

Research Article

Ethyl-acetate and aqueous fractions of *Moringa oleifera* Lam (Moringaceae) leaf extract possess antidepressant activity in mice

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Background: Depression is one of most costly psychiatric illnesses all over the world that afflicts roughly 21% of the world population. *Moringa oleifera* has been used in traditional folk medicine for treating neurologic disorders and plays a key role in memory, mood elevation, response to stimulus such as stress and pleasure which are common in depression. Crude ethanol leaf extract of *Moringa oleifera* has been scientifically evaluated to have antidepressant activity.

Objective: This work evaluated the antidepressant activities of ethyl-acetate and aqueous fractions of *Moringa oleifera* ethanol leaf extract in mice.

Methodology: Ethanol leaf extract of *Moringa oleifera* was partitioned with ethyl-acetate and water to obtain two fractions. Preliminary phytochemical screening and acute toxicity studies were carried out on both fractions. Antidepressant activity of both fractions at doses of 100, 200 and 400 mg/kg was evaluated using tail suspension (TST), forced swim (FST), and locomotor activity tests in mice.

Results: Glycosides, flavonoids, tannins, saponins and terpenoids were detected in both fractions, while the interperitoneal median lethal dose was estimated to be 1,131.4 mg/kg in mice for the two fractions. The ethyl-acetate and aqueous fractions of *Moringa oleifera* leaf extract significantly ($p < 0.05$) reduced the immobility time of mice compared to the normal saline treated group in the tail suspension test. In the forced swim test, ethyl-acetate fraction (EF) at the tested doses significantly ($p < 0.05$) reduced the immobility time compared to the normal saline treated group, while the aqueous fraction (AF) at 200 and 400 mg/kg doses produced a significant ($p < 0.05$) decrease in the immobility time. In the open field test (locomotor activity test), both fractions did not produce statistically significant difference in the mean number of squares crossed by mice compared to control.

Conclusion: This study shows that ethyl-acetate and aqueous fractions of *Moringa oleifera* ethanol leaf extract possess antidepressant activity without altering motor activity in mice tail suspension and forced swim tests, buttressing the potential of *Moringa oleifera* in the management of depression in the nearest future.

Keywords: Antidepressant, tail suspension, forced swim, Open field, *Moringa oleifera*.

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1. Introduction

Depression is one of the most prevalent and costly psychiatric illnesses all over the world that is predicted to be the second biggest contributor to the global health problems by the year 2020 (Malberg and Blendy, 2005;

WHO, 2017). Approximately 10-30% of women and 7-15% of men are likely to have depression in their life time (Malberg and Blendy, 2005). Depression affects 3.9% of the Nigerian population (WHO, 2017). The cause of depression is multi-factorial, including family history, substance abuse (especially alcohol and

cocaine), physiological factors (deficiency of noradrenaline, serotonin and dopamine) and environmental factors (Louise et al, 2016).

Moringa oleifera (*M. oleifera*) is an important medicinal plant and the most widely cultivated species of the moringaceae family. It is highly valued from time immemorial because of its vast medicinal properties (Garima et al, 2011). The importance of *Moringa oleifera* tree to the development of mankind can never be overemphasized as almost every part of the tree possesses product useful to man. *M. oleifera* is commonly known as drumstick tree (English), "Sahajan" (Hindi), "Ben aile" (French) and in Nigeria is popularly known as "Zogale ganji" in Hausa, "Adagba malero" in Yoruba, "Odudu oyibo" in Igbo and "Konamarede" in fulfulde. It has been reported to be used traditionally for the treatment of convulsion and neurologic conditions (Bakre et al, 2013). The leaves are used in folk remedies for tumors (Suphachai, 2014, Yadav et al, 2016) and as a dietary supplement (Makkar and Becker, 1977).

Extracts from *M. oleifera* have been scientifically documented to have antioxidant effects (Richa et al, 2005; Suphachai, 2014), anti-hyperlipidaemic and hypoglycemic effect (Yadav et al, 2016), prevent cyclophosphamide-induced micronucleus formation and DNA damage in mice (Sathya et al, 2010). The aqueous extract enhanced hepatic glutathione restoration (Fakurazi et al, 2008). Prevention of azoxymethane-induced colon carcinogenesis has also been reported (Budda et al, 2011) amongst others. Consumption of *Moringa oleifera* leaves powder supports brain health, mental alertness and play a key role in memory, mood elevation, organ function, response to stimulus such as stress and pleasure which are common in depression (Brenda, 2015; Zaku et al, 2015). Crude ethanol extract has been documented to have antidepressant activity (Ginpreet et al, 2015; Yadav et al, 2016). The present work was undertaken to evaluate the antidepressant activity of two fractions of *Moringa oleifera* leaves.

