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Anxiogenic Like activity of *Sarcocephalus latifolius* Fruit Extract in Mice

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Abstract:

The use of pharmacological agents in the treatment of anxiety disorders have fallen out of favour as their unwanted side effects have become evident. The present challenges call for an inward look into harnessing the full potential of medicinal plants that abound around us. The present study evaluates the anxiogenic activity of ethanolic fruit extract of *Sarcocephalus latifolius* in mice. The prepared extract at 200, 400, and 600 mg/kg as well as 2.5 mg/kg of diazepam, the reference standard was administered orally. The anxiogenic activity of the extract was evaluated using elevated plus maze and open field models. In the elevated plus maze, the extract showed an anxiogenic effect in all the experimental dosage levels by decreasing the time spent and number within the open arms. Animals in the extract and physiological saline groups spent more time in the enclosed arms to avoid the open arms, probably to avoid falling off. The reference standard showed a significant (P<0.001) anxiolytic effect, evidenced by the increased number of entries into the open arms and prolonged time spent in the open arms and centre axis. In the open field model, decreased horizontal locomotor activity was observed in the extract groups. The degree of horizontal locomotion was less in the extract groups compared to the reference standard which had the highest horizontal locomotor activity. Also, there were reductions in the number of rearings at extract doses of 400 and 600 mg/kg. In conclusion, data from the present study suggested that the ethanolic fruit extract of *Sarcocephalus latifolius* is not only devoid of anxiolytic activity, but it appears to exert anxiogenic like effects.

Keywords: *Sarcocephalus latifolius, diazepam, anxiogenic activity, elevated plus maze, and open field models*

1. Introduction:

Anxiety disorders represent the most common forms of psychiatric illnesses,[1] prevalent 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 lead to severe distress over a period of time,[4-5] and disrupt the lives of individuals suffering from them.[4-5] The intensity and frequency 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 like uncontrollable worry, tension, fear, phobias, limited abnormalities of thought, and previous traumatic flashbacks present themselves often.[8] Anxiety disorders are managed with psychotherapy and medication. Psychotherapy techniques are generally considered as a first line of treatment 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 rates 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 has fallen out of favour as their unwanted side effects have become evident.[11] These challenges call for an inward look into harnessing the full potential of medicinal plants that abound around us.

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

2. Materials and Methods:

Plant Material

The fresh fruits of *Sarcocephalus 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^{o}C for two weeks, then crushed into coarse powder.

Extraction of Plant Materials

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

Phytochemical Screening

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

Experimental Animals

Swiss albino mice of either sex, weighing 20-28 grams were obtained from Benue State University, Nigeria. The mice were acclimatized for two 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 a temperature of 20±0.60C, fed with standard rodent chow and allowed free access to potable water. All experiments were carried out in accordance with the experimental procedures of the Animal Unit of the University.

Oral Acute Toxicity Study

Modified Lorke's method was used in the LD_{50} study[6] of ethanolic fruit extract of *Sarcocephalus 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 for the first four hours and subsequently daily for 7 days for any behavioural sign of toxicity. The same procedure as used in first one was adopted in phase two**,** but with different dosage levels of 1600, 2900 and 5000 mg/kg.

Ethic

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

Elevated Plus-Maze Model

The elevated plus-maze model was utilized by the method described by Lister.[18] The elevated plus-maze consists of two

open arms (25×10cm each), and two closed arms (25×10×10cm each) with an open roof. All four arms were radiated from a central platform (10×10cm). The maze is elevated to a height of 60 cm in a dimly lit room. Physiological 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 5 mice each. One hour post treatment, each mouse was placed in the centre 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 the 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 × 45 × 40cm), made of a wooden floor and glass sides. The floor was carved into 9 equal sized squares (15 × 15 cm). An hour before dropping the individual mice into one of the corners of the box (i.e. 60 min. prior), the different groups were administered with respective treatments (Physiological saline, diazepam 2.5 mg/kg, extract doses of 200 mg/kg, 400 mg/kg and 600 mg/kg) locomotion and number of rearings were recorded for 5min.

