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Research Article

Evaluation of Anti-depressant Activity of Ethanolic Extract of *Moringa oleifera* Leaves in Mice

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Abstract

The use of *Moringa oleifera* in the food preparations can help to meet the future demands of nutraceuticals and functional foods. The present work is aimed to evaluate the antidepressant activity of ethanolic extract of *Moringa oleifera* Leaves (EEMOL) in mice. The overnight fasted mice will be divided into 04 groups, each group consisting of 06 animals. The *EEMOL* will be given in various doses (10, 100, 500 and 1000 mg/kg) by oral route with a gavage. After administration of the extract, the animal will be observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and also will be monitored up to 14 days for the toxic symptoms and mortality. The mice of *Moringa oleifera* extract (300 & 600 mg/kg/p.o) treated group showed significantly ($p<0.05$) increased in body weight, feed intake and body water intake as compared to the control group. Treatment with Fluoxetine (10 mg/kg p.o.) the body weight, feed intake and body water intake significantly increased as compared to normal group. The observation of this study suggests that ***Moringa Oleifera*** has antidepressant activity. In conclusion, *Moringa oleifera* extracts possess a broad spectrum of activity against a panel of factors responsible for the most common psychosis diseases.

Keywords: Anti-depressant Activity, Ethanolic Extract of *Moringa oleifera Leaves,* Fluoxetine

INTRODUCTION

Depressed mood is a symptom of some mood disorders such as major depressive disorder or dysthymia; Depression can be defined as the most common of the affective disorders, It is characterized as mood problems as opposed to mental or cognitive difficulties; symptoms can vary from a very mild illness that verges on normalcy to severe psychotic depression that is accompanied by delusions and hallucinations. The need to treat depression and other mental health issues is growing on a worldwide scale.[1] The demand for curbing depression and other mental health conditions is on the rise globally. A recent World Health Assembly called on the World Health Organization and its member states to take action in this direction[2].

Antidepressants are medications that work by modifying the chemical imbalances of neurotransmitters in the brain to help lessen the symptoms of depressive illnesses. Chemical imbalance in the cells is the cause of the change in behavior and mood.

Reuptake is the process by which neurotransmitters like dopamine, serotonin, and noradrenalin or epinephrine are taken up by the other nerve after being released from the exonic end of the first. Antidepressants increase the concentration of a certain neurotransmitter around the brain's neurons by blocking the reuptake of neurotransmitters through specific receptors. Selective serotonin reuptake inhibitors (SSRIs), which modify serotonin levels in the brain, are one type of antidepressant. Antidepressants may recover the signs of depression, but also exert some side-effects.[3]

The drumstick tree, or Moringa oleifera (MO), is native to South Asia, primarily the Indian foothills of the Himalayas. It has been planted and allowed to naturally occur in a number of other nations, including Afghanistan, Nepal, Bangladesh, Sri Lanka, South and Central America, the West Indies, the Philippines, and Cambodia. It is small, simple to grow, grows rapidly, and its leaves don't fall off during the dry season. Its leaves are also very nutrient-dense, full of vitamins, minerals, amino acids, and naturally occurring antioxidants. [4-7]

Botanical Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Violes
Family	Moringaceae
Genus	*Moringa*
Species	*oleifera.*

MATERIAL AND METHODS

Source of data: Every experiment was designed to provide data from the laboratory research, meaning that it was carried out in accordance with the guidelines provided by references, experimental studies published in journals, and textbooks that were available from the college, the SSIP library in Jalandhar, and other organizations.

Methods of collection of data

Study through research articles, research data based like Medline.

The data related to physicochemical details of the drug will be collected from drug information centre, various standard books, journals and other sources like literature data bases such as science direct etc.

The data was collected based on laboratory animal experimentation.

Experimental Animals[8]

Because of its small size, adaptability, docility, cheap husbandry expenses, fertility, well-defined health and genetic backgrounds, and relative simplicity of genetic modification, the domestic mouse, Mus musculus and related subspecies, is a popular choice for mammalian research models. The use of mice as study subjects has significantly risen with the introduction of genetic engineering techniques that allow for the insertion of foreign genes (transgenes) into the mouse genome and the deletion of genes, resulting in "knockout" mice. Since these advances, countless mutant genotypes of mice have been developed, ranging from subtle defects in immune function to full-fledged, inherited diseases virtually homologous with those of higher mammals.

