Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 2 Issue 10 October 2018

Research Article

Preliminary Phytochemical Analysis and Antidepressant Activity of N-Hexane Fraction of *Moringa oleifera* Ethanol Leaf Extract in Mice

Suleiman Yunusa[1]*, Aminu U Kura[1], Ahmad A Ladan[2] and Shehu Y Magaji[3]

[1]*Department of Pharmacology, Faculty of Basic Health Science, Bauchi State University Gadau, Bauchi, Nigeria*

[2]*Department of Human Physiology, Faculty of Basic Health Science Bauchi State University Gadau, Bauchi, Nigeria*

[3]*Department of Pharmacology, Abubakakar Tafawa Balewa University, Bauchi State, Nigeria*

***Corresponding Author:** Suleiman Yunusa, Department of Pharmacology, Faculty of Basic Health Science, Bauchi State University Gadau, Bauchi, Nigeria.

Received: July 27, 2018; **Published:** September 18, 2018

Abstract

This work was carried out to ascertain the bioactive components and antidepressant effect of n-hexane fraction of *Moringa oleifera* Lam using mice. Depression is a neurological disorder characterized by apathy, loss of energy, decreased mood, feeling of guilt, loss of interest, retardation of thinking and activity as well as profound feelings of gloominess, despair and suicidal ideation. About 21% of the world population is largely affected by depression. Many people in the world advocate the use of *Moringa oleifera* to treat variety of central nervous system illnesses. In our present study, we have selected method described by Pranshant., *et al.* [1] for the phytochemical analysis and behavioral despair models for antidepressant screening namely forced swimming test (FST) and tail suspension test (TST). In this study, alkaloids, cardiac glycosides, flavonoids, tannins and saponins were detected from n-hexane fraction of *Moringa oleifera* leaf extract, whereas steroids were absent. N-hexane fraction of *Moringa oleifera* ethanol leaf extract showed antidepressant activity in both antidepressant models using mice. Freshly collected leaves of *Moringa oleifera* were dried under shade with adequate ventilation, it was then grounded to fine powder and extracted using ethanol (70% v/v) following cold maceration methods and then filtered using Whatman filter paper No 1 which was finally concentrated in oven regulated at 500C before air drying. The resultant ethanol extract was then partitioned using n-hexane and the n-hexane fraction (NMOLE) was then kept in the desiccators until use. Forced Swim Test (FST) and Tail Suspension Test (TST) animal models were used to screen the antidepressant effect of this fraction (NMOLE) at the doses of 100, 200 and 400 mg/kg using swiss albino mice. Both Imipramine and NMOLE produced a statistical reduction in the immobility time in both models as compared to normal saline treated group, indicating the antidepressant potential of *Moringa oleifera* leaves.

Keywords: Phytochemistry; Forced Swim Test; Tail Suspension Test; *Moringa oleifera* Lam; Depression and NMOLE

Introduction

Depression constitute the major global cause of disabilities affecting people from all walks of life [2]. Women suffers depression more as compared to men [3]. High incidence of depression in women may be the major reason for poor growth in young children [4]. Incidences of suicide increases every year accounting to thousands suicides daily [5]. Depression occur due to hypo-functioning of monoamine neurotransmitters in the brain. The major neurotransmitters that are implicated in depressive episodes are noradrenaline, serotonin and/or dopamine (Fred-Jaiyesimi and Oredipe, 2013). Clinically used antidepressants are associated with many side effects and the mechanism through which they exert their mode of action is still at the center of debating [6].

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, English common names include: *Moringa*, drumstick tree (from the appearance of the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which

resembles horseradish). It has now naturalized in West Africa, especially in Nigeria where it is cultivated for its nutritional as well as medicinal values.

A growing number of herbal medicines are being introduced into psychiatric practice, many of which have comparable efficacy to prescription medications with lower side effects, and this makes herbal therapies as desirable alternative treatment for severe depression [7].

Figure 1: Moringa Oleifera in its natural habitat, from Misau, Bauchi state Nigeria.

Statement of Research Problem

Depression is one of the leading causes of disability and decrease in productivity amongst the population and it is a significant contributor to the global burden of disease and affects people in all walks of life globally [5].

