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Evaluation of Antidepressant Effect of Ethanol Extract and Chloroform Fraction of *Moringa oleifera* Lam. (Moringaceae) Leaf in Mice

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Abstract

Background: Depression remains the major cause of global burden of disease and affects individuals in all communities across the world. More than 300 million individuals worldwide suffer from depression.

Objective: The objective of the present study was to evaluate the antidepressant activity of ethanol extract and chloroform fraction of *Moringa oleifera* leaf in mice.

Methodology: Ethanol Leaf Extract of *Moringa oleifera* (ELEMO) was prepared from the freshly collected leaves of *Moringa oleifera,* the leaves were shade dried, pulverized and extracted using 70% v/v ethanol by cold maceration for three days with occasional stirring and agitation. The filtrate was filtered using Whatman filter paper No 1 and then concentrated in electric oven at 50°C until dried extract (97 g) was obtained which was then kept in desiccators until use. Significant quantity of the extract (85 g) was then partitioned with chloroform and the resultant fraction was kept in air tight container maintained at 21 ± 1°C until used. Preliminary phytochemical screening and acute toxicity studies of the extract and the fraction were carried out using the standard methods. Antidepressant screening of the extract at the doses of 100, 200 and 400 mg/kg body weight was conducted using Tail Suspension Test (TST) and Forced Swim Test (FST) while the Chloroform Fraction (CF) was evaluated for the antidepressant activity at the doses of 50, 100 and 200 mg/kg body weight.

Results: Alkaloids, glycosides, flavonoids, tannins, saponins, phenols, sterols, carbohydrate and terpenoids were detected from the extract while glycosides, flavonoids, tannins, sterols and terpenoids gave a positive reaction from the Chloroform Fraction (CF). The result of the acute toxicity studies revealed the LD_{50} values of 1131.4 mg/kg body weight in mice for the ethanol extract and 471.0 mg/kg for the chloroform fraction. Ethanol leaf extract of *Moringa oleifera* (ELEMO) at all the tested doses significantly ($p<0.05$) and dose dependently reduced the duration of immobility of mice in tail suspension test compared to control group. The extract at the doses of 200 and 400 mg/kg body weight also exhibited significant ($p<0.05$) reduction in the immobility time when compared to the normal saline treated group in mice FST. Chloroform fraction at the dose of 200 mg/kg body weight significantly ($p<0.05$) reduced the duration of immobility in mice tail suspension test as compared to control group, however in forced swim test, the fraction at all the tested doses did not affect the immobility time compared to control.

Conclusions: The results of our study suggest that ethanol leaf extract of *Moringa oleifera* (ELEMO) possess antidepressant activity in both TST and FST in mice while chloroform fraction possesses antidepressant activity in mice TST only.

Keywords: Antidepressant; Tail suspension test; Forced swim test; *Moringa oleifera*; Chloroform fraction

Introduction

Depression is a serious mood disorder characterized by apathy, anhedonia feeling of helplessness and worthlessness leading to suicidal attempt [1]. It is an illness characterized by persistent sadness, loss of interest and ability to perform daily activities for a period of over two weeks; at worst leading to suicides which is the second cause of death to people aged 15-29 years globally [1]. Several million individuals in the world do experience depression in their lifetime and this translates to about 21% of the world population [2]. Depression affects many African populations and the incidence is more pronounced in Nigeria [3]. Depression occurs due to the default in receptor-neurotransmitter relationships leading to functional deficit in these neurotransmitters (Noradrenalin, 5-Hydroxytryptamine or Dopamine) in the limbic system as well as prefrontal cortex, hippocampus and amygdale areas of the brain [4,5]. The burden of depression is 50% higher for females than males [6]. In fact, depression is the leading cause of disease burden for women in high-income, low and middle-income countries [6]. Research in developing countries suggests that maternal depression may be a risk factor for poor growth in young children [7]. Almost a million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day [8]. Most of the classical antidepressants in clinical use possess undesirable side effects and their mechanisms of action have not yet been satisfactorily resolved [2]. Drugs used in the treatment of depression are collectively known as "Antidepressants" Examples: Amitriptyline, imipramine, fluoxetine, sertraline, selegylin, pargylin, mianserin, etc.