2. Methods

2.1 Animals

Swiss albino mice (18 to 25 g) of either sex were procured from the animal facility, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria, Kaduna State Nigeria. The animals were maintained under standard laboratory condition and had free access to food and water.

The experiments was conducted in accordance with principles of laboratory animal care (NIH publication, 1985).

2.2 Drugs, Solvents and Equipment

Fluoxetine HCL (Ranbaxy Pharma., Nigeria), imipramine HCL (Assos Pharma., Turkey), ethanol, ethyl-acetate, normal saline (Sigma chemical co. St Louis, USA) and distilled water. Glass mercury thermometer, plexy glass tanks of 30 cm height and 20 cm diameter, stopwatch, digital camera, PC device (computer) and electric oven.

2.3 Collection of plant material and extract preparation

Fresh leaves of *Moringa oleifera* were collected in the early month of January, 2017 at Tarauni Local Government Area, Kano State Nigeria. The plant material was identified and authenticated by Mal. Baha'udden of the Herbarium unit, Department of plant biology, Bayero University Kano (BUK) Nigeria and a voucher specimen BUKHAN 0011 was deposited for reference purpose. The plant material was washed, air-dried, grounded to fine powder and extracted using 70% v/v ethanol by cold maceration for three days with occasional stirring and then filtered using Whatman filter paper No 1. The extract was then concentrated using electric oven at 50 °C. The extract was then partitioned with ethyl-acetate and water. The two fractions were kept in air tight containers maintained at 21±1 °C until use.

2.4 Phytochemical screening

Phytochemical screening of the two fractions was carried out using the method described by Prashant et al, (2011).

2.5 Acute toxicity study

The median lethal dose (LD₅₀) of the two fractions was determined using the method described by Lorke (1983).

2.6 Antidepressant study

Tail suspension test (TST) in mice

The method described by Steru et al, (1985) was employed. Mice were randomly divided into five groups of six mice each. Three groups were administered graded doses of the ethyl-acetate fraction (100, 200 and 400 mg/kg *i.p.*). Animals in the control group received normal saline 10 ml/kg and the last group received imipramine (10 mg/kg, *i.p.*). Thirty minutes later, each mouse was suspended by the tail on the edge of a shelve 50 cm above a table top by means of an adhesive tape placed approximately 1 cm from the tip of the tail. The length of their immobility was recorded during a 6 min period, after discarding activity in the first 2 min, during which an animal tries to escape. Mice were considered immobile when they hung passively and completely motionless. A decrease in immobility time was considered antidepressant like activity. The same procedure was repeated with the aqueous fraction.

Forced swim test (FST) in mice

The method described by Porsolt and Bertin (1977) was adopted. Mice were randomly divided into five groups of six mice each. Three groups were administered graded doses of the ethyl-acetate fraction (100, 200 and 400 mg/kg *i.p.*). Animals in the control group received normal saline 10 ml/kg and the last group received imipramine (10 mg/kg, *i.p.*). Thirty minutes later, animals were placed individually in an open cylindrical and transparent container (30 cm height and 20 cm wide) containing 15 cm height of water maintained at 24±1°C. Mice were allowed to swim and after an initial 2 min period of vigorous activity, each animal assumed

a typical immobile posture. The total duration of immobility were recorded for the next 4 min of a total 6 min testing period. Mice were judged immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads and body above water. A decrease in the duration of immobility was considered antidepressant like effect. The same procedure was repeated with the aqueous fraction.

Open field test in mice

Locomotor activity test was carried out as described by Santosh et al, (2011). Thirty mice were randomly divided into five groups of six mice each. The first group received normal saline (10 ml/kg *i.p*); the second, third and fourth groups were pre-treated with the graded doses of the ethyl-acetate fraction (100, 200 and 400 mg/kg *i.p*) while the fifth group was pre-treated with the standard antidepressant drug fluoxetine (20 mg/kg body weight *i.p*). Thirty minutes after, animals were individually placed in the central square of the open field. Five minutes observation was followed based on the number of central as well as peripheral squares crossed by mice. More number of square crossings indicates better locomotor activity. The same protocol was repeated for the aqueous fraction.