Depressive episodes accounts for the most debilitating as well as life-threatening disorders with high incidences of morbidity and mortality [8].

Most of the available antidepressants in clinical use especially the TCA's are associated with unbearable side effects including urinary retention, constipation, dry mouth, nausea and infertility, which needs urgent intervention [9].

Justification of the Study

The most effective method of identifying medicinal plants today is ethno-pharmacological studies [10].

WHO encourages the addition of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries [11].

Aim and Specific Objectives

To ascertain the bioactive components and antidepressant effect of n-hexane fraction of Moringa oleifera Lam leaves using mice.

Materials and Methods

Materials

Equipment used include Cylinders, sample containers, syringes and needle (1 ml, 5 ml, 10 ml and 20 ml), stirrers, electric ovum, whatman filter paper No 1, refrigerator, glass mercury thermometer, plexy glass tanks of 30 cm height and 20 cm diameter, water source, video recording device (SAMSUNG ES95 model), PC device (computer), stopwatch, table of about 50 cm height, medical tape and hand gloves. All chemicals and drugs were locally procured.

Plant material collection and extract preparation

Fresh leaves of *Moringa oleifera* were collected early in the morning from a garden in Bauchi State Nigeria. The collection was carried out in the presence a botanist. The collected sample was authenticated by a specialized botanist from the Herbarium unit, Department of Biological Science, Bayero University Kano Nigeria and the voucher specimen with the voucher number BUKHAN001 was compared with the existing one.

The collected leaves were dried under shade, after which they were blended using mortar and pestle and sieved until a fine powder that weighed 600 g was obtained. The powdered plant material was then macerated with five (5) litres of 70% V/V ethanol in a container for three (3) days with occasional stirring which was then filtered using Whatman filter paper No 1 to get residue and filtrate and the filtrate was concentrated in an oven at 50°C and air dried. The extract was then partitioned/fractionated with n-hexane using standard fractionation method as described by Hossain., *et al* [12].

Animals Animals

Seventy (70) albino mice weighing 18 to 25g of either sex were obtained from the Animal House Facility of the Department of

Pharmacology and Therapeutics, Bayero University Kano Nigeria. They were housed and fed on a standard pellet food and water and maintained at standard laboratory condition in accordance with principles of laboratory animal care (NIH publication, 1985).

Drugs and Chemicals

The standard drug used for the experiment was imipramine HCL, Batch No: 1608010, EXP. 07/19 (Assos Pharma., Turkey). Ethanol, n-hexane, normal saline were procured from Sigma Che-mical Co. St Louis, USA and distilled water was prepared locally using water distiller. Other reagents were ferric chloride, dragendorff reagent, wagner reagent, sulphuric acid, Sodium hydroxide and dilute hydrochloric acid.

Methods

Phytochemical Analysis

Preliminary Phytochemical analysis were conducted using method described by Pranshant., *et al* [1].

Antidepressant Screening

Forced Swim Test (FST)

As described by Porsolt and Bertin [13]. Thirty mice were divided into five groups of six mice each. The first group was pre-treated with normal saline 10 ml/kg i.p; the second, third and fourth groups were pre-treated with 100, 200 and 400 mg/kg of the n-hexane fraction, i.p while the fifth group was pre-treated with 10 mg/kg body weight imipramine i.p. Thirty minutes (30 minutes) after the pretreatment, depression was produced by forcing the animal to swim individually in a transparent and open glass container of 30 cm height and 20 cm wide containing fresh water of 15 cm height and maintained at 21 ± 0.5°C. Each animal assumed a typical immobile posture after struggling to escape. The immobility time on each mice was recorded using digital camera for the entire six minutes period. Each mouse was considered immobile when given up struggling to escape. Immobility time reduction was taken as antidepressant like action.

Tail Suspension Test (TST)

The method described by Steru., *et al.* [14] was adopted. Thirty mice were divided into five groups of six mice each. The first group was pre-treated with normal saline 10 ml/kg i.p; the second, third and fourth groups were pre-treated with 100, 200 and 400 mg/kg of the n-hexane fraction of *Moringa oleifera*, i.p while the fifth group was pre-treated with the standard antidepressant drug (imipramine) 10 mg/kg body weight i.p. Thirty (30) minutes after the pre-treatment, animals were individually suspended 50 cm above the floor by means of an adhesive tape placed approximately 1 cm from the tip of the tail. Duration of immobility was measured for the entire testing period of 6 minutes. Each mouse was considered inactive when hung passively motionless.

