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Comparative in-*vivo* anxiolytic efficacy of aqueous and methanol *Tapinanthus globiferus* leaf extracts

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ABSTRACT

Anxiety disorders have been known to be highly prevalent and to exert heavy disease burden on the health delivery system. The increasing prevalence is not helped by the fewness and individual drawbacks of the currently prescribed anxiolytic drugs. This situation calls for discovery of new anxiolytic agents. This study investigated the in-vivo anxiolytic efficacy of intraperitoneal injection of 50, 150 and 500 mg/kg aqueous and methanol leaf extracts of *T. globiferus*, 0.5 mg/kg diazepam and 10 ml/kg distilled water in mice using standard test battery of open-field and elevated zero-maze tests. While treatment with the aqueous *Tapinanthus globiferus* leaf extract caused significant ($p<0.05$) alterations compared to the negative control in all the four rodent anxiety parameters evaluated which comprised percentage centre zone time (%CZT), rears, percentage open segment time (%OST) and unprotected head dips (UHDs); its methanol counterpart only caused significant alterations in only three parameters which comprised percentage centre zone time (%CZT), rears, percentage open segment time (%OST). Overall, these results indicate though both aqueous and methanol leaf extracts of *T. globiferus* grown on *Azadirachta indica* possess anxiolytic effects, the former however, exhibited superior anxiolytic potency over the latter and these extracts need be developed further for potential anxiety-alleviation use.

Keywords: In-*vivo*, anxiolytic efficacy, *Tapinanthus globiferus*, test battery, mice, optimization

INTRODUCTION

Anxiety disorders are pathological anxiety states characterized by sustained pervasive feelings of unpleasantness and unease. These disorders, in the various forms as selective mutism, social anxiety, generalized anxiety, separation anxiety, agoraphobia and panic disorder, have been reported to be a highly prevalent class of mental disorders that may be affecting up to a third of the general population.[1] Pharmacotherapy remains the cornerstone of management of these disorders. But drug treatment is still faced with delayed or sub-optimal efficacy, or intolerable toxicities in the long term.[2,3] The gap thus created by the foregoing indicates a need to search for new anxiety-alleviating agents. Research has shown that a number of medicinal plants (e.g. *Passifloraincarnata*, *Albiziajulibrissin*, *Passifloraincarnata*, *Sonchusoleraceus*, *Withaniasomniferum*, *Uncariarhynchophylla*,) have exhibited significant anxiolytic potential in previous human and animal studies.[4] *Tapinanthus globiferus* is one of the numerous *Tapinanthus* species commonly known as African mistletoes (evergreen dioecious plants belonging to the Loranthaceae family).[5-7] It is a parasitic plant commonly seen on Neem and other trees and it is regarded traditionally as an all-cure plant with reported ethmo-medicinal efficacy in nervousness, raised blood pressure, seizures, neoplasms and hyperglycemia.[8]

Reported pharmacological activities of *Tapinanthus globiferus* include antioxidant and anticonvulsant,[9] anti-inflammatory, hepatoprotective[10] and antimicrobial[11] effects. Despite its traditional use to alleviate anxiety in Sokoto and the surrounding towns and villages; and a reported anxiolytic effect in an animal study of a leaf extract of *Azardirachta indica* (neem)[12] (i.e., the host tree of the *Tapinanthus globiferus* under investigation), there has not been any scientific report on the anxiolytic activity of the extracts of this plant. Hence, this study aims to investigate the anxiolytic activity of the aqueous and methanol leaf extracts of the plant in Swiss Albino mice using standard rodent behavioural battery of open-field and elevated zero-maze tests.

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MATERIALS AND METHODS

Fresh leaves of *Tapinanthus globiferus* grown on a Neem tree located along Shuni road, Mabera, Sokoto; Sokoto State, Nigeria, were collected in the month of March 2019. They were then briefly washed, dried under a shade, ground to a powder and kept dry in opaque plastic containers for later use. The leaves were identified by a Botanist. Specimens were vouchers kept at the Herbarium of the Department of Botany, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria. The plant part was extracted in the laboratory of the Pharmacognosy Drug Development Department, and the behavioural experiments took place in the behavioural room of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna state.