2.7 Statistical analysis

Results were expressed as Mean \pm Standard Error of the Mean (SEM) in form of text/charts. Statistical analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p < 0.05$ were considered significant.

3. Results

Phytochemical analyses of the ethyl-acetate fraction of *Moringa oleifera* leaf extract gave positive reactions for alkaloids, glycosides, flavonoids, tannins, saponins, phenols, sterols and terpenoids while the aqueous fraction gave positive reactions for glycosides, flavonoids, tannins, saponins, carbohydrates and terpenoids.

The intraperitoneal median lethal dose (LD_{50}) of both ethyl-acetate and aqueous fractions of *Moringa oleifera* leaf extract were estimated to be 1130 mg/kg body weight in mice.

In the mice tail suspension test, the ethyl-acetate fraction (EF) at all tested doses significantly ($p \leq 0.05$) and dose dependently reduced the duration of immobility of mice compared to control group (normal saline). A reduction in immobility time indicates antidepressant like effect. The activity observed with the fraction at 400 mg/kg was greater than the standard drug imipramine 10 mg/kg (**Figure 1**). The aqueous fraction of *Moringa oleifera* leaf extract produced a dose dependent and significant ($p \leq 0.05$) reduction in the immobility time of mice compared to control group.

Decreased immobility time demonstrates antidepressant action. The activity at 400 mg/kg was observed to be slightly greater than the standard drug imipramine 10 mg/kg (**Figure 2**).

In the mice forced swim test, the ethyl-acetate fraction of *Moringa oleifera* leaf extract at the tested doses significantly ($p \leq 0.05$) and dose dependently reduced the duration of immobility of mice in the forced swim test compared to control group. Reduction in immobility time indicates antidepressant like effect (**Figure 3**). The immobility time of mice was significantly ($p \leq 0.05$) reduced by the aqueous fraction of *Moringa oleifera* leaf extract at the doses of 200 and 400 mg/kg body weight compared to control in forced swim test. The lower the immobility time, the better the antidepressant like action (**Figure 4**).

The ethyl-acetate fraction of *Moringa oleifera* leaf extract did not show any statistical increase in mean number of squares crossed by mice compared to the control group in the open field test (**Figure 5**). The higher the number of squares crossed, the better the locomotor activity. The aqueous fraction of *Moringa oleifera* leaf extract did not show any statistical difference in mean number of squares crossed by mice (locomotor activity) compared to the control group in the open field test (**Figure 6**). Greater number of squares crossed, indicates general motor stimulation.

4.0 Discussion

Moringa oleifera is a plant with numerous medicinal values that is grown extensively in many parts of the world for its nutritional and health benefits. Earlier studies have shown that the crude ethanol leaf extract possessed antidepressant activity in mice tail suspension and forced swim paradigms (Ginpreet et al, 2015; Yadav et al, 2016). This study was carried out to evaluate the antidepressant effects of ethyl-acetate and aqueous fractions of *Moringa oleifera* ethanol leaf extract using tail suspension, forced swim and open field tests in mice.

Phytochemical results revealed that ethyl-acetate fraction contains alkaloids, glycosides, flavonoids, tannins, saponins, phenolic compounds, phytosterols and terpenoids, while aqueous fraction revealed the presence of glycosides, flavonoids, tannins, saponins, carbohydrates and terpenoids. Some secondary metabolites especially flavonoids, polysaccharide, alkaloids, saponins and polyphenols have been reported to have antidepressant activity (Sai et al, 2011; Fred-Jaiyesimi and Oredipe, 2013; Hamid et al, 2017).

The main symptoms of depression are due to functional deficiency in the levels of monoaminergic transmitters noradrenaline, 5- hydroxytryptamine and dopamine in the brain (Meyers, 2000). The major antidepressant therapies aim for an enhancement in the transmitters' levels in the neurons and thus normalize the neurotransmission (Jithan and Chinnalalaih, 2009).

