

Absence of anxiolytic activity of *Sarcocephalus latifolius* fruit extract

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Abstract

Introduction: The use of pharmacological agents in the treatment of anxiety disorders have fallen out of favor as their unwanted side-effects have become evident. These presenting challenges call for an inward look into harnessing the full potential of medicinal plants that abound around us. **Aim:** This study aimed at evaluating the anxiolytic activity of ethanolic fruit extract of *Sarcocephalus latifolius* in mice. **Materials and Methods:** The prepared extract at 200, 400, and 600 mg/kg as well as 2.5 mg/kg of diazepam reference standard was administered orally. Anxiolytic activity of the extract was explored using elevated plus-maze and open-field models. **Result:** In the elevated plus-maze, the extract possessed insignificant ($P > 0.05$) anxiolytic effect by decreasing the time spent in open arms and entries into the open arms. However, the time spent in the closed arms increased significantly in the extract treated groups compared to the reference standard. In the open-field model, no significant ($P > 0.05$) locomotor activity was observed in the extract groups. The number of locomotion was less in the extract groups compared the reference standard having the highest locomotive activity. Furthermore, there were reduction in the number of rearing at extract doses of 400 and 600 mg/kg compared with the normal saline and reference standard. **Conclusion:** The results of this study showed that the ethanolic fruit extract of *S. latifolius* lack anxiolytic activity.

Key words: Anxiolytic activity, diazepam, elevated plus-maze, open-field models, *Sarcocephalus latifolius*

INTRODUCTION

Anxiety disorders represent the most common forms of psychiatric illnesses,^[1] prevalence among children, and adults. Its prevalence changes during childhood and adolescence,^[2] affecting about one-eighth of the total world population.^[3] Anxiety disorders may cause severe distress over a period of time,^[4,5] and disrupt the lives of individuals suffering from them.^[4,5] The frequency and intensity of anxiety involved in these disorders is often debilitating.^[6] The causes of anxiety

disorders still remain unclear, but studies have implicated genetic, environmental factors, psycho-stimulating drugs,^[7] mental illnesses, and brain injury as possible causes. Anxiety disorders are suspected whenever features such as uncontrollable worry, tension, fear, phobias, limited abnormalities of thought, previous trauma flashbacks present itself often.^[8] Anxiety disorders are managed with psychotherapy and medications. Psychotherapy techniques is generally considered as the first line treatment most especially in children and adolescents,^[9] which may be used alone or in conjunction with medications such as antidepressants and sedative anti-anxiety agents in severe anxiety disorders.^[9] High relapse rate have been reported with the use of pharmacological agents in the treatment of anxiety disorders.^[10] The use of pharmacological agents in the treatment of anxiety disorders have fallen out of favor as their unwanted side-effects have become evident.^[11] These presenting challenges call for an inward look into harnessing the full potential of medicinal plants that abound around us.

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Sarcocephalus latifolius commonly called African peach is a multi-stemmed shrub with irregular and dense foliage that grows up to 12 m. It is predominantly found in Africa and some parts of Asia. *S. latifolius* have a wide range of medicinal applications, which includes: cough remedy, diabetes, malaria treatment,^[12] diarrhea, and central nervous system diseases such as epilepsy.^[13,14] Decoction root extract of *S. latifolius* has been reported to have anticonvulsant, anxiolytic, and sedative properties.^[14] The aim of this study was to evaluate the anxiety activity of the ethanolic extract of *S. latifolius* on animal anxiety models.

MATERIALS AND METHODS

Plant material

The fresh fruits of *S. latifolius* were obtained from Makurdi, Benue State, Nigeria. The plant was identified and authenticated by Ikechukwu Chijioke of Federal College of Forestry Jos. The collected fruits were sliced, washed and air-dried at 25°C for 2 weeks, then crushed into coarse powder.

Extraction of plant materials

A total of 80 g of the powdered fruit was measured and dissolved in sufficient quantity of ethanol for 24 h with mechanical shaking (4 h/day), at the end of 24 h; the mixture was filtered with ashless filter paper. The extract was concentrated using rotary evaporator at a temperature of 4°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was later stored in the refrigerator at 4°C.