Two hundred and fifty grams (250g) of fine powders of *Tapinanthus globiferus* leaves was soaked and allowed to macerate in 1 L of distilled water or 70% methanol for 24 hours. They were then filtered using Whatman's paper (150 mm) and separately evaporated in rotatory water bath at 45-50 degree Celsius. The drying process produced 30.53 g of brownish aqueous dry extract (12.21%) and 31.45 g of greenish methanol paste (12.58%) of *Tapinanthus globiferus* leaf powder.

The behavioural anxiolytic efficacy study on these extracts was carried out in a way similar to the procedure earlier adopted by Schmitt and Hiemke[13] (modified by reducing the inter-test delay to almost zero). Forty-five minutes after intraperitoneal injection of 50, 150 or 500 mg/kg of plant extracts, 0.5 mg/kg diazepam or 10 ml/kg distilled water, randomized groups (n = 10) of male mice under good laboratory practices, were each subjected to a test battery of open-field and elevated zero-maze in opaque cubicles having 100-lux illumination. Each run of behavioural assay was begun with by placing each mouse in the centre of the open field and allowed to freely move and explore the field for 5 minutes. At the end of this period, each mouse was again transferred to the mid-point of an open segment of the elevated zero-maze with the mouse facing one of the closed segments. The animal was allowed to freely explore its new environment for 5 minutes. Animals that jumped off the open segment of the zero-maze during the procedure were gently returned immediately to the test. Video recordings were made of the behavioural studies in addition to visual monitoring. Data were analyzed using analysis of variance (ANOVA) followed by Turkey post hoc test. *P*-values less than 0.05 were considered as significant.

Figure 1: *Tapinanthus globiferus* (with leaves and fruit berries) grown on Neem tree
(Source: Corresponding author)

RESULTS

Anxiolytic activity of aqueous and methanol *Tapinanthus globiferus* leaf extracts in mice

Administration of the aqueous *Tapinanthus globiferus* (ATG) leaf extract caused significant ($p < 0.05$) alterations compared to the negative control in all the four rodent anxiety parameters evaluated (Tables 1-4); its methanol counterpart (MTG) only caused significant alterations in only three parameters. On the percentage centre zone time (%CZT), ATG leaf extract at 150 mg/kg dose exhibited best mean value of 11.67±4.96 compared to the methanol *T. globiferus* (MTG) with an 8.06±4.91best mean value at the same dose level. On the number of rears, MTG exhibited superior dose-dependent and significant ($p < 0.05$) reductions in this parameter at all dose levels compared to ATG with significant ($p < 0.05$) anxiolytic activity only on the 150 and 500 mg/kg dose levels. On the number of rears, MTG exhibited superior dose-dependency and significant ($p < 0.05$) reductions in this parameter at all the three dose levels compared with ATG with significant anxiolytic effect on this parameter at only 150 and 500 mg/kg dose levels.

On the percentage open segment time (%OST), while ATG treatment caused significant ($p < 0.05$) increases in this parameter on the 150 and 500 mg/kg dose levels, MTG only caused significant increase in mean %OST only at the 150 mg/kg dose level. Additionally, on this same parameter, ATG's best mean %OST of 44.27±6.24 was superior to MTG's best of 38.50±5.74. On the unprotected head dips (UHDs), ATG increased incidence of head dips that was significant ($p < 0.05$) at 150 mg/kg dose level, while MTG did not record any significant ($p > 0.05$) anxiolytic activity on this parameter at any dose level. ATG's mean UHDs of 10.33±2.33 at the 150 mg/kg dose was comparable to 9.25±1.61 mean UHDs of 0.5 mg/kg diazepam treatment. Overall, these results indicate that though both leaf extracts of *T. globiferus* grown on *Azadirachta indica* possess anxiolytic effects, the former however, exhibited superior anxiolytic potency over the latter.

Table 1: Per cent centre zone time of mice on open-field test

Treatments groups	Extracts' doses		
	50mg/kg	150mg/kg	500mg/kg
D/water (10ml/kg)	2.12 ± 0.73	2.50 ± 0.51	1.81 ± 0.35
Aqueous *T. globiferus*	7.03 ± 2.99*	11.67 ± 4.96	5.28 ± 0.84*
Methanol *T. globiferus*	4.45 ± 1.53	8.06 ± 4.91*	2.21 ± 0.29
Diazepam (0.5mg/kg)	6.79 ± 1.47*	13.64 ± 3.33*	6.21 ± 1.15*

Data were entered as mean ± S.E.M. of mice; *Statistically significant ($p < 0.05$)

