Trop J Nat Prod Res, February 2018; 2(2):87-91 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Antidepressant Effect of Methanol Stem Bark Extract of *Adansonia digitata* L. (Malvaceae) in Mice

Aishatu Shehu[1]*, Mohammed G. Magaji[1], Jamilu Yau [1], Bukhari Mahmud[1], Abubakar Ahmed[2]

[1]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Nigeria*
[2]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Nigeria*

ARTICLE INFO

Article history:
Received 22 December 2017
Revised 24 January 2018
Accepted 30 January 2018
Published online 07 February 2018

ABSTRACT

Adansonia digitata (Malvaceae) is a plant native to Africa with all its parts used extensively since ancient times in traditional medicine for different purposes including psychiatric disorders. The plant has been used ethnomedicinally as a remedy for depression. The aim of the study was to investigate the antidepressant activity of the methanol stem bark extract of *A. digitata*. Phytochemical screening and acute oral toxicity (LD_{50}) study were done using standard procedures. Antidepressant activity of the extract (250-1000 mg/kg) was evaluated using tail suspension test (TST) and forced swim test (FST) in mice. Tests for motor co-ordination deficit and stimulant activity were evaluated using beam walking assay (BWA) and open field test (OFT), respectively. The effect of the methanol stem bark extract of *A. digitata* was tested using the novel object recognition task (NORT). The phytochemical screening of the extract showed the presence of steroids, tannins, flavonoids, alkaloids and saponins. The LD_{50} was found to be greater than 5000 mg/kg orally. The extract significantly ($P < 0.01$) and dose-dependently decreased the duration of immobility in the TST and FST. There was no significant change in the number of lines crossed and on the number of foot slips in the OFT and BWA, respectively. The extract showed no significant effect on cognition in the NORT. In conclusion, the methanol stem bark extract of *A. digitata* possesses antidepressant activity.

Keywords: *Adansonia digitate*, depression, tail suspension test, forced swim test.

Introduction

Depression is one of the common psychiatric disorders affecting nearly 17% of the world population and the existing antidepressant drugs like selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs) are associated with numerous adverse effects such as dry mouth, constipation, urinary dysfunction, infertility, drowsiness and nausea which are distressing.[1] As a result, there is the need to search for new agents especially from natural sources for the treatment of depression. *Adansonia digitata* L. (family Malvaceae) is a multi-purpose tree native to Africa. Different parts of the plant have been used extensively since ancient times in traditional medicine for different purposes including the treatment of depression,[2] in addition to its use as food and non-food products such as fuel, timber, and fodder.[3] The plant is commonly called baobab tree, dead rat, monkey bread, upside down and cream of tartar tree. In Hausa, it is called 'Kuka'.[2] Every part of the baobab tree is reported to be edible and useful to treat ailments such as diarrhoea, malaria and microbial infections. It is an excellent anti-oxidant due to high vitamin C content (ten times higher than the content in oranges). Biological properties such as antimicrobial, antiviral, anti-oxidant and anti-inflammatory effects have been linked to Baobab.[4-8]

*Corresponding author. E mail: pharmaishatu@gmail.com
Tel: +234-803-6028298

Citation: Shehu A, Magaji MG, Yau J, Mahmud B, Ahmed A. Antidepressant Effect of Methanol Stem Bark Extract of *Adansonia digitata* L. (Malvaceae) in Mice. Trop J Nat Prod Res. 2018; 2(2):87-91. doi.org/10.26538/tjnpr/v2i2.6

Phytochemical investigation has shown the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals.[7] The present study, therefore, is aimed at evaluating the antidepressant activity of the methanol stem bark extract of *Adansonia digitata*.

Materials and Methods

Plant Collection and Extraction

The plant materials including leaves, fruits and stem were collected in Zaria Local Government Area of Kaduna State in December 2016. The plant material was identified and authenticated by Namadi Sanusi of the Department of Biological Sciences, Ahmadu Bello University Zaria. A voucher specimen (2512) was deposited in the Herbarium of same department.