Albino mice (25-30 gm) of either sex were used in the present study and were housed under standard conditions of light and dark cycle in the central animal house facility of St. Soldier Institute of Pharmacy, Jalandhar, Punjab in different polypropylene cages with husk bedding and were maintained at standard laboratory pellet chow diet and water *ad libitum*. The animals were acclimatized to the laboratory conditions prior to the experimental study. All experiments were performed between 08:00 and 16:00 hr in semi sound proof laboratory conditions. The experimental protocol was approved by the Ministry of Environment and Forests, Government of India's Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), and it was carried out in compliance with its guidelines. (Reg. No. 2011/PO/Re/S/18/CPCSEA and date of registration is 1/5/2018) for the use and care of experimental animals. Adequate measures were taken to minimize pain or discomfort with animal's experimental procedure. Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2023/PR-039).

Drugs and Reagents/ Chemical:

All the chemicals and biochemical reagents used in this study were of analytical grade and were freshly prepared before use. All chemicals of analytical grade were procured from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

Procurement of the extract:

Materials: **-**

Moringa oleifera Leaves ethanolic extract was collected from the dealer of Shreedha Phyto extracts Jaipur – 302019. The same group also provided a certification of the Plant's identify and quality.

Experimental animals:

24 mature, either sex mice weighing 25–30g were acquired from the pharmacy department's animal home. ST. Soldier institute of pharmacy, Jalandhar- Amritsar Bypass NH-I , Behind NIT Jalandhar , Punjab India – 144011 .

Animals:

Healthy, adult Swiss albino mice of either sex weighing (25-30 g), maintained under standard laboratory conditions, at temperature 25 ± 2°C and a 12 hr light-12 hr dark period will be employed for the experimentation. Food and water will be provided for free.

Acute oral toxicity study[9]

Acute toxicity study for the ethanolic extract of *Moringa oleifera* Leaves done according to the OECD guidelines No: 423 and low, medium and high dose will be selected for treatment.

Method:

The overnight fasted mice will be divided into 04 groups, each group consisting of 06 animals. The *EEMOL* will be given in various doses (10, 100, 500 and 1000 mg/kg) by oral route with a gavage. After administration of the extract, the animal will be observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and also will be monitored up to 14 days for the toxic symptoms and mortality.

Treatment:

GROUPS (where N=4)	Treatment
Group I	Naive animal, received standard pellet diet and tap water ad libitum daily.
Group II	Standard group received 10mg/kg Fluoxetine orally daily.
Group III	Test group-I received 300mg/kg Ethanolic extract of *Moringa oleifera* Leaves orally daily.
Group IV	Test group –II received 600mg/kg Ethanolic extract of *Moringa oleifera* Leaves orally daily.

Parameters for depression in mice :

The behavioral effects of an acute or sub acute (14 day course) will be orally administered. *Moringa oleifera* Leaves (300, 600 mg/kg) ethanolic extract will be evaluated in male and female Swiss mice in forced swim test (FST) and locomotor activity (Actophotometer). The animals were housed in plastic cages in groups of six per cage, at room temperature about 21 + 1 ~ C, and with free access to water and food. They were kept on an artificial 12 h/12 h day/night cycle.

Tail suspension test [10] :

In this model, groups of 6 animals are treated with the test compounds or the vehicle by orally 30 min prior to testing. For the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1cm from the tip of the tail. Mice is considered immobile when they hang passively and completely motionless for at least 1 min.

Actophotometer [10]:

Locomotors activity-: The locomotors activity can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell in cut off by the animal, a count is recorded. An actophotometer could have either circular or square across in which the animal moves. Both mice and rats may be used for testing in this equipment.