Phytochemical screening

Phytochemical screening of the ethanolic fruit extract of *S. latifolius* was carried out using standard procedure described by Trease and Evans.^[15]

Experimental animals

Swiss albino mice of either sex weighing 20-28 g were obtained Benue State University, Nigeria. The mice were acclimatized for 2 weeks to laboratory conditions in the Animal Unit of the University of Jos, Nigeria. The mice were housed in plastic cages in a ventilated room at temperature of 20°C ± 0.6°C fed with standard rodent chow and allowed free access to potable water. All experiment was carried out in accordance to the experimental procedure of the Animal Unit of the University.

Oral acute toxicity study

Modified Lorke's method was used in the LD₅₀ study,^[16] of ethanolic fruit extract of *S. latifolius*. This test was carried out in two phases. In the first phase, nine mice

randomized into three groups of three mice each, were given 10, 100, 1000 mg/kg of the prepared extract orally. The mice were observed at the very first 4 h and subsequently daily for 7 days for any behavioral sign of toxicity. The same procedure as used in first one was adopted in phase two but with different dose levels of 1600, 2900, and 5000 mg/kg.

Ethic

The anxiolytic study was carried out to the "Principles of Laboratory Animal Care,"^[17] and in accordance to standard experimental procedure approved by the Ethical Committee of Animal House, Department of Pharmacology University of Jos after filling of the ethic form.

Elevated plus-maze model

The elevated plus-maze model was carried-out using the method described by Lister.^[18] The elevated plus-maze consists of two open arms (25 cm × 10 cm each), and two closed arms (25 cm × 10 cm × 10 cm each), with an open roof. All four arms were radiated from a central platform (10 cm × 10 cm). The maze is elevated to a height of 60 cm in a dimly lit room. Normal Saline (10 ml/kg, orally), plant extract (200, 400 and 600 mg/kg, orally) and diazepam (2.5 mg/kg, orally) were administered to groups of five mice each. One hour posttreatment, each mouse was placed in the center of the elevated plus-maze, facing one of the closed arms. During a 5 min test period, the following parameters were taken: The number of entries and time spent in the open and enclosed arms. Entry into an arm was recorded when the mice cross the demarcation of respective arm with its four paws, and was considered to be on the central platform whenever two paws were on it.

Open-field model

Each mouse was placed in an open-field apparatus (45 cm × 45 cm × 40 cm), made of wooden floor and glass sides. The floor was carved into nine equal sized squares (15 cm × 15 cm). An hour before dropping the individual mice in one of the corner of the box (i.e., 60 min prior), the different groups were administered with respective treatments (normal saline, diazepam 2.5 mg/kg, extract doses of 200 mg/kg, 400 mg/kg and 600 mg/kg) and then locomotion (number of both central and peripheral crossings), number of rearing were recorded for 5 min.

Statistical analysis

The results obtained from the study were expressed as mean ± standard error mean (SEM). Statistical significance was determined by one-way ANOVA followed Dunnett post-hoc test and values of $P < 0.05$ were considered as significant.

RESULTS

Phytochemical screening

Phytochemical screening of ethanolic extract of *S. latifolius* revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, cardiac glycosides, steroids, saponins, and anthraquinones.

Acute toxicity study

The LD₅₀ was estimated to be >5000 mg/kg body weight. No mortality was recorded at all the entire experimental dose levels used in the acute toxicity study.

Effect of *Sarcocephalus latifolius* on various parameters in the elevated plus-maze model

Extract doses of 200, 400, and 600 mg/kg used in the study possessed insignificant ($P > 0.05$) anxiolytic effect by decreasing the time spent in open arms and entries into the open arms. However, the time spent in the closed arms increased significantly in the extract treated groups compared with the reference standard. More so, the time spent in the center was less in the extract treated groups compared with the normal saline and the reference standard [Table 1].

Effect of *Sarcocephalus latifolius* on various parameters in the open-field model

No significant ($P > 0.05$) locomotor activity was observed in the extract groups. The number of locomotion was less in the extract groups compared to the reference standard having the highest locomotor activity. Furthermore, there was the reduction in the number of rearing at extract doses of 400 and 600 mg/kg compared to the normal saline and reference standard [Table 2].