Statistical analysis

The data obtained from the study was expressed as a mean±S.E.M (standard error mean). Statistical significance was determined by one-way ANOVA followed Dunnett post-test and values of $P<0.05$ were considered significant. The analysis was performed using instant graphpad prism (version 5.02)

3. Results and discussions

Phytochemical Screening

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

Acute Toxicity Study

The LD_{50} was estimated to be greater than 5000 mg/kg body weight. No mortality was recorded at all of the entire experimental dosage 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 showed significant ($P<0.05$) anxiogenic effects, increasing the time spent in closed arms and entries into the closed arms compared to the reference standard. More so, the time spent in the centre was less in the extract treated groups compared to the physiological saline and the reference standard.

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

Treatment	Time spent in seconds			Number of entries	
	Open arms	Closed arms	Centre	Open arms	Closed arms
Physiological Saline (5 ml/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 2.5mg/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)

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

Decreased horizontal locomotor activity was observed in the extract groups. The degree of horizontal locomotion was less in the extract groups compared to the reference standard having the highest horizontal locomotor activity. Also, there was a reduction in the number of rearings at extract doses of 400 and 600 mg/kg compared to the physiological saline and reference standard.

Table II: Effect of aqueous root extract of *Sarcocephalus latifolius* and diazepam on open field model

Treatment	No. of horizontal locomotion	No. of rearings
Physiological saline (5 ml/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)	23.40±2.15	5.40±0.64

Values expressed as mean±SEM, n=5, ** (P<0.01)

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

Discussion

Anxiety disorders present a pattern response that display two emotional states: fear and anxiety.[19] The distinction between the two emotional states lies in the concept that the former is a response to an actual threat while the latter is an anticipatory response to a potential threat.[20-21]Animal anxiety models are widely employed in the screening of anxiolytic and anxiogenic like agents, with the view of analyzing the pathological state of anxiety and assumption that some anxiety states are essential mechanisms for survival and are a feature of all mammals.[22] The present study was aimed at evaluating the anxiogenic activity of the ethanolic extract of *Sarcocephalus latifolius* in mice with the use of elevated plus maze and open field models.

The anxiogenic makers commonly associated with anxiogenic agents in the elevated plus maze model decrease the time spent in the open arms as well as decrease the frequency of crossing the intersection.[23] These makers are important parameters that validate test agents with anxiogenic properties. The extract showed anxiogenic effects in all the experimental dosage levels by decreasing the time spent and number of in the open arms. Animals in the extract

and physiological saline groups spent more time in the closed arms avoiding the open arms, probably to avoid falling off.[11] Avoidance of the open arm is also an index that measures anxiety in rodents.[24] Also, avoidance of the open arms clearly demonstrates a fear response,[25] with the display of anxiety related behaviours.[23] However, the extract caused a 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 reflects the anxiogenic effects of the extract.

The reference standard showed a significant (P<0.001) anxiolytic effect evidenced in the increased number of entries into the open arms and prolonged time spent in the open arms and centre axis. Anxiolytic agents can increase this effect and increase the number of entries in the open arm.[23] Reduction in open arm activity in the extract treated group elucidates the highest level of anxiety in rodents. The decrease in the time spent in the open arm predictably illustrates the anxiogenic activity of the extract. Decoction preparation of the root of *Sarcocephalus latifolius* has been reported to possess anticonvulsant, anxiolytic and sedative properties,[14] but in this present study the ethanolic fruit extract showed anxiogenic properties

The horizontal locomotor activity as well as the number of rearings in the open field model decreased in the extract treated groups compared to the reference standard, with 200 mg/kg having the highest number of rearings (table II). The reference standard produced the highest horizontal locomotor activity. Locomotor activity is considered as an indicator of alertness and decreases could lead to sedation as a result of reduction in the central nervous system excitability.[26] A decrease in the locomotor activity of the extract treated group elucidates the manifestation of anxiety and lack of anxiolytic activity of the extract.

Conclusion

Data from the present study suggests that the ethanolic fruit extract of *Sarcocephalus latifolius* is not only devoid of anxiolytic activity, but it appears to exert anxiogenic like effects.

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