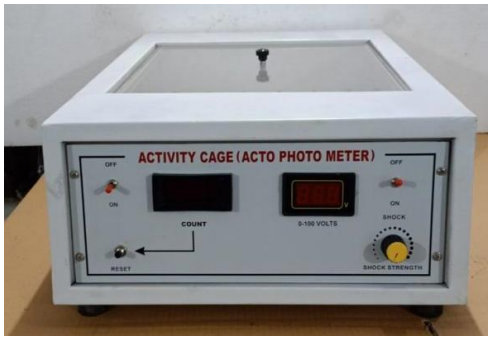


Figure 1: Actophotometer and TST

Biochemical estimation[11]:

Collection of Blood Samples

On 15th day, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10 min using refrigerated centrifuge (Paramount scientific works, Ambala cantt, India) to separate the plasma, which was used for estimation of corticosterone levels.

Estimation of plasma corticosterone levels:

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10 % solution of p-nitroso-N,N- dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 cc of 0.10 N sodium hydroxide was added. After plugging the tubes with cotton wool and shielding them from light for five hours, they were allowed to stand at 0°C. To the above solution, 2.0 ml of buffer for pH 9.8, 5.0 ml of 0.10 % solution of phenol in ethanol and 0.50 ml of 1.0 % aqueous solution of potassium ferricyanide were added. For ten minutes, the tubes were maintained at 20±2°C in a water bath. The solution was read at 650 nm using UV-visible spectrophotometer (UV 3200 UV-VIS Spectro-photometer, Somajiguda, Hyderabad).

Note: All the parameters will be performing with suitable time interval to prevent unwanted stress in animals.

Statistical Analysis

Each and every result was given as Mean ± SEM. The data of all the groups were analyzed by One way ANOVA followed by Turkey's test using software Graph pad prism In Stat (Graph Pad Software Inc., USA). A value of $p<0.05$ was considered to be significant.

RESULTS

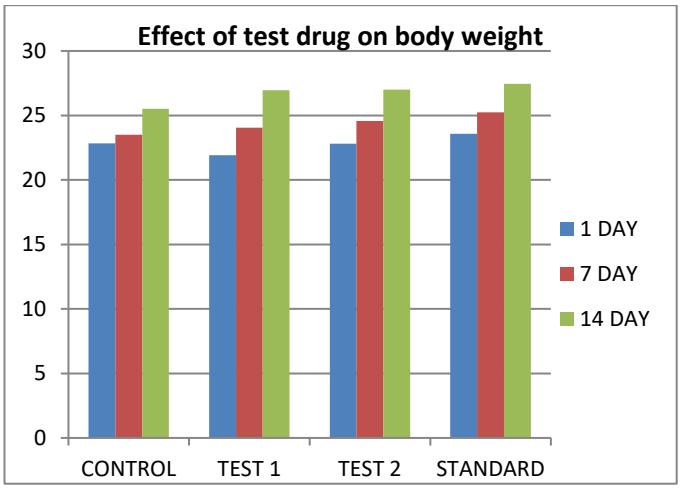
Phytochemical properties of MO Leaves

SL No	Phytochemical	Result
1	Reducing sugars	-
2	Tannins	+
3	Saponins	-
4	Glycoside	+
5	Phenolic compounds	+
6	Flavonoids	+
7	Alkaloids	-

+ refers present and – refers absent

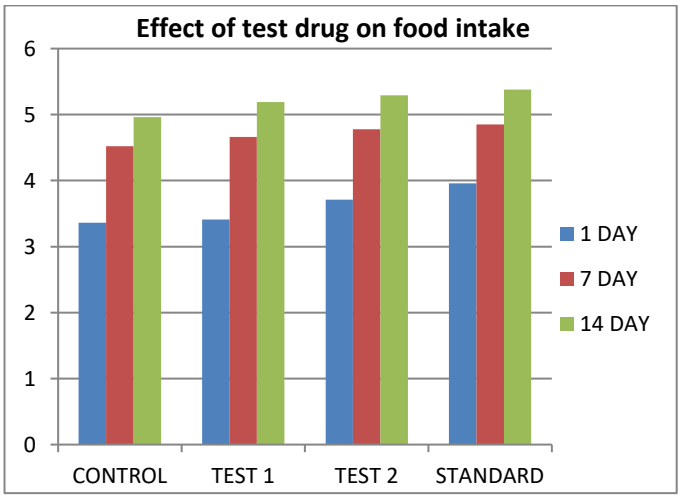
All the following parameters were performed with suitable time interval to prevent unwanted stress in animals.