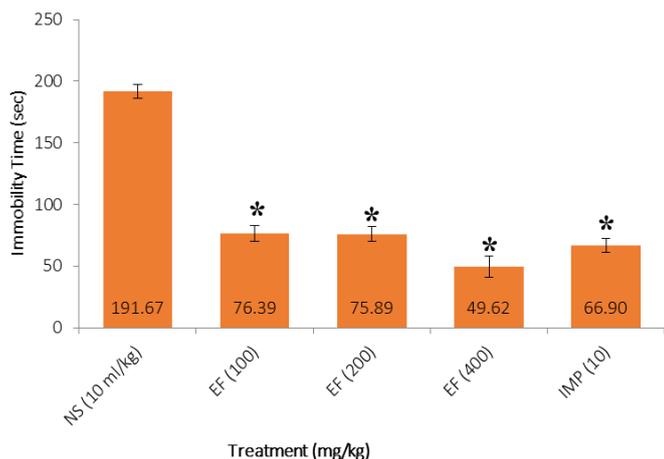


Figure 1: Effect of ethyl-acetate fraction (EF) of *Moringa oleifera* ethanol leaf extract on immobility period in mice tail suspension test.

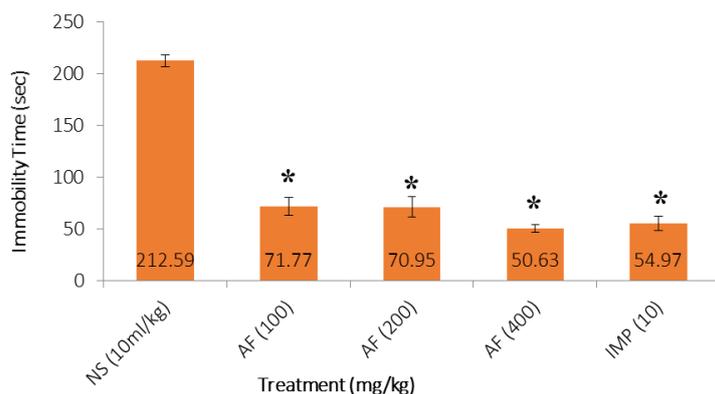


Figure 2: Effect of aqueous fraction (AF) of *Moringa oleifera* ethanol leaf extract on immobility period in mice tail suspension test

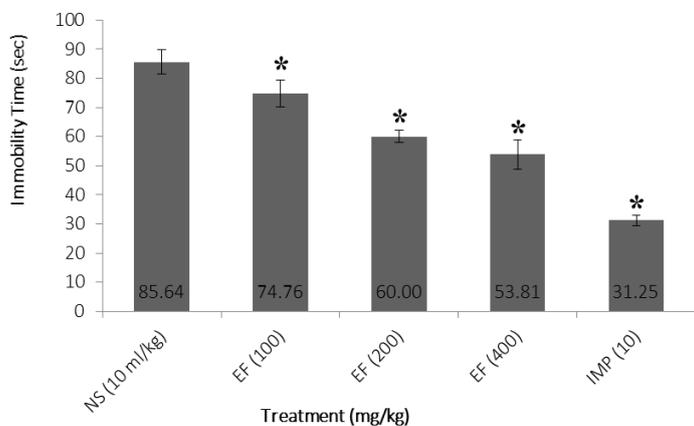


Figure 3: Effect of ethyl-acetate fraction (EF) of *Moringa oleifera* ethanol leaf extract on immobility period in mice forced swim test

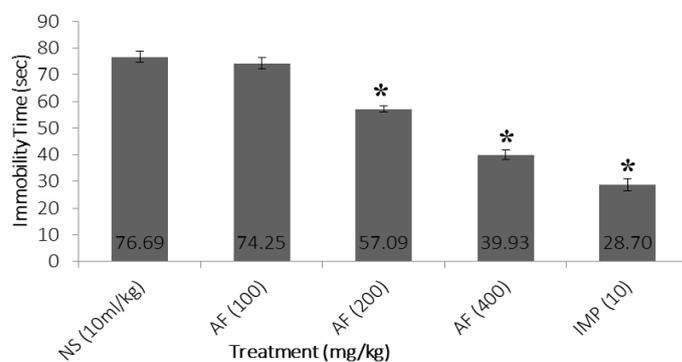


Figure 4: Effect of aqueous fraction (AF) of *Moringa oleifera* ethanol leaf extract on immobility period in mice forced swim test