The stem bark of the plant was shade dried and pounded into a coarse powder using mortar and pestle. About 1000 g of the coarsely powdered material was extracted with 3.5 L of methanol in a soxhlet apparatus. The methanol extract was concentrated over a water bath at 45℃ after which the extract was stored in a desiccator.

Animals

Swiss albino mice (both sex) were obtained from the Animal House Facility of Pharmacology and Therapeutics Department, Ahmadu Bello University, Zaria. They were housed in propylene cages under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. All experimental protocols were as approved by the University Animal Ethics Committee with reference number ABUCAUC/2017/PG/014.

Drugs

Imipramine (Tofranil GSK brand), Diazepam (Roche, France), Methanol (Fluka-Aldrich)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Phytochemical Screening
The methanol stem bark extract was screened for the presence of various phytochemicals.[9]

Acute Toxicity Study
LD_{50} was determined using Organization for Economic Co-operation and Development (OECD 420) guidelines in mice. Two groups of three mice (average weight 22 g) each were fasted 3 h prior to dosing in the experiment and doses calculated according to the fasted body weight. Food was further withheld for 1-2 h after which the aqueous solution of the extract was administered. The limit test was conducted in two stages. In the first stage, 5000 mg/kg was used for one mouse and observed for 48 h. On survival, the second stage was carried out with two additional mice. Mice were observed during the first 30 minutes of treatment and then occasionally within 24 h, and finally daily for 14 days. Animals were monitored for tremors, convulsions, salivation, diarrhoea, sleep, behavioural changes and coma.

Antidepressant Screening
Tail suspension test in mice: About 40 mice weighing 18-22 g were transported to the laboratory and adapted for 1 h. They were divided into five groups of eight mice each. The first, second and third groups were treated with 250, 500 and 1000 mg/kg of the extract, respectively one hour before the test. The fourth and fifth groups were treated with distilled water (10 mL/kg) and imipramine (15 mg/kg), respectively. During the test, mice were suspended on the edge of a shelf 58 cm high placed on a table clipped by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility was recorded over 6 minutes' period.[10]

Forced swim test: Forty mice divided into five groups of eight animals each. Groups 1, 2 and 3 were treated orally with 250, 500 and 1000 mg/kg of the extract, respectively. Groups 4 and 5 were treated with distilled water (10 mL/kg) and imipramine (15 mg/kg), respectively. One-hour post-treatment, each mouse was forced to swim inside a vertical glass cylinder of height 24 cm and width 12 cm, which contained water 12 cm high maintained at 25℃. The duration of immobility was measured over 5 minutes' period. A mouse was considered immobile when it remained floated passively in the water with its nose just above the surface to keep its breath.[11]

Open field test: Forty mice divided into 5 groups of eight mice each were used. Groups 1, 2 and 3 received 250, 500 and 1000 mg/kg of the extract orally 1 h prior to test. Groups 4 and 5 were treated with distilled water (10 mL/kg) and imipramine (15 mg/kg), respectively. 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 min. the arena was cleaned with 10% ethanol before and after subjecting each mouse to the test.[12]

Beam walking assay (Test for motor co-ordination deficit)
A ruler of length 80 cm and width 3 cm elevated 30 cm above a bench which directed to a goal box was used to train mice thrice. Mice that passed the training were randomly grouped into five groups of eight mice each. The first, second and third groups received 250, 500 and 100 mg/kg of the extract orally. The fourth and fifth groups received distilled water (10 mL/kg) and diazepam (0.25 mg/kg) orally, respectively. One-hour post-treatment, each mouse was placed on the beam (60 cm long, 8 mm in diameter, elevated as above) at one end and allowed to walk to the goal box. Mice that fell were returned to point of fall, with a maximum time of 60 s allowed on beam. The number of foot slips (one or both hind limbs) was recorded.[13]

