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Original Research Article

Antidepressant Activity of Methanol Extract of *Tapinanthus globiferus* (A. Rich) Tiegh in Swiss Mice

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ABSTRACT

Depression has been a global public health issue for a while and has prompted many researches to develop safer and more effective treatment of depressive illness. It affects how an individual feel, think, relate to others and handle daily activities. The aim of this study is to evaluate the antidepressant activity of the methanol extract of *Tapinanthus globiferus* in acute model of depression in mice. Phytochemical screening was carried out to identify the different phytochemicals present in the extract. Acute toxic effect of the extract was determined using OECD method. The antidepressant activity of the extract was evaluated using tail suspension test, forced swim tests and confirmed with the open field test.
The methanol extract of *T. globiferus* contains some phytochemicals some of which have been shown to have antidepressant activity, it was also shown to be practically nontoxic using the OECD guidelines. The extract significantly reduced the immobility time in Tail suspension test but not in the forced swim test. In addition, locomotor activity in open filed test was not significantly affected implying that the extracts activity is not due to locomotor stimulation
The result of the study revealed that the methanol extract of *T. globiferus* has antidepressant activity.

***Keywords*:** *Tapinanthus globiferus*, depression, antidepressant, immobility, forced swimming, tail suspension.

Introduction

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, tendencies, feelings and sense of well-being.[1] It is a common but serious mood disorder.[2] It causes severe symptoms that affect how an individual feel, think, and handle daily activities, such as sleeping, eating, or working.[3] Depression is one of the most prevalent and life-threatening forms of mental illness that affects about 21% of the world's population.[4] The pathophysiology of depression has been difficult to elucidate due to the heterogeneity of its clinical presentation and aetiology. Hence, many theories have been put forward which focuses on psychological stress, stress hormones, neuro-circuitry and neurotropic factors, circadian rhythm and neurotransmitters.[5] The multiple etiological factors have made finding the right antidepressant difficult, as the prevalence of depression continues to go up despite dramatic increase in antidepressant use.[6] *Tapinanthus globiferus* is the most common mistletoe that grows on *Vitellaria paradoxa* tree (host) in West Africa.[7] It is a plant with glabrous pendulous stems up to 1.2 m long with roots that mostly grow on the branches of the host tree species such as *Vitellaria*, Kola, Citrus, Acacia etc. The leaf is known for its use in traditional medicine to treat inflammations, malaria, and headaches, bacterial infections, diabetes mellitus etc.[8] Shehu *et al.*, reported its use as an antidepressant in Northwestern Nigeria.[9] The aim of this study was to evaluate the antidepressant activity of the methanol extract of *Tapinanthus globiferus* in mice.

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Materials and Methods

Drugs and chemicals
Fluoxetine tablets 20 mg (V.S International Pvt Ltd. Dabhel, India), Normal Saline (Sigma-Aldrich, USA), Methanol (Sigma-Aldrich, USA), T. globiferus extract.

Laboratory animals
Mice of both sexes weighing 18-23g used were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria. The animals were kept in well-ventilated and hygienic cages, maintained under normal environmental conditions and fed *ad libitum* with laboratory rodent pellet and water. The experimental protocols adopted in this study were reviewed by the Ahmadu Bello University Committee on Animal Use and Care and ethical approval was given (ABUCAUC/2020/029).

Plant collection and identification
The plant sample comprising of the leaves attached to the stalk and fruits was collected from a bushy area behind *Samaru* community in Sabon-Gari Local Government Area of Zaria, Kaduna State on 31st January 2019. The plant was identified in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. A voucher specimen number of 09186 was assigned to the plant.

Plant preparation and extraction
The whole plant was shade-dried for 2 weeks to a constant weight and the dried plant was powdered using wooden mortar and pestle and subjected to cold maceration using 3 L of 70% methanol. The extract was concentrated to dryness in a water bath and subsequently termed methanol extract of *T. globiferus* (TG extract).

Phytochemical screening
Phytochemical screening was carried out on the plant extract based on the method described by Trease and Evans.[10] Thin layer

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chromatography was conducted to further ascertain the presence of phytoconstituents reported in the previous test.

Acute Toxicity Study

The median lethal dose of the extract was estimated using the OECD 425 guideline.[11] A fixed dose of 5000 mg/kg of the extract was administered to one mouse and observed for signs and symptoms of toxicity and death in four hours. As the mouse survived, two more mice were dosed with the same 5000 mg/kg of the extract and were observed for another 24 hours for death and other signs and symptoms of toxicity.