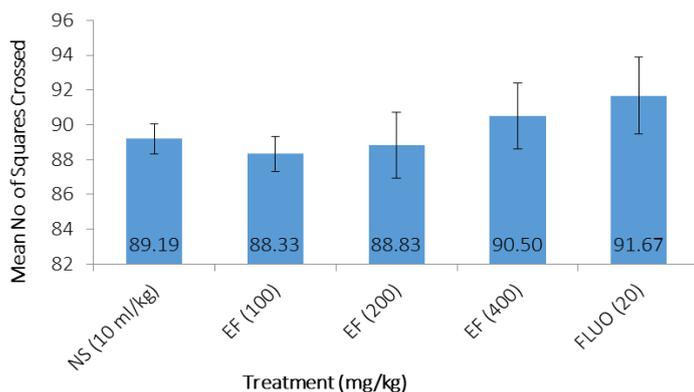


Figure 5: Effect of ethyl-acetate fraction (EF) of *Moringa oleifera* ethanol leaf extract on locomotor activity of mice in open field test

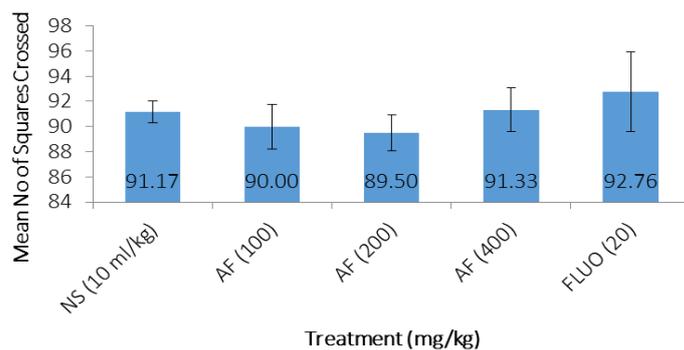


Figure 6: Effect of aqueous fraction (AF) of *Moringa oleifera* ethanol leaf extract on locomotor activity of mice in open field test

* = significantly different ($p \leq 0.05$) compared to control. One way Analysis Of Variance (ANOVA) followed by Dunnett's post hoc analysis, n=6. NS= Normal saline, EF= Ethyl-acetate fraction of ethanol leaf extract of *Moringa oleifera*, AF= Aqueous fraction of ethanol leaf extract of *Moringa oleifera*, IMP = Imipramine, FLUO = Fluoxetine.

Tail suspension (TST) and forced swim (FST) tests are the most commonly used antidepressant screening models that produce a state of behavioral despair which is similar to human depression (Willner, 1984; Sharma et al, 2009). Both models are considered to be sensitive and relatively specific to all major classes of antidepressant drugs like tricyclics, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (Porsolt et al, 1977; Detke et al, 1995). Clinically effective antidepressants reduce the immobility time displayed by rodents after active and unsuccessful attempts to escape during forced swim or when suspended by the tail. In this study, both ethyl-acetate and aqueous fractions of *M. oleifera* leaf extract reduced the immobility time of mice dose dependently in the two protocols used. A decrease in the immobility time indicates antidepressant like effect (Vikram and Swati, 2011). The activity of the fractions at highest dose (400 mg/kg) was observed to be greater than the standard drug (imipramine 10 mg/kg) in the tail suspension test. These results suggest that both fractions possess antidepressant activity in mice TST and FST. The mechanisms by which the fractions produced antidepressant effects are not completely understood. However, going by our results, the pattern of behaviors exerted by the fractions especially in the TST was similar to those of imipramine which suggests that fractions act probably by enhancement of norepinephrine neurotransmission.

Open field test is a popular and most widely used animal model which can be employed to assess whether the observed antidepressant effect was due to stimulation of general motor activity (Vikram and Swati, 2011; Santosh et al, 2011). It has been established in the literature that, agents that alter locomotor activity such as caffeine, amphetamine, theophylline etc might produce a false positive/negative antidepressant activity (Novas, 1988; Santosh et al, 2011). In this study, both ethyl-acetate and aqueous fractions of *M. oleifera* leaf extract did not produce statistically significant difference in the mean number of squares crossed when compared to control, suggesting that both fractions exhibited antidepressant activity without altering locomotor activity. The higher the number of squares crossed, the better the locomotor activity.

5.0 Conclusion

Ethyl-acetate and aqueous fractions of *Moringa oleifera* ethanol leaf extract contain bioactive constituents that possess antidepressant effect in both mice tail suspension and forced swim tests suggesting the potential use of *Moringa oleifera* as an adjuvant in the treatment of depression.

Conflict of Interest declaration

The authors declare no conflict of interest.

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