Trop J Nat Prod Res, February 2024; 8(2):6446-6450 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

Evaluation of Antidepressant Activity of Ethanol Leaf Extract of *Entada africana* Guill. & Perr. (Fabaceae) in Mice

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ARTICLE INFO

Article history:
Received 27 October 2023
Revised 05 February 2024
Accepted 05 February 2024
Published online 01 March 2024

ABSTRACT

Depression remains the major cause of global disease burden and affects individuals in all communities around the world. More than 300 million individuals worldwide suffer from depression. The objective of the present study was to evaluate the antidepressant activity of ethanol leaf extract of *Entada africana* (ELEEA) in mice. Preliminary phytochemical screening and acute toxicity studies of the extract were carried out using standard methods. Antidepressant activity screening of the extract was conducted using Tail Suspension Test (TST), Forced Swim Test (FST), and Open Field Test (OFT) in mice. Phytochemical screening shows the presence of terpenoids, steroids, cardiac glycosides, tannins, saponins, flavonoids and carbohydrates in the extract. The result of the acute toxicity studies revealed an LD_{50} value of 28.3 mg/kg body weight in mice. ELEEA at all the tested doses significantly ($p < 0.05$) reduced the duration of immobility of mice in TST compared to the normal saline (control) group. In the FST, the extract at doses of 2, 4 and 8 mg/kg body weight exhibited significant ($p < 0.01$) reduction in the immobility time when compared to the control group. However, in the OFT, ELEEA at doses of 2, 4 and 8 mg/kg body weight and diazepam (0.5 mg/kg) had no significant effect ($p > 0.05$) on the number of lines crossed by the mice compared to the control group. The results of our study suggest that the ethanol leaf extract of *Entada africana* has potential for use as an antidepressant agent.

***Keywords*:** Antidepressant, Tail suspension test, Forced swim test, Open field test, *Entada Africana*

Introduction

Depression is a chronic mental disorder that causes changes in mood, thoughts, behaviour and physical health.[1] The hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest in activities that were rather pleasurable in the past (anhedonia) for a duration of at least two weeks. These symptoms must also be accompanied by at least four of the following manifestations such as changes in appetite or weight, sleep patterns, altered psychomotor activity, feelings of worthlessness or guilt, difficulty concentrating or making decisions and recurrent thoughts of death or suicidal ideation.[2, 3] There are different types of depression such as MDD, dysthymic disorder (DD), melancholic depression, seasonal affective disorder (SAD), post-partum depression (PPD) and psychotic depression (PD).[4, 5] This state of health has been attributed to hypo-functioning of monoamine neurotransmitters in the brain. The major neurotransmitters that are implicated in depressive episodes are noradrenaline, serotonin and dopamine,[6] even though, the exact mechanism remains unclear.[7] Depression is a significant contributor to the global burden of diseases and life-threatening disorder that affects hundreds of millions of people in all communities across the world.[8] The lifetime prevalence for major depression is reported to be as high as 14-17% and the one-year prevalence is 4-8%.[1]

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Citation: Bebeji MY and Yaro AH. Evaluation of Antidepressant Activity of Ethanol Leaf Extract of *Entada africana* Guill. & Perr. (Fabaceae) in Mice. Trop J Nat Prod Res. 2024; 8(2):6446-6450. http://www.doi.org/10.26538/tjnpr/v8i2.37

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Depression is also a significant cause of morbidity worldwide, according to the world health report; about 350 million people suffer from depression across all age categories.[9] In Africa, it affects many populations and the incidence is more pronounced in Nigeria.[10] Depression is one of the most important reasons for disability and it is predicted to be the leading cause of disease burden globally by the year 2030, based on disability-adjusted-life-year.[11] Depression significantly contributes to the increasing suicide rate in the 21st century.[12] Almost a million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day.[13] Most of the classical antidepressants in clinical use possess undesirable side effects and their mechanisms of action have not yet been satisfactorily elucidated.[14] Drugs used in the treatment of depression are collectively known as "Antidepressants". Examples are amitriptyline, imipramine, fluoxetine, sertraline, selegeline, pargyline, mianserin, etc.