Novel object recognition test: An open field arena of size 44 × 44 × 17 cm^2 kept in a reduced sound room with a low level of light was used. Novel object recognition test was carried out over three consecutive days. On the first day, mice were exposed to open field arena for 30 minutes (habituation). On the second day, mice were exposed to a 10 minutes' familiarization trial in the presence of two cotton filled identical cylindrical glassware objects of diameter and height (3 cm x 8 cm), placed 12 cm from the walls in the opposite corner of the apparatus. On the third day, mice were subjected to a 5 minutes' choice trial in the presence of familiar object (F) and a novel object (N). Each mouse was placed in the centre of the box facing the wall and allowed to freely explore the apparatus and the objects. The time spent exploring the two objects (F) and (N) was manually scored for 5 min. After each cycle, the arena and objects were cleaned with 70% ethanol solution.[14]
Object exploration is considered as the orientation of the nose to the object at a distance ≤ 2 cm and placing the forepaws on the objects. Results for this test was expressed as (1) exploration time of each object during the test session, measured as time spent exploring familiar or novel object divided by total time spent exploring both objects and (2) a discrimination index (DI) between objects, taken as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects [DI = (N–F)/(N+F)].

Statistical Analysis
Results obtained were expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test using SPSS version 20.0. P-value < 0.05 was considered significant.

Results and Discussion

The methanol stem bark extract of *Adansonia digitata* decreased the duration of immobility in treated mice in both forced swimming test (FST) and tail suspension test (TST). Significant response was obtained at all the tested doses ($P \leq 0.01$) for the TST (Figure 1) and significant ($P \leq 0.001$) and dose-dependent for FST (Figure 2). Forced swimming test (FST) and tail suspension tests (TST) are behavioural distress tests developed based on the assumption that rodents when exposed to unavoidable stress, they make efforts to escape but eventually lost hope and exhibit immobility which was extrapolated to reflect a level of behavioural despair.[15] They are valuable tools in drug discovery for high-throughput screening of prospective antidepressant compounds. These tests have been extensively used to screen for antidepressant activity due to exposure of the animals to stress, which was reported to play a role in the likelihood of depression.[16] In addition, the FST and TST were shown to share some of the behaviours altered by depression in humans such as changes in food consumption, sleep alterations and anhedonia.[17] Duration of immobility is the indicator behaviour observed in both tests.[18] The methanol stem bark extract of *A. digitata* (MEAD) significantly decreased the immobility time indicating its antidepressant activity when assessed in FST and in TST. In addition, the extract MEAD significantly ($P \leq 0.001$) increased swimming time but not climbing behaviour, unlike the standard drug imipramine which significantly ($P \leq 0.001$) increased climbing behaviour with no effect on the swimming time (Figure 3). The behaviour of mice in the FST has been divided into swimming and climbing, each of which correlates with a particular neuromodulatory characteristics.[19] Both behaviours are reported to be enhanced by different classes of antidepressants. Agents such as imipramine that selectively inhibits noradrenaline uptake enhances the climbing behaviour whereas the selective serotonin reuptake inhibitors (SSRIs) like fluoxetine enhances swimming but not climbing behaviour. In addition, dopaminergic pathways were also recently shown to be activated in climbing behaviour. The extract markedly modified swimming but not climbing behaviour, unlike the imipramine which enhanced climbing time only. Thus, the antidepressant activity of MEAD may be as a result of activation of serotonergic pathways.
The control animals, when placed in the open field arena exhibited ambulatory activities marked by the number of lines crossed (108.75 ± 8.39) which dropped with MEAD treatment at dose of 1000 mg/kg. However, the inhibitory effect of diazepam on the locomotion was more pronounced ($P \leq 0.01$) than that of the plant extract (Figure 4). In addition to the above, psychostimulants were also shown to reduce immobility in FST and TST as well as enhanced motor activities.[20] This is contrary to antidepressants, which do not enhance motor stimulation.[21] The inability of the extract to neither increase nor decrease the number of line crosses suggest that it does not stimulate ambulatory behaviour nor have central depressant activity in the animals. Thus, the reduction in the duration of immobility of animals treated with the extract MEAD may not be attributed to any stimulant potential.
The extract MEAD did not impair motor coordination deficit at all doses tested. The standard drug diazepam however significantly impaired motor coordination ($P \leq 0.001$) at the dose of 10 mg/kg orally (Figure 5). This indicated that the antidepressant-like profile of MEAD is without sedative side effect as observed in the beam walking assay.
Rodents are likely to interact more with a novel object than with a familiar object described by behavioural scientists in attempts to study learning and memory.[22]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Phytochemical constituents of the methanol extract of *Adansonia digitata*