Results

Phytochemical constituents	Inference
Alkaloid	+
Cardiac glycoside	+
Flavonoids	+
Steroids	-
Tannins	+
Saponins	+

Table 1: Phytochemical Constituents of n- hexane fraction of ethanol leaf extract of Moringa oleifera.

Key: (+): Present; (-): Absent

S/N	Treatment	Dose	Immobility period (sec)
1	N/SALINE	10 ml/kg	130.65 ± 1.79
2	NMOLE-1	100mg/kg	122.32 ± 2.46
3	NMOLE-2	200mg/kg	72.44 ± 5.99*
4	NMOLE-3	400mg/kg	54.88 ± 7.56*
5	Imipramine	10mg/kg	40.07 ± 2.11*

Table 2: Effect of n- hexane fraction of ethanol leaf extract of Moringa oleifera (NMOLE) in mice tail suspension test.

Values represents the mean ± SEM (N = 6),*P < 0.05 when compared to vehicle treated animals.

S/N	Treatment	Dose	Immobility period (sec)
1	N/SALINE	10 ml/kg	147.55 ± 5.88
2	NMOLE-1	100 mg/kg	92.32 ± 8.77*
3	NMOLE-2	200 mg/kg	69.56 ± 5.11*
4	NMOLE-3	400 mg/kg	39.18 ± 3.56*
5	Imipramine	10 mg/kg	41.88 ± 2.11*

Table 3: Effect of n-hexane fraction of ethanol leaf extract of Moringa oleifera (NMOLE) in mice forced swim test.

Values represents the mean ± SEM (N =6),*P < 0.05 when compared to vehicle treated animals.

Discussion

In this study, alkaloids, cardiac glycosides, flavonoids, tannins and saponins were detected from n-hexane fraction of *Moringa oleifera* leaf extract, whereas steroids were absent. Our phytochemical results were in concordance with the report of Mishra., *et al* [15].

Forced swim test (FST) and Tail suspension Test (TST) remain the most applicable animals models for antidepressant screening owing to their ease of operation are require little resources. These protocols are quite specific to almost all classical antidepressants in clinical use [15]. Tail suspension and Forced swim tests showcase a state of behavioral despair in rodents which reflects depression in humans [16]. Clinically used antidepressants decrease the immobility time significantly in both FST and TST [17].

In this study, NMOLE has been found to significantly ($p < 0.05$) reduced the duration of immobility in both Forced Swimming Test and Tail Suspension Test animal models as compared to normal saline treated group. The activities observed in the extract (NMOLE) at the highest tested dose (400 mg/kg) was comparable to the one observed in the already established antidepressant agent (Imipramine) 10 mg/kg following TST. It is worthy to state that, in FST, the activity recorded with the extract (NMOLE) at the highest tested dose (400 mg/kg) was even greater than the activity observed with Imipramine at the dose of 10 mg/kg. The activity observed conforms to the report of Yadav., *et al* [18].

Hamid., *et al*. [19] reported the antidepressant like effect of some secondary metabolites such as alkaloids, flavonoids, saponins and polyphenols. Thus, these bioactive component (s) present in this fraction may be responsible for the observed antidepressant activity.

The results of this study therefore supports the use of *Moringa oleifera* for treating many central nervous system disorders in our locality [20]. Antioxidant activity has been reported with Moringa oleifera extract Torres-Castillo., *et al.* [21] and that could strongly support its antidepressant activity. In this work, the exact mechanism through which this fraction exhibited its antidepressant like effect has not been exploited, however, the pattern of immobility reduction by the fraction exhibited in both TST and FST was similar to that of imipramine, suggesting its activity via adrenergic pathway [22].

Conclusion

N-hexane fraction of *Moringa oleifera* poses secondary metabolites that has antidepressant effects in both Forced Swim Test and Tail Suspension Test using mice.