Effect of *Moringa oleifera* extract on body weight (g) of mice



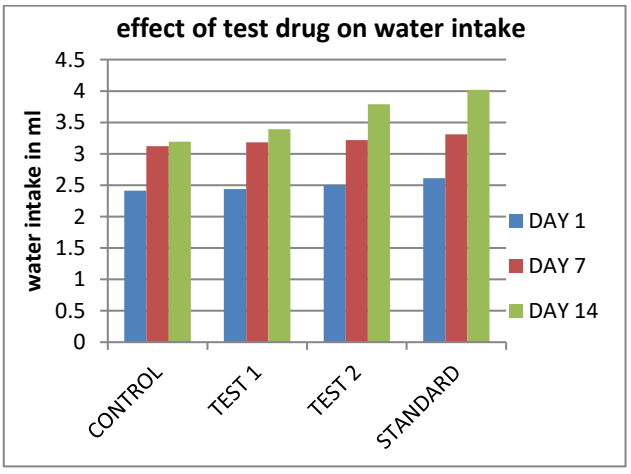
Graph Showing Effect of *Moringa oleifera* extract on body weight (g) of mice.

Effect of *Moringa oleifera* extract on Feed intake (g) of mice



Graph Showing Effect of *Moringa oleifera* extract on Feed intake (g) of mice

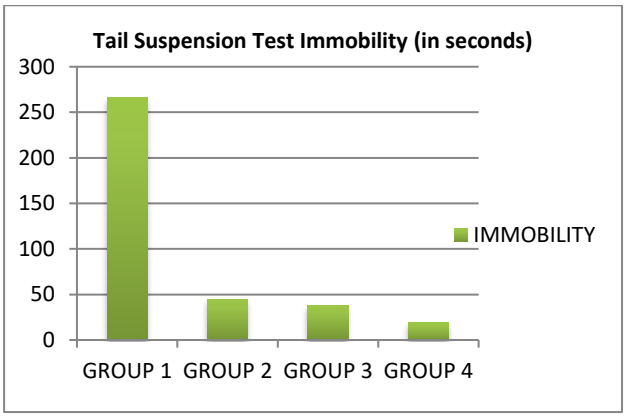
Effect of *Moringa oleifera* extract on Water intake (ml) of mice



Graph Showing Effect of *Moringa oleifera* extract on Water intake (ml) of mice

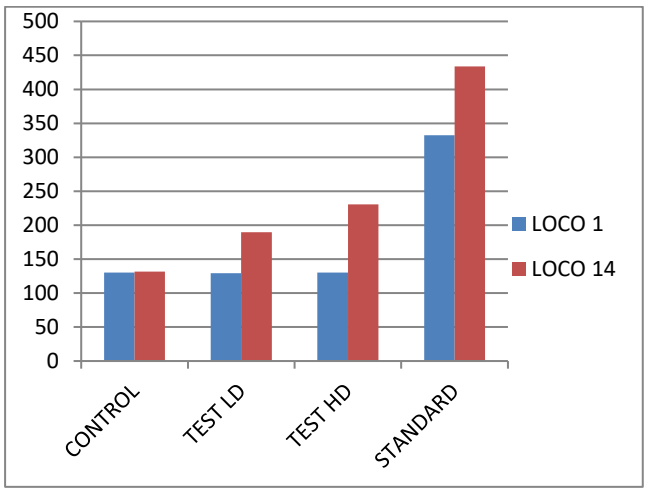
Evaluation of antidepressant effect of *Moringa Oleifera* leaves ethanolic extracts in TST & Actophotometer Models:

Tail Suspension Test (TST)