Moringa oleifera a member of Moringaceae family has been used in traditional folk medicine for treating numerous central nervous system disorders including convulsion and hysteria [9,10]. *Moringa oleifera* is a small, fast-growing and evergreen tree that usually grows as high as 9 m, with a soft and white wood and the bark is gummy. The name "Horse Radish Tree" emanates from the taste of the root. Leaves of *Moringa oleifera* are tripinnate in nature and contained significant quantity of amino acids, natural vitamins especially Retinol (Vitamin A) and Ascorbic acid [11], the leaves are longitudinally cracked, reaching 30-75 cm long and its branch jointed, leaflets are glabrous and entire [11]. It has a finely hairy leaves and the upper surface is nearly hairless [12,13]. It is commonly called; Moringa, Drumstick tree (from the appearance of the long, slender, triangular seed-pods), Horseradish tree (from the taste of the roots, which resembles horseradish), Ben oil tree (from the oil which is derived from the seeds) and in Nigeria is popularly known as "Zogale" (Hausa), "Adagba malero" (Yoruba), "Odudu oyibo" (Igbo) and "Konamarede" (Fulani). *Moringa oleifera* has been used in traditional folk medicine for treating central nervous system disorders as well as memory enhancing agent [10]. It has also been known to treat epilepsy and neurologic conditions [9], Daily consumption of *Moringa oleifera* leaves powder support 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 and psychosis [14,15]. The leaves of *Moringa oleifera* are used for the treatment of variety of disorders, including diabetes, liver diseases, obesity, infections, inflammation, and convulsion [16,17]. The leaves were used in folk remedies for tumors [18] and widely recognized as a food source for human [19]. The plant is also reported to be used in the treatment of ascites, infection, hiccough, influenza, internal abscess, rheumatism, venomous bites as well as cardiac and circulatory stimulant [20,21]. It is also used in many countries to treat malnutrition and malaria [22].

Forced Swim Test (FST) and Tail Suspension Test (TST) are animal models of depression that are widely used to screen new antidepressant drugs [23]. They are quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, Monoamine Oxidase (MAO) inhibitors and atypical antidepressants [24]. FST was first described by [25] while the TST was first described by [23].

Materials and Methods

Animals

One hundred and thirty three (133) Swiss albino mice (18 to 25 g) of either sex were procured from the animal facility, Department of Pharmacology and Therapeutics, Bayero University Kano (BUK) Nigeria. The animals were maintained at 25 ± 1°C, 12-hour light and dark cycle and had free access to food and water. The experiment was conducted in accordance with principles of laboratory animal care [26] and all procedures were approved by the institutional ethical committee.

Drugs, solvents and equipment

Imipramine (Assos Pharm., Turkey), ethanol, chloroform, normal saline (Sigma chemical co. St Louis, USA) and distilled water. Electric oven, Whatman filter paper, refrigerator, glass mercury thermometer, transparent cylindrical containers (30 cm height and 20 cm diameter), video recording machine (digital camera), tripod, PC device (computer), stopwatch, table (50 cm height) and hand gloves.

Collection of plant material and extract preparation

Fresh leaves of *Moringa oleifera* were collected in the month of January, 2017 from a garden located at Tarauni Local Government Area, Kano State Nigeria. The plant material was identified and authenticated by a botanist in the herbarium unit, Department of plant biology, Bayero University Kano (BUK) Nigeria and the voucher specimen BUKHAN 0011 was deposited as reference. The plant material was washed, shade-dried, pulverized and extracted with 70% v/v ethanol by cold maceration for three days with occasional stirring and agitation. The mixture was then filtered using Whatman filter

paper No 1 and the filtrate was later concentrated using electric oven at 50°C and the resultant dried extract (97 g) was kept in a desiccator until used. A significant quantity (85 g) of the extract was then partitioned with chloroform and the chloroform fraction (CF) was kept in a separate air tight container (21 ± 1°C) until use.

Phytochemical screening

Preliminary phytochemical screening of the extract and the fraction was carried out using the method described by Tiwari P et al. [27].