DISCUSSION

Anxiety disorders present a pattern response that display two emotional state: Fear and anxiety.^[19] The distinction between the two emotional state lies in the concept that the former is a response to the actual threat while the latter is anticipatory response to the potential threat.^[20,21] Animal anxiety models are widely employed in the

screening of anxiolytic agents with the view of analyzing the pathological state of anxiety in the assumption that some anxiety states are essential mechanism for survival and are the feature of all mammals.^[22] This study aimed at evaluating the anxiolytic activity of the ethanolic extract of *S. latifolius* in mice with the use of elevated plus-maze and open-field models.

The anxiolytic makers commonly associated with anxiolytic agents in the elevated plus-maze model are increase in time spent in the open arms as well as increase in the frequency of crossing the intersection.^[11] These makers are important parameters that validate test agents with anxiolytic property. The extract showed insignificant ($P < 0.05$) anxiolytic effect in all the experimental dose levels on the time spent in the open arms as well as the number of entries into the open arms when compared with the reference standard. Animal in the extract treated and normal saline groups spent more time in the closed arms avoid the open arms probably to avoid falling off.^[11] Avoidance of the open arm is also an index that measure anxiety in rodents.^[23] Furthermore, avoidance of the open arms clearly demonstrates fear response,^[24] with the display of anxiety related behaviors.^[25] However, the extract caused significant ($P < 0.05$) increase in the time spent in the closed arms and entries into the closed arms at extract doses of 400 and 600 mg/kg. This increase clearly demonstrated the lack anxiolytic activity of the extract.

The reference standard showed anxiolytic effect with significant ($P < 0.001$) increase in the anxiolytic makers, compared to the extract treated and normal saline with a decrease in time spent in the closed arms and entries. Anxiolytic agents can increase this effect and increase number entries in the open arm.^[25] Reduction in open arm activity in the extract treated group elucidate the highest level of anxiety in rodents. The decrease in the time spent in the open arm predictably illustrates the absence of Anxiolytic property of the extract. Decoction preparation of the root of *S. latifolius* have been reported to possess anticonvulsant, anxiolytic and sedative properties,^[14] but in this present study the ethanolic fruit extract lack anxiolytic property.

Table 1: Effect of ethanolic fruit extract of *Sarcocephalus latifolius* on elevated plus-maze

Treatment	Time spent in seconds			Number of entries	
	Open arms	Closed arms	Centre	Open arms	Closed arm
Normal saline 5 mg/kg	42.00±16.93	215.20±21.59	42.80±5.44	3.80±1.16	7.80±1.28
200 mg/kg	38.20±20.37	256.00±21.06	8.80±3.31**	0.80±0.20	1.40±0.24***
400 mg/kg	15.20±3.92	271.80±3.11*	12.80±1.83**	1.40±0.40	1.40±0.51***
600 mg/kg	7.40±3.50	281.40±9.40*	9.20±5.95**	1.00±0.32	2.00±0.55**
Diazepam 2.5 mg/kg	186.00±17.26***	80.00±11.40***	33.40±9.56	5.60±1.36	8.40±1.21

Values expressed as mean± SEM, n=5. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ compare to control (one-way ANOVA followed Dunnett test).

Table 2: Effect of ethanolic fruit extract of *Sarcocephalus latifolius* and diazepam on the open-field model

Treatment	No. of locomotion	No. of rearing
Normal saline 5 mg/kg	10.00±6.12	5.60±0.93
200 mg/kg	13.80±4.31	6.60±0.81
400 mg/kg	3.40±1.78	1.40±0.50**
600 mg/kg	7.20±2.75	2.80±0.96
Diazepam (2.5 mg/kg)	2340±2.15	5.40±0.64

Values expressed as mean±SEM, n=5. ** $P < 0.01$ compare to control (one-way ANOVA followed Dunnett test).

The extract had no significant ($P < 0.05$) effect on the locomotor activity of the mice in the open-field model. The locomotor activity as well as the number of rearing decrease in the extract treated groups compared to the reference standard with 200 mg/kg having the highest number of rearing. The reference standard produced the highest locomotor activity. Locomotor activity is regarded as index of alertness and decrease lead to sedation as a result of reduced excitability of the central nervous system.^[26] Decrease in the locomotor activity in the extract treated group elucidate manifestation of anxiety and lack of anxiolytic activity of the extract.

CONCLUSION

The results of the study showed that ethanolic fruit extract of *S. latifolius* lack completely anxiolytic effect on the animal anxiety models used and does not substantiate it used in the treatment of anxiety disorders.

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