Antidepressant study

Tail Suspension Test (TST)

Mice were pretreated one hour before the test with methanol extract of *T. globiferus.* The duration of immobility following tail suspension was measured according to the method described for evaluating potential antidepressants.[12] Mice were assigned to 5 different groups (n = 6 for each group). Group 1 received normal saline (10 mL/kg), groups 2, 3 and 4 received TG extract (1500, 750, 375 mg/kg) respectively while group 5 received fluoxetine (20 mg/kg). Mice were suspended individually on the edge of a table, 50 cm above the floor with the help of an adhesive tape of 10 cm placed approximately 1 cm from the tip of the tail. Immobility time was recorded during 6 minutes' period in different groups. The animal was considered to be immobile when it did not show any movement of the body and hanged passively.

Forced swimming test (FST)

Forced Swim Test as described by Porsolt was carried out.[13] Mice were assigned to 5 different groups (n = 6 for each group). Group 1 received normal saline (10 mL/kg), groups 2, 3 and 4 received TG extract (1500, 750 and 375 mg/kg) respectively while group 5 received fluoxetine (20 mg/kg). Mice were dropped one at a time into a Plexiglas cylinder (25 cm height, diameter 10 cm containing water to a height of 10 cm at 23–25°C) and observed for 6 minutes. A mouse was judged immobile if it floated in the water in an upright position and made only slight movements to prevent sinking. The total duration of immobility was recorded during the last 4 minutes of the 6 minutes test.

Open field test

The method was carried out as described by Rex *et al*,.[14] Each mouse was placed in an open field apparatus (70 × 70 × 35 cm, length × breadth × height) that had a transparent front view. The floor of the apparatus had 16 visible squares (15×15 cm) with one central square. Peripheral and central square crossing was recorded for 5 minutes. The arena was cleaned with 10% ethanol before and after placing each mouse into the set up to remove all faecal pellets and wipe up all spots of urination.

Statistical analysis

All data are presented as mean ± SEM. Data were analyzed by One Way Analysis of Variance (ANOVA) and followed by Bonferroni post hoc test (where necessary) using SPSS® software version 24.0. The level of significance for all tests was set at $p < 0.05$.

Results and Discussion

Acute toxicity test

The median lethal dose (LD_{50}) of *T. globiferus* methanol extract in mice was found to be greater than 5000 mg/kg body weight *P.O.*

Phytochemical screening

Preliminary phytochemical analysis of *T. globiferus* extract revealed the presence of carbohydrates, saponins, steroids, terpenoids, alkaloids, glycosides, flavonoids, and tannins.

Effect of Extract on Immobility Duration in Tail Suspension Test

The extract produced a significant ($p < 0.05$) reduction in immobility time at the highest dose (1500 mg/kg) when compared to normal saline treated group. The standard antidepressant drug (fluoxetine 20 mg/kg) also produced a significant ($p < 0.05$) effect compared to the negative control (Table 1)

Effect of extract on immobility duration in forced swim test

The extract produced an insignificant ($p>0.05$) reduction in immobility time compared to Normal saline group. On the other hand, fluoxetine (20 mg/kg) produced a significant reduction in immobility time compared to normal saline group (Table 2).

Effect of extract on open field test

The extract did not produce a significant difference ($p>0.05$) in line crossing at different doses and also in comparison with both fluoxetine (20 mg/kg) and normal saline group (Table 3).

T. globiferus has been used for hypertension, diabetes mellitus, inflammatory conditions, depressive illness etc. [15] Both forced swim test (FST) and tail suspension test (TST) are used for primary antidepressant screening.[16] The tail suspension test is best used to validate the antidepressant efficacy of drugs, as well as effects of environment, neurobiological, and genetic manipulations on behaviour of rodents and has no risk of hypothermia due to submersion in water in contrast to the forced swim test.[17]

Table 1: Effect of Methanol Extract of *T. globiferus* on Immobility Duration of Mice in Tail Suspension Test

Treatment	Dose (mg/kg)	Mean immobility Time (seconds)
N/S	10 mL/kg	183.34 ± 4.20
TG	1500	122.27 ± 3.25*
TG	750	141.73 ± 4.16
TG	375	145.00 ± 3.17
Fluoxetine	20	109.70 ± 2.16*

Immobility time are presented as Mean ± SEM, n= 6. Data was analysed using One-way ANOVA followed by Bonferroni post-hoc test. * = $p < 0.05$ vs Normal saline. Key: N/S = Normal Saline; TG = Methanol Extract of *T. globiferus*