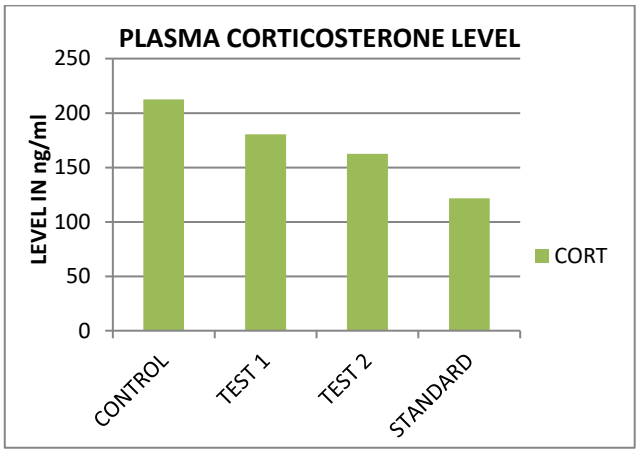
Graphical representation of effects of different doses of *Moringa Oleifera* ethanolic extract on antidepressant effect as compared to standard (Fluoxetine).

Effect of *Moringa oleifera* extract on locomotors activity of mice



Graph Showing Effect of *Moringa oleifera* extract on locomotors activity of mice.

Effect of Ethanolic extract of *Moringa oleifera* leaves on plasma corticosterone levels (CORT)



Graph showing Effect of *Moringa oleifera* extract on plasma corticosterone levels

DISCUSSION

Progress in unraveling the neuro-chemical mechanism is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. Although animals cannot replicate human psychopathology in every detail, they should be properly conceived as experimental systems in which selected any specific questions can be investigated in ways impossible to do in humans. A good animal model permits the understanding of a specific behavior or a set of behaviors in terms of developmental and social contacts as well as pathophysiological. Therefore the animal models have played role in pre clinical evaluation of psychiatric drugs.

The mice of *Moringa oleifera* extract (300 & 600 mg/kg/p.o) treated group showed significantly (p<0.05) increased in body weight as compared to the control group. Treatment with FXT (10 mg/kg p.o.) the body weight significantly increased as compared to normal group.

The mice of *Moringa oleifera* extract (300 &600 mg/kg/p.o) treated group showed significantly (p<0.05) increased in feed intake as compared to the control group. Treatment with FXT (10 mg/kg p.o) the feed intake significantly (p<0.05) increased as compared to control group group.

The mice of *Moringa oleifera* extract (300 &600 mg/kg/ p.o.) treated group showed significantly (p<0.05) increased in body water intake as compared to the control group. Treatment with FXT(10 mg/kg p.o.) the water intake significantly (p<0.05) increased as compared to another group.

Treatment with Fluoxetine significantly decreased the duration of immobility time (P< 0.001) in TST. Ethanolic extract of *Moringa Oleifera* treated mice also exhibited dose dependent significant decreased the duration of immobility time. The duration of immobility was also significantly reduced as compared to the vehicle treated group. But there is no significant difference between *Moringa Oleifera* extracts treated animals & Fluoxetine treated animal. The above observation suggests that *Moringa Oleifera* has antidepressant activity.

The mice of *Moringa oleifera* extract (300 & 600 mg/kg/p.o) treated group showed significantly (p<0.05) difference in locomotors activity as compared to control group. Treatment with FXT (10 mg/kg p.o.) also showed significant change as compared to control group.

The mice treated group of *Moringa oleifera* extract (300 & 600 mg/kg/p.o.) showed increased plasma corticosterone level (p<0.05) as compared to normal control mice. However, pretreatment FXT (10 mg/kg i.p)14 days showed significant increased (p<0.05) in plasma corticosterone significantly.

The LD_{50} was estimated in Acute oral toxicity study.

The median lethal dose (LD_{50}) of Ethanolic extract of *Moringa oleifera* Leaves (EEMOL) was determined in accordance with the Organization for Economic Co-operation and Development (OECD, 425) guidelines using five mice which were fasted overnight before dosing with ethanolic extract of *EEMOL* separately at maximum dose level up to 1000 mg/kg orally starting from dose of 100, 250 , 500 mg/kg. It was observed for the first 24 h and then for 14 d for signs of toxicity (changes in mucous membranes, skin, fur and eyes, circulatory, respiratory, somato-motor activity and behaviour pattern) and mortality. It has been observed that no change in behavioral responses and observation shows any acute oral toxicity.