Entada africana Guill & Perr., belongs to the family *Fabaceae,* it is commonly known as "*Tawatsa*" in Hausa Language and "*Ogurobe*" in Yoruba.[15] In African traditional medicine, *Entada africana* (*Ea)* is used for the treatment of dysentery, cataract, wound, stomach ache, fever and liver related diseases.[16] In northern Nigeria and northern part of Ghana, an infusion of the leaves or stem bark is used as tonic and in the treatment of stomach ache; the leaves possess a good wound healing effect and it also prevents suppuration.[17, 18] Other traditional uses of *Ea* include treatment of cutaneous and subcutaneous parasitic infections, venereal diseases, fabrifuges, paralysis, epilepsy, convulsions, spasm among others.[19, 20] However, studies conducted on this plant have shown that the plant possesses anti-hepatotoxic,[21] antioxidant[22] and antimicrobial properties.[23] It has been reported that *Entada africana* leaves have been used ethno-medicinally in the treatment of mental disorders like depression.[24]

Forced Swim Test (FST) and Tail Suspension Test (TST) are animal models of depression that are widely used to screen new antidepressant drugs.[25] They are quite very sensitive and relatively specific to all major classes of antidepressant drugs including serotonin-specific reuptake inhibitors, monoamine oxidase (MAO) inhibitors and atypical

antidepressants.[26] FST was first described by Porsolt *et al*. (1977) while TST was first described by Steru *et al*. (1985). The Open Field Test (OFT) is one of the most popular procedures used to measure behaviors in animal models. It is relatively an easy test that provides a variety of behavioral information ranging from locomotor activity to emotionality in rodents.[27] It is generally conducted to avoid false positive results in TST and FST and was originally described by Hall (1934). The present study therefore seeks to investigate the antidepressant activity of *Entada africana* leaves using the FST, TST as well as the OFT in mice.

Materials and Methods

Animals

One hundred and twenty four (124) Swiss albino mice (18 - 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 12-hour dark cycle and had free access to food and water. The experiment was conducted in accordance with the principles of laboratory animal care[28] and all procedures were approved by the institutional ethical committee with ethical approval number BUK/ACUREC/CAP/PG14.

Drugs, solvents and equipment

Imipramine (Assos Pharm., Turkey), diazepam (Roche Pharm., Switzerland), ethanol, normal saline (Sigma chemical co. St Louis, USA) and distilled water. Electric oven, weighing balance, 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), white wooden open field apparatus (70×70×35 cm, length × breadth × height) and hand gloves.

Collection of plant material and extract preparation

Fresh leaves of *Entada africana* were collected in the month of May, 2022 from Kudingi forest, Giwa Local Area, Kaduna state, Nigeria. The plant material was identified by a taxonomist (Namadi Sunusi) in the Herbarium Section, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria and herbarium specimen with voucher number ABU90040 was deposited as a reference. The plant material was washed, shade-dried, pulverized and extracted using 70% v/v ethanol by cold maceration for three days with occasional stirring and agitation. The extract was filtered using Whatman filter paper No 1 and then concentrated in electric oven at 50°C until dried extract (600 g) was obtained. The extract was kept in a desiccator until ready for use.

Phytochemical screening

Preliminary phytochemical screening was carried out using the method described by Trease and Evans (1989).

Acute toxicity study

Acute toxicity study of the extract was conducted using the method described by Lorke (1983). The method consisted of two phases and a total of 13 mice of both sexes were used. In phase 1, three groups of three mice each were administered the extract intraperitoneally at doses of 10, 100 and 1000 mg/kg body weight and observed for signs and symptoms of toxicity including death for 24 hours. In the second phase, which was determined by the results of the first phase, three groups of one mouse each were administered the extract intraperitoneally at specific doses of 20, 40 and 1600 mg/kg. Mice were observed for signs and symptoms of toxicity including death for 24 hours. The LD_{50} was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

Evaluation of antidepressant activity

Tail Suspension Test (TST) in mice

The method described by Steru *et al*. (1985) was adopted. Mice were randomly divided into six groups of six mice each, (n = 6) and pretreated as follows; group I received normal saline (10 mL/kg ip), groups II, III, IV and V received graded doses of ethanol extract of *Entada africana* leaves (1, 2, 4, 8 mg/kg ip, respectively) while group VI 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 total 6 min testing period. Mice were considered immobile when they hung passively and completely became motionless. A decrease in the immobility period was recorded as potential antidepressant activity.