S/No	Phytoconstituents	Inference
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponin glycoside	+
5	Cardiac glycoside	+
6	Unsaturated Steroids	+
	and Triterpenes	+
7	Anthraquinones	+
8	Carbohydrates	+

+= present, -=absent

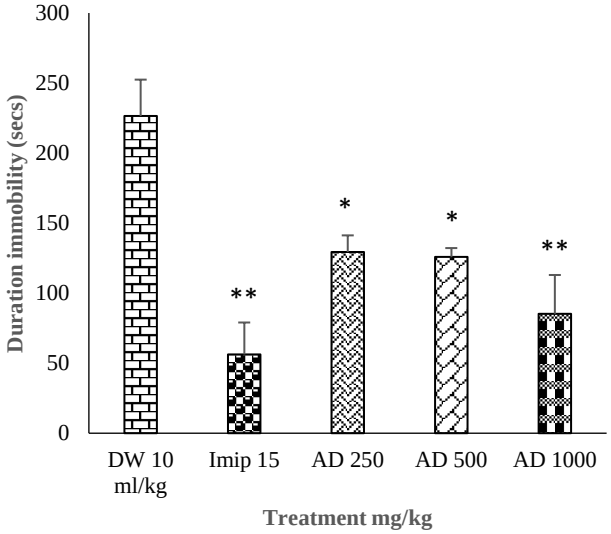

Figure 1: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on the Tail Suspension Test in Mice.
Data represents Mean ± S.E.M. (n = 8). AD = *A. digitata*, DW= Distilled water, Imip = Imipramine. *P ≤ 0.01, **P ≤ 0.001 significantly different from distilled water treated group.

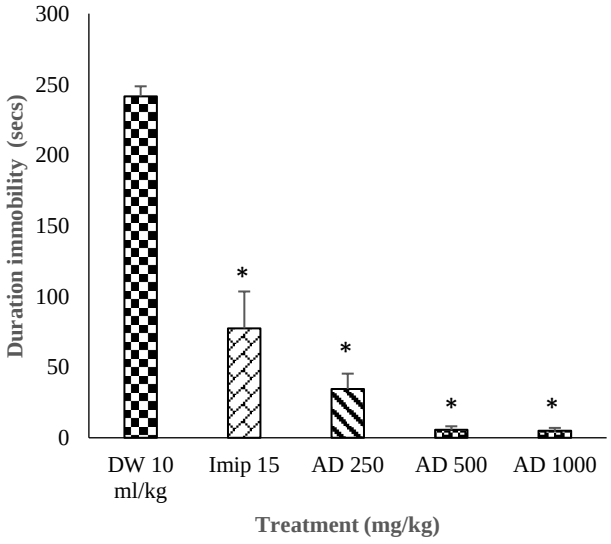

Figure 2: Effect of Methanol Stem Bark extract of *Adansonia digitata* on the Forced Swim Test in Mice.
Data represents Mean ± S.E.M. (n = 8). AD = *A. digitata*, DW= Distilled water, Imip = Imipramine. *P ≤ 0.001 significantly different from distilled water treated group.

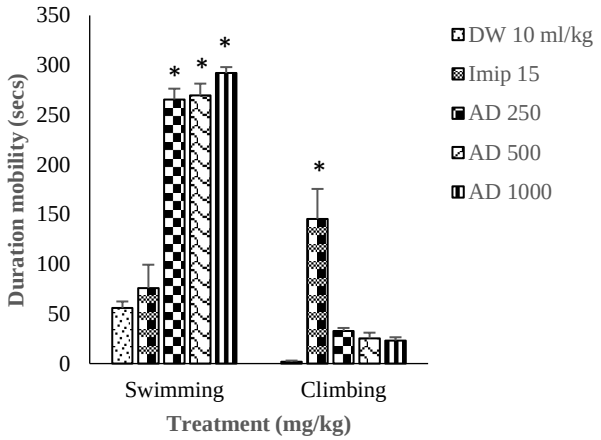

Figure 3: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Swimming and Climbing Behaviour in Forced Swim Test in Mice. Data represents Mean ± S.E.M. (n = 8). AD = *A. digitata*, DW= Distilled water, Imip = Imipramine. *P ≤ 0.05 significantly different from distilled water treated group.