Table 2: Number of rears of mice on open-field test

Treatments groups	Extracts' doses		
	50mg/kg	150mg/kg	500mg/kg
D/water (10ml/kg)	18.73 ± 4.06	22.33 ± 5.63	17.67 ± 3.49
Aqueous *T. globiferus*	9.00 ± 2.53	7.33 ± 3.09*	5.50 ± 1.52*
Methanol *T. globiferus*	6.18 ± 2.11*	5.42 ± 1.06*	5.36 ± 2.11*
Diazepam (0.5mg/kg)	4.64 ± 1.38*	6.92 ± 2.23*	7.15 ± 1.76*

Data were entered as mean ± S.E.M. of mice; *Statistically significant ($p < 0.05$)

Table 3: Per cent open segment time of mice on elevated zero-maze test

Treatments groups	Extracts' doses		
	50mg/kg	150mg/kg	500mg/kg
D/water (10ml/kg)	18.61 ± 2.76	21.45 ± 3.54	19.08 ± 3.87
Aqueous *T. globiferus*	24.67 ± 8.16	44.27 ± 6.24*	39.92 ± 7.56*
Methanol *T. globiferus*	24.82 ± 6.42	38.50 ± 5.74*	33.72 ± 10.09
Diazepam (0.5mg/kg)	36.75 ± 9.20*	43.25 ± 5.39*	42.39 ± 7.98*

Data were entered as mean ± S.E.M. of mice; *Statistically significant ($p < 0.05$)

Table 4: Number of unprotected head dips (UHDs) on elevated zero-maze test

Treatments groups	Extracts' doses		
	50mg/kg	150mg/kg	500mg/kg
D/water (10ml/kg)	4.36 ± 0.94	3.58 ± 0.99	3.83 ± 1.07
Aqueous *T. globiferus*	7.36 ± 1.98	10.33 ± 2.33*	5.83 ± 1.33
Methanol *T. globiferus*	7.72 ± 2.15	5.83 ± 1.40	3.92 ± 0.87
Diazepam (0.5mg/kg)	9.00 ± 1.88*	9.25 ± 1.61*	13.33 ± 3.34*

Data were entered as mean ± S.E.M. of mice; *Statistically significant ($p < 0.05$)

DISCUSSION

The existing and increasing gap between the high prevalence and attendant huge health costs of anxiety disorders on one hand, and the fewness and individual drawbacks of the synthetic anxiolytic drugs in current use on the other, is a clarion call for renewed efforts targeted at discovering new anxiolytic agents. It is in this light that this study carried out an in-*vivo* anxiolytic efficacy screening of the aqueous and methanol of *Tapinanthus globiferus* leaf extracts in mouse anxiety tests of open-field and elevated zero-maze with the overall goal of finding potential anxiolytic factors. The effective and indispensable roles of the rodent anxiety tests especially the widely used open-field and elevated zero-maze paradigms in anxiolytic drug discovery have been severally reported.[14-17]

In this study both aqueous and methanol *Tapinanthus globiferus* leaf extracts exhibited significant anxiolytic activities compared to the negative control on all the rodent anxiety parameters evaluated. The four rodent parameters i.e. percentage centre zone (% CZT) and rears in the open-field test and the percentage open segment time (% OST) and number of unprotected head dips (UHDs) have been shown to be fundamental anxiety parameters in rodents (with decreased rears, increased % CZT, % OST and UHDs indicating reduced anxiety levels).[18,19] Although both extracts have demonstrated significant anxiolytic effect in mice in this study, the finding of superior anxiolytic activity of the aqueous over the methanol extract suggests that the former has greater quantity of the active chemical entities than the latter.

This finding may justify the traditional use of water decocts from this plant for anxiety relief. The outcome of this research may constitute the first report of anxiolytic activity on the leaf extracts of *Tapinanthus globiferus* grown on *Azadirachta indica*. Antidepressant effects have earlier been associated with a methanol extract of the same plant in mice.[20] The finding is also similar to the outcome of a previous study in which a crude aqueous stem bark extract of another specie (*Tapinanthus dodoneifolius*) was found to exhibit anxiolytic and antidepressant activity.[21]

CONCLUSION

This study has shown that aqueous and methanol leaf extracts of *Tapinanthus globiferus* grown on *Azadirachta indica* possess anxiolytic property and need to be further investigated for potential use as anti-anxiety agents.

Source of support

Nil.

Conflict of interest

None declared.

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