Conflict of Interest

Authors declare no conflict of interest whatsoever.

Acknowledgment

Authors are indebted to the staff of Pharmacology Laboratory and Chemistry Lab. Bayero University Kano (BUK), for the technical assistance offered towards the success of this research work.

Bibliography

1. Pranshant T., *et al*. "Phytochemical screening and Extraction: A Review". *Internationale Pharmaceutica Sciencia* 1.1 (2011): 98- 106.
2. World Health Organization. WHO Regional Director for Africa Message: "Depression, let's talk" to draw the attention to the global burden of this common mental disorder. World Health Day (2017).
3. World Health Organization. The Global Burden of Disease 2004 update (2008).
4. Rahman A., *et al*. "The neglected 'm' in MCH pro- grammes–why mental health of mothers is important for child nutrition". *Tropical Medicine and International Health* 13.4 (2008): 579-583.
5. World Health Organization, World suicide prevention day (2012).
6. Xiaosu GY., *et al*. "Antidepressant-like effects of auraptenol in mice". *Scientific Reports* 4 (2014): 4433.
7. Thachil AF., *et al*. "The evidence base of complementary and alternative therapies in depression". *Journal of Affective Disorders* 97.1-3 (2007): 23-35.
8. Sai VD., *et al*. "Antidepressant activity of hydro-alcoholic extract of fruits of momordica cymbalaria hook. f in animal models". *Journal of Chemical and Pharmaceutical Sciences* 4.4 (2011): 158-162.
9. Shehu A., *et al*. "Antidepressant Effect of Methanol Stem Bark Extract of Adansonia digitata L. (Malvaceae) in Mice". *Tropical Journal of Natural Product Research* 2.2 (2018): 87-91.
10. World health organization. Traditional medicine fact sheet. Number 134 (2003).
11. Amos S., *et al*. "Neuropharmacological effect of the aqueous extract of Sphoeranthus senegalensis in mice". *Journal of Ethnopharmacology* 78.1 (2001): 33-37.
12. Hossain M., *et al*. "Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in Sultanate of Oman". *Asian Pacific Journal of Tropical Biomedicine* 4.1 (2014): 368-372.

13. Porsolt RD and Bertin A. "Behaviour despair in mice A primary screening test for antidepressants". *Archives Internationalesde Pharmacodynamie et de Therapie* 229.2 (1977): 327-361.

14. Steru L., *et al*. "The tail suspension test: A new method for screening antidepressants in mice". *Psychopharmacology* 85.3 (1985): 367-370.

15. Mishra G., *et al*. "Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview". *Der Pharmacia Lettre* 3.2 (2011): 141-164.

16. Sharma V., *et al*. "Anti-Depressant Activity of Zizyphus xylopyrus". *International Journal of Phytomedicine* 1.1 (2009): 12-17.

17. Yunusa S and Musa A. "Evaluation of Antidepressant Effect of Ethanol Extract and Chloroform Fraction of Moringa oleifera Lam. (Moringaceae) Leaf in Mice". *Journal of Drug Research and Development* 4.1 (2018).

18. Yadav J., *et al*. "Evaluation of antidepressant activity of leaves extract of Moringa oleifera by using FST and TST models on Swiss albino mice". *World Journal of Pharmaceutical Research* 5.7 (2016): 967-976.

19. Hamid H., *et al*. "Indole Alkaloids from plants as potential leads for antidepressant drugs: A Mini Review". *Journal of Frontiers in Pharmacology* 8 (2017): 96.

20. Bakre A., *et al*. "Studies on neuropharmacological profile of ethanol extract of Moringa oleifera leaves in mice". *Journal of Ethnopharmacology* 149.3 (2013): 783-789.

21. Torres-Castillo J., *et al*. "Moringa oleifera: phytochemical detection, antioxidants, enzymes and antifugal properties". *International Journal of Experimental Botany* 82 (2013): 193-202.

22. Ginpreet K., *et al*. "Evaluation of antidepressant activity of Moringa oleifera alone and in combination with fluoxetine". *Journal of Ayurveda and Integrative Medicine* 6.4 (2015): 273-279.

Volume 2 Issue 10 October 2018