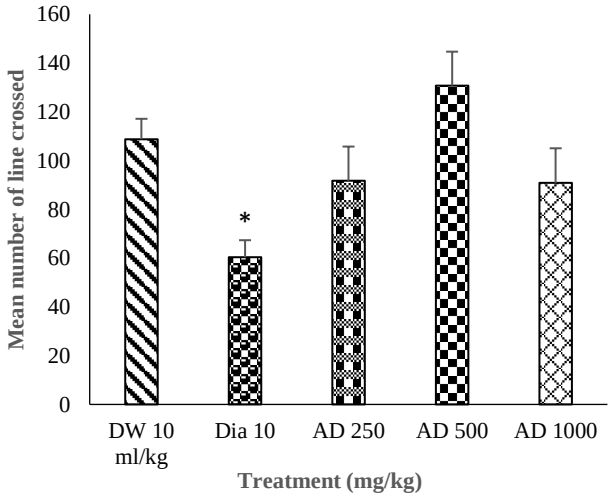

Figure 4: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on the number of lines crossed by Mice in the Open Field Test. Data represents Mean ± S.E.M. (n = 8). AD = *A. digitata*, DW= Distilled water, Diaz = Diazepam. *P ≤ 0.01 significantly different from distilled water treated group.

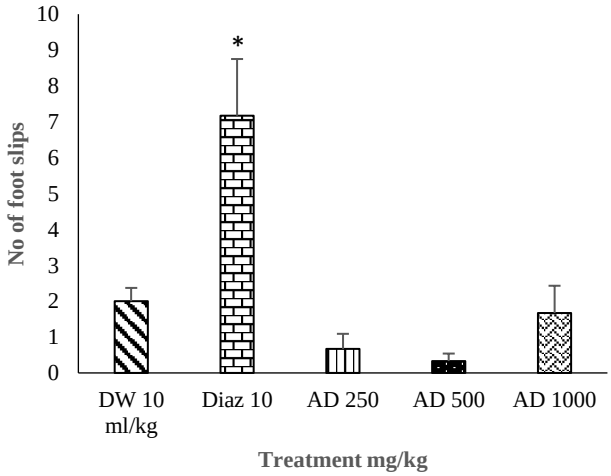

Figure 5: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on motor coordination in mice. Data represents Mean ± S.E.M. (n = 6). AD = *Adansonia digitata*; Diaz = Diazepam. *P ≤ 0.001 significantly different from distilled water treated group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

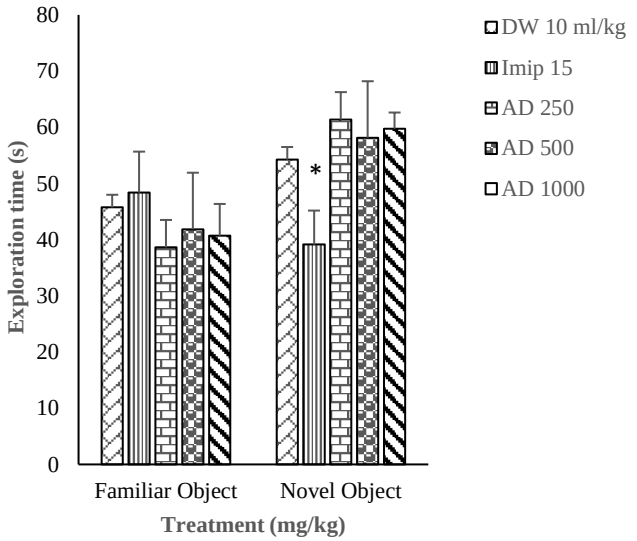

Figure 6: Mean exploration time of identical objects and novel object in the 5-min acquisition phase of the novel object recognition task following acute administration of methanol stem bark extract of *Adansonia digitata* administration in mice. Data represents Mean ± S.E.M. (n = 8). DW = Distilled water; Imip = Imipramine; AD = methanol stem bark extract of *Adansonia digitate*. *p<0.05 significantly different from distilled water treated group.