Acute toxicity study

Acute toxicity study of the extract and the fraction was conducted using the method described by Lorke D [28].

Antidepressant study

Tail Suspension Test (TST) in mice: The method similar to that described by Steru L et al. [23] was adopted. Mice were randomly divided into five groups (n=6) and pretreated as follows; group I received normal saline (10 ml/kg ip), groups II, III and IV received graded doses of ethanol extract (100, 200 and 400 mg/kg ip) while group V received imipramine (10 mg/kg ip). Thirty minutes later, each mouse was individually suspended 50 cm above the floor by means of adhesive tape which was placed approximately 1 cm from the tip of the tail. The time during which the animal assumed immobile posture was measured during the last 4 min of a total 6 min testing period. Mice were considered immobile when they hung passively and completely motionless. A decrease in the immobility period indicates antidepressant activity. The same procedure was repeated for the chloroform fraction at the doses of 50, 100 and 200 mg/kg ip).

Forced Swim Test (FST) in mice: The method described by Porsolt RD et al. [25] was employed. Mice were randomly grouped in to five, (n=6) and the respective treatment were administered: group I received normal saline, (10 ml/kg ip), groups II, III and IV received graded doses of ethanol extract (100, 200 and 400 mg/kg ip) while group V received standard antidepressant agent imipramine (10 mg/kg, ip). Thirty minutes later, 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 25 ± 1°C. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in the next 4 min of a total 6 min testing period. Mice were considered immobile when they ceased struggling to escape and thus, remain floating motionless on water, making only those movements necessary to keep their heads and body above the water. A decrease in the immobility time was considered antidepressant like effect. The same procedure was repeated for the chloroform fraction at the three graded doses (50, 100 and 200 mg/kg ip).

Statistical analysis

Data were analyzed using SPSS statistical software version twenty (V.20). Results were expressed as mean ± SEM. 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 statistically significant.

Results

Preliminary qualitative phytochemical analyses of *Moringa oleifera* ethanol leaf extract revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, phenols, sterols, carbohydrates and terpenoids while the chloroform fraction gave a positive reaction to glycosides, flavonoids, tannins, sterols and terpenoids (Table 1).

The intraperitoneal median lethal dose (LD_{50}) of *Moringa oleifera* ethanol leaf extract was estimated to be 1131.4 mg/kg body weight in mice and the chloroform fraction revealed the LD_{50} value of 471.0 mg/kg body weight in mice.

Effect of ELEMO and CF on immobility time in mice Tail Suspension Test (TST)

Ethanol leaf extract of *Moringa oleifera* (ELEMO) at all the tested doses and chloroform fraction (CF) at the dose of 200 mg/kg significantly ($p<0.05$) reduced the duration of immobility of mice in tail suspension test compared to control group. The activity was dose dependant with ELEMO (Figures 1 and 2).

Effect of ELEMO and CF on immobility time in mice Forced Swim Test (FST)

Ethanol leaf extract of *Moringa oleifera* (ELEMO) at the doses of 200 and 400 mg/kg body weight exhibited a significant ($p<0.05$) decrease in the immobility time of mice while the chloroform fraction (CF) did not produce any significant effect compared to control group in forced swim test (Figures 3 and 4).

Discussion

It has been reported that *Moringa oleifera* leaves powder has been used ethno-medicinally to support brain health and mental alertness that play a vital role in depression instances [14,15]. The results of our preliminary phytochemistry shows that ethanol leaf extract of *Moringa oleifera* contained alkaloids,

Table 1: Phytochemical constituents of the Ethanol Extract (ELEMO) and Chloroform Fraction (CF) of *Moringa oleifera* leaf extract

Phytochemical constituent	Inference ELEMO	CF
Alkaloids	+	-
Glycosides	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	-
Phenolic acid	+	-
Sterols	+	+
Carbohydrates	+	-
Terpenoids	+	+