Forced Swim Test (FST) in mice

The method described by Porsolt *et al*. (1977) was employed. Mice were randomly grouped into six groups of six mice each, (n = 6) and treatments were administered: group I received normal saline (10 mL/kg ip), groups II, III, IV and V received graded doses of ethanol extract of *Entada africana* leaves (1, 2, 4 and 8 mg/kg ip, respectively) while group VI received standard antidepressant agent imipramine (10 mg/kg ip). Thirty minutes later, depression was produced by forcing the animal to swim individually in transparent open glass container of 30 cm length and 20 cm in diameter containing fresh water of 15 cm depth 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.

Open Field Test (OFT) in mice

The OFT was performed according to the method described by Prut and Belzung (2003). Animals were randomly divided into five groups of six mice each (n = 6). Group 1 was treated with normal saline (10 mL/kg ip), groups II, III, and IV received graded doses of the ethanol extract of *Entada africana* leaves (2, 4, and 8 mg/kg ip, respectively) and group V was treated with diazepam (0.5 mg/kg ip). After thirty minutes of administration, each mouse was placed in white wooden open field apparatus (70×70×35 cm, length × breadth × height). The exploratory behavior of each mouse in the apparatus was recorded for 5 min. The apparatus was cleaned with 10% ethanol before and after subjecting each mouse to the test.

Statistical analysis

Data were analyzed using SPSS statistical software version twenty (V.20). Results were expressed as mean ± SEM. Difference between means were analyzed using one way Analysis of Variance (ANOVA) followed by Dunnet's post hoc test. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Phytochemical constituents of Entada Africana leaves

The result of the preliminary phytochemical analyses shows that the ethanol leaf extract of *Entada africana* contained terpenoids, steroids, cardiac glycosides, tannins, saponins, flavonoids and carbohydrates (Table 1). This was in line with the findings of Yusuf and Abdullahi (2019).[29] Phytochemical components especially alkaloids, saponins, flavonoids, phenols, terpenoids and carbohydrates have been reported to have antidepressant activity.[30, 31]

Median lethal dose (LD_{50}) of Entada Africana leaf extract

The intraperitoneal median lethal dose (LD_{50}) of *Entada africana* ethanol leaf extract was estimated to be 28.3 mg/kg body weight in mice.

Antidepressant activity of ethanol leaf extract of Entada africana

Animal models serve as an essential tool for studying the pathophysiology of depression and developing promising therapeutic leads as antidepressant agents.[25, 32, 33] The models (TST and FST) of behavioral despair mimic human depressive symptoms; these models are well-established and provide a fast and efficient way to screen

potential antidepressant drugs.[25, 32] In these animal models, mice usually struggled against the condition, and some of them gradually developed despair and gave up the struggle with an increased immobility time that represents increased despair and depression.[34]

Hence, immobility duration is considered the core index in the use of TST and FST in evaluating antidepressant effects. These tests are the most widely utilized models for screening potential antidepressant drugs. The time spent in an immobile state by the animal during the 6 minutes is a measure of escape-related behavior.[2] The decrease in immobility time is used as the main index for the antidepressant effects of the test agents in TST and FST models.[35] These tests are quite sensitive to major antidepressant drugs, such as selective serotonin reuptake inhibitors (SSRIs), tricyclics and monoamine oxidase inhibitors (MAOIs), which can effectively reduce the immobility time and increase activity.[25, 32] In this study, the ethanol leaf extract of *Entada africana* (ELEEA) at all tested doses in the TST significantly ($p < 0.05$) decreased the duration of immobility when compared to the control (normal saline at 10 mL/kg) group. Similarly, the standard drug, imipramine (10 mg/kg), also significantly ($p < 0.01$) decreased the duration of immobility and this is a clear indication that the extract possesses antidepressant activity in this model (Table 2). In the FST, the extract at doses of 2, 4 and 8 mg/kg body weight significantly ($p < 0.05$) decreased the duration of immobility when compared to the control group. Similarly, the standard drug, imipramine (10 mg/kg), also significantly ($p < 0.01$) decreased the duration of immobility time when compared to the control group. This suggests that, the extract possesses antidepressant activity (Table 3). The better activity observed in the TST compared to the FST could be due to the fact that TST is less stressful than FST and therefore may have better pharmacological compassion.[36] Although the predictive validity of TST and FST in animal models of depression has been confirmed, and there are some defects in these models.[2] Some psycho-stimulant drugs, such as caffeine, amphetamine, and methylphenidate, that stimulate the CNS, also increase locomotor activity but lack antidepressant properties.[37] Hence, to avoid the possibility of false-positive results, the effect of ELEEA on locomotion and exploration was assessed in an open-field arena. The ethanol leaf extract of *Entada africana* at doses of 2, 4 and 8 mg/kg body weight and diazepam (0.5 mg/kg) had no significant effect ($p > 0.05$) on the number of lines crossed by the mice compared to the normal saline (10 mL/kg) (control) group (Table 4). The extract did not increase spontaneous motor activity in mice but relatively inhibited locomotor activity which suggest that it possesses general CNS depressant potential. Overall, it is evident that the antidepressant action of ELEEA has no relationship with CNS stimulation. Moreover, several well-known antidepressants decrease locomotor activity.[38, 39]