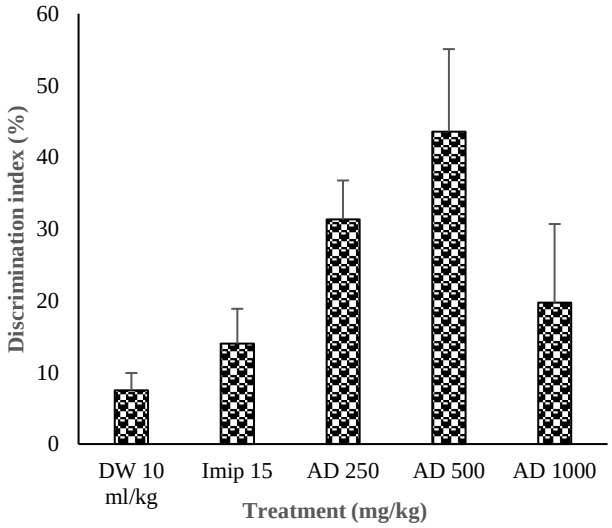

Figure 7: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on the Discrimination Index in the Novel Object Recognition Task. Data represents Mean ± S.E.M. (n = 8). DW = Distilled water; Imip = Imipramine; AD = Methanol stem bark extract of *Adansonia digitata*

The exact mechanisms underlying this 'recognition memory' has been used to study mutation, ageing deficits, nootropic activity, teratological drug exposure and novelty seeking.[23] The extract MEAD neither increases nor decrease exploration time in the novel object recognition task (NORT). However, imipramine (15 mg/kg) significantly decrease the exploration time in the NORT (Figure 6). In addition, MEAD had no effect on discrimination index (Figure 7). Thus, extract MEAD showed an insignificant increase or decrease in recognition memory. This showed that the plant neither alters nor increase memory and learning capabilities. Plants with neuroactive principles were reported to be toxic.[20] This makes toxicity studies of relevant. The acute lethal dose of MEAD orally was found to be above 5000 mg/kg indicating its relative safety as described by Lorke.[24]

Phytochemical constituents present in MEAD extract include glycosides, carbohydrates, flavonoids, tannins, anthraquinones and alkaloids (Table 1). Most pharmacological actions in medicinal plants have been linked to the presence of phytoconstituents.[25, 26] Flavonoids, saponins and alkaloids were reported to show antidepressant activity. Thus, the antidepressant activity shown by MEAD extract may be due to the presence of these phytoconstituents.

Conclusion

This study showed that the methanol stem bark extract of *Adansonia digitata* possesses antidepressant activity and provided some scientific basis for the folkloric use of the plant in depressive illnesses.

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.

Acknowledgements

The technical help of Mr Aliyu Ahmed of the Department of Pharmacology and Therapeutics, Bayero University Kano is acknowledged.