Key: (+)=Present, (-)=Absent

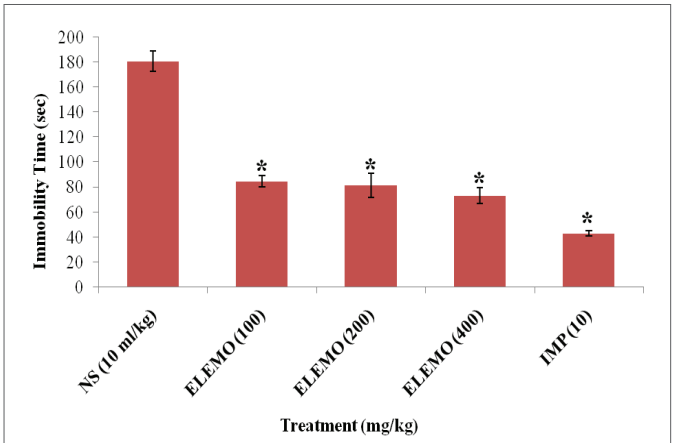

Figure 1: Effect of Ethanol Leaf Extract of Moringa oleifera (ELEMO) on Immobility Time in Mice Tail Suspension Test (TST)
One way ANOVA followed by Dunnett's post hoc. *P<0.05. NS: Normal saline; IMP: Imipramine

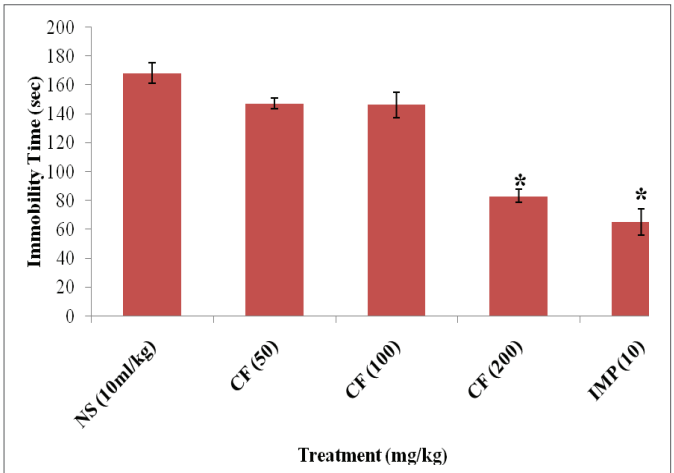

Figure 2: Effect of Chloroform Fraction of Moringa oleifera (CF) on Immobility Time (Sec) in Mice Tail Suspension Test (TST)
One way ANOVA followed by Dunnett's post hoc. *P<0.05. NS: Normal saline; IMP: Imipramine

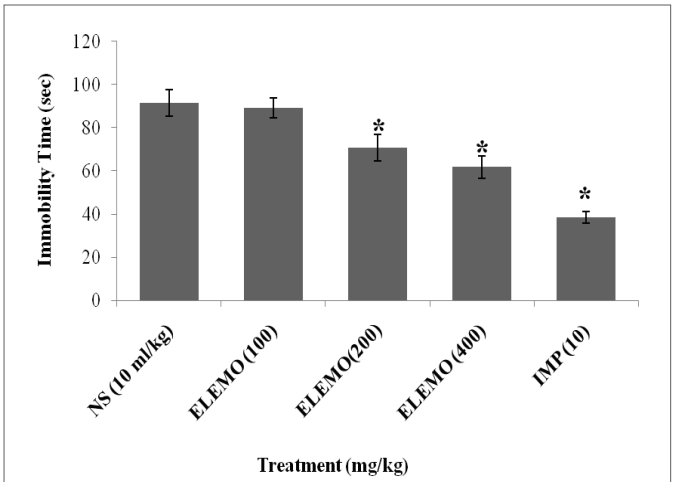

Figure 3: Effect of Ethanol Leaf Extract of Moringa oleifera (ELEMO) on Immobility Period in Mice Forced Swim Test (FST)
One way ANOVA followed by Dunnett's post hoc. *P<0.05. NS: Normal saline; IMP: Imipramine