Progress in unraveling the neuro-chemical mechanism is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. There is no known animal condition corresponding to the inherited condition of depression in humans, but various procedures have been described that produce in animals behavioral states typical of human depression.[9]

After selection of *Moringa oleifera*, acute oral toxicity was detected with ethanolic extracts (EEMOL) having dose (100, 250, 500, 1000 mg/kg) via oral route, shows no change in behavioral responses and observation shows no acute oral toxicity. Hence depending upon it, Dose was selected 300 mg/kg & 600 mg/kg for our experimental work. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethanol provided more consistent activity compared to those extracted by water. The results of antidepressant activity of plant *Moringa oleifera* against the investigated Locomotion and TST parameters are shown in table. The higher dose extract produced more effect comparative to lower extract in both parameters. This might have resulted from the lack percentage of the active constituents in ethanol extract showed some degree of antidepressant activity. Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy.[12]

In locomotion (Actophotometer), Group - Control 0.9% w/v sodium chloride Normal saline (10 ml/Kg) or (1ml / 100gm, p.o.) administration have shown their immobility and number of photocell crossed is significant.

When Ethanolic extract of *Moringa oleifera* (300 mg/kg) orally administered, have shown immobility and number of photocell crossed is significant. When Ethanolic extract of *Moringa oleifera* (300 mg/kg) orally administered, have shown immobility and number of photocell crossed is significant. When standard dose of Fluoxetine (10 mg/kg) was orally administered, have shown immobility and number of photocell crossed is more significant.

The Ethanolic extract of *Moringa oleifera* 600 mg/kg showed the most remarkable activity. This plant can be further subjected to isolation of the therapeutic Antidepressant compound and carry out further pharmacological evaluation.

SUMMARY AND CONCLUSION

It has been used traditionally to cure many diseases and is also an important ingredient of Ayurveda herbs. Animal based studies have proved its effectiveness to prevent and cure diseases and hence strengthened its claim of herbal medicine. The use of *Moringa oleifera* in the food preparations can help to meet the future demands of nutraceuticals and functional foods. Medicinal plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants.

The ethanol extract of *Moringa oleifera* showed the most remarkable activity due to its rich chemical constituents. The percentage Yield of ethanolic Extract of *Moringa oleifera* was calculated. Preliminary Phytoprofile &Preliminary Phytochemical Studies of Extract of *Moringa oleifera* were performed. Antidepressant active plant principles such as phenolic compounds, Proteins, tannins, glycosides, Carbohydrate, Starch, Vitamins & Minerals were observed in the extract.

From our investigation of screening of *Moringa oleifera*, the results obtained confirm the therapeutic potency of plant used in traditional medicine. In addition, these results form a good basis for selection of candidate plant species for further Phytochemical and pharmacological investigation.

The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antidepressant properties that can be used as Antidepressant agents in new drugs for the therapy of neuropsycho diseases caused by various factors. The most active extracts can be subjected to isolation of the therapeutic Antidepressant and undergo further pharmacological evaluation.

In conclusion, *Moringa oleifera* extracts possess a broad spectrum of activity against a panel of factors responsible for the most common psychosis diseases. These promissory extracts open the possibility of finding new clinically effective antidepressant compounds. The ethanolic extracts of *Moringa oleifera*, investigated individually for Antidepressant activity by locomotion and TST method at the dose level of 300 & 600mg/ml. The ethanolic extract higher dose of *Moringa oleifera* showed considerably high activities than lower dose. These results were compared with standard antidepressant Fluoxetine. But the exact active components of the extract that showed this effect were not isolated. In conclusion, although active components were not isolated, but antidepressant active plant principles were observed in the both doses of ethanolic extracts.

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Authors Contributions: All the authors have contributed equally.

Source of Support: Nil

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethics approval: Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2023/PR-039).

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