References

1. Jun S, Junjian Z, Min D, Yue L, Yuan H, Lei Z. The Antidepressant Effect of *Angelica sinensis* Extracts on Chronic Unpredictable Mild Stress-Induced Depression Is Mediated via the Upregulation of the BDNF Signaling Pathway in Rats. Evid Based Comp Alt Med. 2016; 1-8.
2. Shehu A, Magaji MG, Yau J, Abubakar A. Ethnobotanical survey of medicinal plants used for the management of depression by Hausa tribes of Kaduna state, Nigeria. J Med Plants Res. 2017; 11(36):562-567.
3. Donatien K, Hagrétou S, Bréhima D, Clarisse SC, Mamoudou HD, Mogens J. A review of baobab (*Adansonia digitata)* products: Effect of processing techniques, medicinal properties and uses. Afr J Food Sci. 2011; 5(16):833-844.
4. Kamatou GPP, Vermaak I, Viljoen AM (2011). An updated review of *Adansonia digitata*: A commercially important African tree. S. Afr. J Bot. 2011; 77(4):908-919.
5. Yusha'u M, Hamza MM, Abdullahi N. Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates. Int J Biomed Health Sci. 2010; 6:129-135.
6. Nguta JM, Mbaria DW, Gakuya- Gathumbi PK, Kiama SG. Antimalarial herbal remedies of Msambweni, Kenya. J Ethnopharmacol, 2010; 128:424-432.
7. De Caluwé E, Halamová K, Van Damme, P. Baobab (*Adansonia digitata* L.): A review of traditional uses, phytochemistry and pharmacology. In Juliana HR, Simon J, Ho CT (eds) African natural plant products: new discoveries and challenges in chemistry and quality. Am Chem Soc Symposium Series 2009; 1021(4):51-84.
8. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmcol. 2006; 104:68-78.
9. Evans WC. Trease and Evans Pharmacognosy London Villiere Tindal UK, 1996; 57 p.
10. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol. 1985; 85:367-370.
11. Alpermann HG, Schaut U, Usinger P, Hock FJ. Pharmacological effects of Hoc 249: A new potential antidepressant. Drug Dev Res. 1992; 25:267-282.

12. Rex A, Voigt JP, Voits M, Fink H. Pharmacological evaluation of a modified open field test sensitive to anxiolytic drugs. Pharmacol. Biochem. Behav. 1998; 59:677-683.
13. Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS. The mouse beam walking assays offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol. 2005; 19(3):221-227.
14. Sahay A, Kimberly NS, Alexis SH, Colin MO, Mazen AK, Nesha SB, Andre AF, Alex D, Rene H. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 2011; 472(7344):466-470.
15. Roni YY, Motty F, Avrham H, Ravid D. The Forced Swim Test as a Model of Depressive-like Behavior. J Vis Exp. 2015; (97):52587.
16. Adem C, David TD, Chantelle ET, Sean CP, Shambhu B, Todd DG. The Tail Suspension Test. J Vis Exp. 2012; 59:3769-3778.
17. Hsu LKY, Cheng H, Chang C, Lin Y, Cheng Y, Hsieh M, Peng WH. Antidepressant-like activity of the ethanolic extract from *Uncaria lanosa* Wallich var appendiculata Ridsd in the forced swimming test and the tail suspension test in mice. Evid Based Comp Alt. Med. 2012; 1-13.
18. Ngoupaye GT, Bum EN, Daniels WMU. Antidepressant-like effects of the aqueous macerate of the bulb of *Gladiolus dalenii* Van Geel (Iridaceae) in a rat model of epilepsy-associated depression. BMC Comp Alt Med. 2013; 13:272.
19. David AS, John FC. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc. 2012; **7**:1009–1014.
20. Mannan A, Abir AB, Rahman R. Antidepressant like effects of methanolic extract of *Bacopa monniera* in mice. BMC Comp Alt. Med. 2015; 15:337.
21. Ya'u J, Malami S, Abugi MA, Ngura HE. Antidepressant Activity of Ethanol Extract and Residual Aqueous Fraction of *Carissa edulis* (Apocynaceae) Root Bark in Mice. Trop J Nat Prod Res. 2017; 1(3):129-132.
22. Bevins RA, Besheer J. Object recognition in rats and mice: a one trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc. 2006; 1:1306-1311.
23. Du B, Tang X, Liu F, Zhang C, Zhao G, Ren F, Leng X. Antidepressant-like effects of the hydroalcoholic extracts of *Hemerocallis citrina* and its potential active components. BMC Complement Altern. Med. 2014; 14: 326-331.
24. Lorke DA. A new Approach to Practical acute toxicity testing. Arch Toxicol. 1983; 45: 275-87.
25. Haixia D, Ying C, Xinmin L, Qiong W, Liwei W, William J and Yuqing W. Antidepressant effects of ginseng total saponins in the forced swimming test and chronic mild stress models of depression. Prog Neuropsychopharmacol Bio Psych. 2009; 33:1417-1424.
26. Mohit KB, Mahendra B and Shrinivas KK. Antidepressant-like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioural, biochemical and neurochemical changes. Pharmacol Biochem Behav. 2009; 92:39-43.