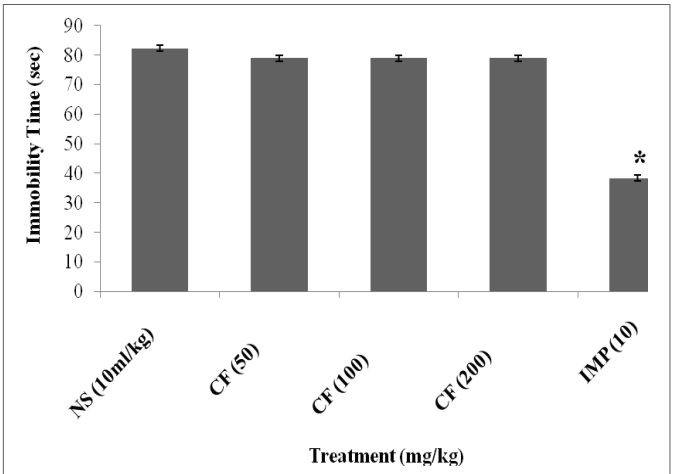

Figure 4: Effect of Chloroform fraction of Moringa oleifera (CF) on Immobility Time in Mice Forced Swim Test (FST)
One way ANOVA followed by Dunnett's post hoc. *P<0.05. NS: Normal saline; IMP: Imipramine

glycosides, flavonoids, tannins, saponins, phenolic compounds, phytosterols, carbohydrates and terpenoids, this conforms with the findings of [29]. Glycosides, flavonoids, tannins, sterols and terpenoids were detected in the chloroform fraction. Phytochemical components especially alkaloids, saponins, flavonoids, phenols and carbohydrates have been reported to have antidepressant activity [30,31].

Forced swim test (FST) and Tail suspension test (TST) are the most commonly used models for the screening of new antidepressant drugs. Almost all the major classes of antidepressants have been found sensitive and relatively specific to these protocols [21]. Both models predispose rodents to the state of behavioral despair which is comparable to human depression [32]. Depressive symptoms manifest due to functional deficiency of noradrenalin, serotonin or dopamine neurotransmitters in the limbic system, prefrontal cortex, hippocampus and amygdale areas of the brain [4,5]. The major target of antidepressants is therefore to increase the level of these neurotransmitters in the brain and hence reverse the symptoms of depression [33]. Clinically useful antidepressants such as imipramine and fluoxetine reduce the immobility time in both tail suspension test and forced swim test. In this study, the ethanol extract significantly ($p<0.05$) and dose dependently reduced the duration of immobility of mice in TST, suggesting that the extract possess antidepressant activity in this model. The extract also reduced immobility time at higher doses of 200 and 400 mg/kg body weight in mice FST suggesting that the extract possess antidepressant activity in mice FST. The better activity observed in TST as compared to FST could be due to the fact that TST is less stressful than FST and therefore may have better pharmacological compassion [34]. Our results for the antidepressant screening with this extract conforms to the findings of [10,17] who independently reported that ethanol leaf extract of *Moringa oleifera* possess antidepressant activity in mice TST and FST.

In this work, the chloroform fraction significantly ($p<0.05$) reduced the immobility time in TST at the highest tested dose (200 mg/kg body weight) only, suggesting that the fraction possess antidepressant like effect in mice TST. In Forced Swim Test (FST) however, the fraction did not produce significant difference in immobility time at all the tested doses indicating that the fraction does not possess antidepressant like effect in this model. This result might be as a result of the absence of some secondary metabolites such as alkaloids, saponins, phenolic compounds and carbohydrates which have been reported to have antidepressant activity [35,36].

Plants with antidepressant activity that contain flavonoids, polysaccharide, alkaloids, saponins and polyphenols include *Morusme sozygia* [30], *Momordica symbalaria* [37], *Passiflora foetida* [38] and *Eclipta alba* [21]. Therefore, the observed antidepressant effect observed with ELEMO and CF could be due to the presence of one or more of these secondary metabolites. In this work, the exact mechanism of antidepressant activity of ethanol extract and chloroform fraction of *Moringa oleifera* leaf was not very clear, therefore we suggest further work to ascertain their possible mechanisms of action.

Conclusions

The results of our study suggests that ethanol leaf extract of *Moringa oleifera* possess antidepressant activity in both FST and TST while chloroform fraction possess activity in mice TST only suggesting the potential use of *Moringa oleifera* in the treatment of depression.

Conflict of Interest Statement

Authors declare no conflict of interest.

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