Numerous works have reported a number of phytochemical constituents like tannins, saponins and flavonoids to have neuroprotective and antidepressant effect.[40-43] Therefore, the observed antidepressant effect in ELEEA could be due to one or more of the detected secondary metabolites. In this work, the exact phytochemical constituent(s) responsible for the activity as well as the mechanism of antidepressant activity seen in ELEEA is not very clear, we therefore suggest further studies to specifically determine the phytochemical constituent(s) responsible for the observed antidepressant activity and ascertain the possible mechanism(s) of action.

Conclusion

The results of the present study suggest that ethanol leaf extract of *Entada africana* possesses antidepressant activity by reducing behavioural despair in acute depression animal models. Therefore, *Entada africana* has the potential for use in the treatment of depression.

Conflict of Interest

The authors declare no conflict of interest.

Table 1: Phytochemical constituents of ethanol leaf extract of *Entada africana*

Phytochemical	Inference
Carbohydrates	+
Saponins	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Anthraquinones	-
Steroids	+
Terpenoids	+
Alkaloids	-

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

Table 2: Effect of ethanol leaf extract of *Entada africana* on Tail Suspension Test in mice

Treatment	Immobility time (sec)
Control	233.17 ± 08.95
Imipramine (10 mg/kg)	145.17 ± 08.86**
ELEEA (8 mg/kg)	191.50 ± 05.49*
ELEEA (4 mg/kg)	157.83 ± 05.98**
ELEEA (2 mg/kg)	171.17 ± 03.08**
ELEEA (1 mg/kg)	182.67 ± 08.04**

Values are presented as Mean ± SEM, n = 6. * = P < 0.05, ** = P < 0.01 compared to control group. ELEEA = Ethanol leaf extract of *Entada africana*, Control = Normal saline 10 mL/kg.

Table 3: Effect of ethanol leaf extract of *Entada africana* on Forced Swim Test in mice

Treatment	Immobility time (sec)
Control	138.00 ± 07.71
Imipramine (10 mg/kg)	29.00 ± 03.68**
ELEEA (8 mg/kg)	47.33 ± 06.67**
ELEEA (4 mg/kg)	35.50 ± 05.61**
ELEEA (2 mg/kg)	35.50 ± 05.61**
ELEEA (1 mg/kg)	134.50 ± 10.51

Values are presented as Mean ± SEM, n = 6. ** = P < 0.01 compared to control group. ELEEA = Ethanol leaf extract of *Entada africana*, Control = Normal saline 10 mL/kg

Table 4: Effect of ethanol leaf extract of *Entada africana* on locomotor activity in the Open Field Test in mice

Treatment	Mean squares crossed
Control	97.33 ± 2.14
Diazepam (0.5 mg/kg)	66.67 ± 1.41
ELEEA (8 mg/kg)	66.33 ± 7.09
ELEEA (4 mg/kg)	70.17 ± 8.08
ELEEA (2 mg/kg)	65.17 ± 3.92

Values are presented as Mean ± SEM, n = 6. No significant difference ($p > 0.05$) compared to control group. ELEEA = Ethanol leaf extract of *Entada africana*, Control = Normal saline 10 mL/kg.

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.

Acknowledgments

The authors are indebted to all the academic and technical staff of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical sciences, Bayero University Kano for their contributions towards the success of this research work.

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