Table 2: Effect of Methanol Extract of *T. globiferus* on Immobility Duration in Forced Swim Test

Treatment	Dose (mg/kg)	Mean Immobility Time (seconds)
N/S	10 mL/kg	202.20 ± 3.07
TG	1500	162.39 ± 2.23
TG	750	186.31 ± 2.23
TG	375	121. 87 ± 5.31
Fluoxetine	20	170.56 ± 4.40

Immobility time are presented as Mean ± SEM, n= 6. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test. * = $p < 0.05$ Vs Normal Saline. Key: N/S = Normal Saline; TG = Methanol Extract of *T. globiferus*.

Table 3: Effect of Methanol Extract of *T. globiferus* on Line Crossing of Mice in Open Field Test

Treatment	Dose (mg/kg)	Mean Number of Crossings
N/S	10 mL/kg	77.0 ± 2.0
TG	1500	65.0 ± 4.0
TG	750	71.0 ± 3.0
TG	375	78.0 ± 3.0
Fluoxetine	20	71.0 ± 2.0

Number of lines crossed are presented as Mean ± SEM, n= 6. Data was analysed using One-way ANOVA = $p > 0.05$ Vs Normal Saline. Key: N/S =Normal Saline; TG = Methanol Extract of *T. globiferus.*

The forced swimming test is well-known behavioural test in rodents that predicts the efficacy of many types of antidepressants and investigates the probable mechanism underlying their action.[18] Though it has its draw backs, represented by the possibility of obtaining some false positive or negative responses.[19] Psychostimulants were shown to reduce immobility in FST and TST as well as enhance motor activities contrary to antidepressants, which do not enhance motor stimulation.[20]
In the tail suspension test, the extract produced a significant antidepressant activity as compared to normal saline at the highest dose but at a lower mean value than the standard drug (fluoxetine). At the other doses the extract caused a decrease in immobility time compared to the negative control (normal saline) but was not statistically significant. Likewise, the effect of the standard drug (Fluoxetine) was not significant against any dose of the extract (Table 1). In the forced swim test, a dose dependent reduction in immobility time was noted when the groups that received the *T. globiferus* extract were compared to the negative control (normal saline) group. Although, there was no statistically significant difference between the groups when negative control (normal saline) and positive control (fluoxetine) group were compared to those that received the extract (Table 2) the open field test is usually important in the test of an antidepressant in order to rule out non-specific motor stimulation.[21] It is known that antidepressants do not increase the locomotor activity that is otherwise observed in psychostimulant drugs. This is achieved by comparing the number of line crossing of the group of animals given the extract to group given a known antidepressant.[22] In the open field test, the doses of the extract that were able to display antidepressant-like response (i.e. in TST), did not exhibit significant change in locomotion based on the number of line crossing. The mean number of crossings recorded for different doses of the extract and positive control (fluoxetine) were very similar as shown in Table 3. There was no statistically significant difference among the five groups. It is clear that the immobility time was much reduced in TST compared to FST, this can be explained by several factor one of which is that TST is a more sensitive test than FST.[23]
Also, experiments have shown that differences in performance in TST and FST using the same animals exists despite a face value similarity as a result of the difference in neurochemical pathways mediating performance in these two widely used tests.[24]
In this study, *T. globiferus* extract and fluoxetine did not affect locomotion, this indicates that their antidepressant activity is most likely specific and not related to the stimulation of general motor activity as observed with psychostimulants. The methanol extract of *T. globiferus* tested positive for an array of phytochemicals some of which have been suggested to have antidepressant activity like glycosides, carbohydrates and alkaloids.[9] Median lethal dose (LD_{50}) of the extract was found to be greater than 5000mg/kg via oral route. There was no death or sign of toxicity seen on observation. This may suggest that the safety profile of the drug is good and agrees with an earlier finding where non-toxic nature of *T. globiferus was* reported by Umarudeen and Magaji.[25]

Conclusion

The methanol extract of *T. globiferus* contains some phytochemicals with antidepressant activity, and was practically nontoxic using the OECD guidelines. The extract ameliorated anhedonia by reducing the immobility time in tail suspension test but not in the forced swim test. The extracts activity was shown not to be due to locomotor stimulation. The methanol extract of *T. globiferus* possesses antidepressant activity. This provides justification for the use of the plant in the management of depression in traditional medicine.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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