solution of each extract and fraction was prepared by dissolving 2g of the extract in 20ml 0.9% saline to give a100 mg/ml solution for some of the tests. Equal amount of the ethylacetate fraction was dissolved in DMSO (3%) then diluted with 0.9% saline.

Animals for the behavioural Study

Male Swiss mice (20-27g) were used for the experiments. The animals were bred and housed in a well lit and aerated room (temperature 26±1°C, relative humidity 66%) in the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals had free access to drinking water and standard commercial diet (Guinea Feeds Brand, Bendel Feeds and Flour Mills Ltd, Nigeria). The cages were cleaned once a week and five mice were housed in a standard cage. The animal experiments were carried out according to the protocol adopted by the committee on animal use and care of the Obafemi Awolowo University, Ile-Ife, Nigeria.

Chemicals and Drugs

The drugs used for the experiments in this report are: amphetamine sulphate (Sigma Chemical Co, St Louis, USA), R(-) apomorphine hydrochloride hemihydrate (Sigma Chemical Co, St Louis, USA). Methanolic (SCCR) extract, Butanolic (SCBT), Aqueous (SCAQ) and Ethylacetate (SCEA) fractions of S.cayennensis. All drugs and extracts were administered dissolved in 0.9% saline on each day of the experiment, except apomorphine which was suspended in 0.2% Tween 80/water and ethylacetate fraction that was dissolved in 3% DMSO/water.

Acute Toxicity Tests

The method described by Hays [20] was used in determining LD$_{50}$. The experiment was done with graded doses of the methanolic extract, butanol, aqueous and ethylacetate fractions (20-140 mg/kg, i.p.) of the leaves of S.cayennensis in mice (n=10 for each dose level) to see the effect of the extract on some behavioural parameters for the 30 minutes duration of the test for each mouse. Observations of both the activities of the mice during the time of the experiment and the behavioural pattern at all doses employed were noted. The time of death and the pattern of death were noted immediately and over 24 hours. The control group received saline equivolume to the test substances. The LD$_{50}$ was calculated using the arithmetic method of Spearman Karber [20].

Amphetamine and apomorphine-induced stereotyped behaviour in mice

The method of Siqueira et al [21] was used in determining the effect of the test extracts on the amphetamine and apomorphine-induced stereotypy. Mice were allowed a minimum of 30 minutes to acclimatize to the observation cages prior to the experiments. Test drugs (extracts) were given 30 minutes before (d)-amphetamine (35mg/kg, i.p) and R(-) apomorphine hydrochloride hemihydrates (1mg/kg, s.c). Each mouse was individually observed for 2 minutes in perspex observation cages (45 x 25 x 25 cm) at 10, 20, 30 and 45 minutes after (d, l) amphetamine and R(-) apomorphine hydrochloride hemihydrates. Stereotyped behavior was scored as follows: Complete absence of stereotyped behaviour (O); presence of stereotyped movements of the head and intermittent sniffing (1); sniffing and chewing (2), chewing and intense licking (3). The assessment was rated by a skilled independent observer who was blind to the treatment.

REFERENCES

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