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Research Article

# STUDY OF THE ANTIDEPRESSANT ACTIVITY OF THE ETHANOLIC EXTRACT OF THE LEAVES OF *MORINGA OLEIFERA* L. (EEMO) ON ALBINO MICE

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**ABSTRACT**

**Objectives:** The aim of the study is to evaluate the antidepressant activity of the ethanolic extract of the leaves of *Moringa oleifera* L. (EEMO) on albino mice.

**Methods:** The anti-depressant activity was evaluated using forced swimming test (FST) and tail suspension test (TST). Healthy albino mice were individually forced to swim inside a vertical glass cylinder. Climbing, swimming, and immobility were tested and recorded. In TST, mice were suspended on a string held by a metal stand, and immobility time was recorded.

**Results:** In both FST and TST, it was seen that immobility time decreased with increase in the dose of EEMO.

**Conclusion:** Ethanolic extract of *M. oleifera* possess anti-depressant activity in a dose dependent manner.

**Keywords:** Anti-depressant, Forced swimming test, Tail suspension test, *Moringa oleifera*.

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## INTRODUCTION

Depression is characterized by persistent sadness, accompanied by an inability to carryout daily activities, feelings of worthlessness, guilt or hopelessness, and thoughts of self-harm [1].

Depression is a common psychiatric disorder affecting millions of people around the globe. It is a leading cause of worldwide disability and it has major contribution to overall burden of disease across the globe [2]. It is treatable with psychotherapy, also known as counseling, antidepressant medication, or a combination of these [3,4].

Antidepressant drugs are the most effective and widely used forms of the treatment for depression but this does not mean that they are free of adverse drug reactions (ADR) [2]. Along with the therapeutic effect, almost every drug's adverse effect profile is quite vast [5].

Large number of ADRs associated with use of psychiatric medication due to their effect on multiple dopaminergic pathways [6].

*Moringa oleifera* Lam. (Drumstick tree) a plant in a family of Moringaceae and its cultivation in India is very wide. It is used as an active ingredient of the food preparation and medication. Almost all parts of the plant are utilized in traditional medical practices. It is believed to be a miracle herb because of its medicinal properties. The leaves and young buds of the plant can be rubbed on the temples for relieving headache, while the root and root bark are regarded as antiscorbutic and can be used as counter-irritants externally. The extract of leaves mixed with honey is used for the treatment of eye diseases. The leaves of the plants have also been reported for its hypotensive, antioxidant, anti-inflammatory, and diuretic properties [7].

In complementary and alternative medicines, various plants are used worldwide for the management of stress. The vigorous use of safer as well as cheaper herbal medicines has been reported as anti-stress agent because they can reduce stress without affecting the physiology of human body [8].

There is not much information regarding the anti-depressant effect of *M. oleifera*. Considering this, the present study has been undertaken on animal models.

## MATERIALS AND METHODS

Healthy albino mice (Mus musculus) weighing 20–40 g of either sex.

### Drugs

Ethanolic extracts of leaves of *M. oleifera* (EEMO).

Normal saline.

Fluoxetine.

### Equipments

Glass jar.

Metal stand.

Tuberculin syringe.

Marker pen.

### Plant material

Fresh tender leaves of *M. oleifera* collected from Assam Medical College Campus were authenticated by Dr M. Islam, Professor of Life Science, Dibrugarh University, Assam, India. The leaves were air dried at room temperature and ground to fine powder which was soaked in 95% ethanol in a percolator. The extract obtained was concentrated in vacuum using rotary flash evaporator. There was a net yield of 150 g of concentrated extract.

### Preparation of extract

Coarsely powdered leaves were extracted with ethanol-water by maceration in a closed vessel for 72 h. At the interval of 24 h, the used ethanol was changed with fresh amount ethanol. The vessel was shaken

occasionally during the extraction period. The ethanolic extract of the leaves was filtered, pooled, and vacuum concentrated using a rotary evaporator. The dark brown colored sticky residue was collected after the complete removal of the solvent. EEMO thus obtained was used for biological activity [9].

**Animals**

The animals used in the study were procured from the Central Animal House, Assam Medical College and Hospital. The study conducted was in compliance with Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines and the study was also approved by the Institutional Animal Ethical Committee (Registration no.- 634/02/a/CPCSEA). The animals were fed with standard diet and water was provided *ad libitum.* The experimental animals used were healthy albino mice of the species *M. musculus* of either sex weighing 20–30 g.

**Acute toxicity study**

Acute toxicity test was done for the ethanolic extract of *M. oleifera* following OECD 425 guidelines [10]. As the extract was found safe even at doses more than 2000 mg/kg without any sign of toxicity or mortality, an arbitrary dose 500 mg/kg was selected for the study.

**Antidepressant Study**

The animals were divided into four groups of five animals each and treated with the following

- Group A: Control (Normal saline-10 mL/kg)
- Group B: Test drug (EEMO-200 mg/kg)
- Group C: Test drug (EEMO-400 mg/kg)
- Group D: Standard (Fluoxetine-20 mg/kg).

In this acute study, all drugs were given orally 24 h, 5 h and 1 h before the test and the volume of medication was kept constant at 10 mL/kg body weight of the animals.

**Animal models of depression**

*Forced swimming test (FST) in glass jar*

FST in glass jar was performed as described by Porsolt *et al.*

This test consists of two parts, an initial training period of 15 min followed by actual test for 6-min duration 24 h later.

Mice were individually forced to swim inside a vertical glass cylinder (height: 40 cm; diameter: 15 cm; containing 15 cm height of water maintained at 25±1°C).

The activity was recorded from above for 6 min after three doses of drug [11].

Various activities were:

- Climbing
- Swimming
- Immobility

It was seen that mice on antidepressant drug had decreased episodes of immobility while swimming and climbing increased.

*FST*

CLIMBIMG SWIMMING IMMOBILITY

**Statistical analysis**

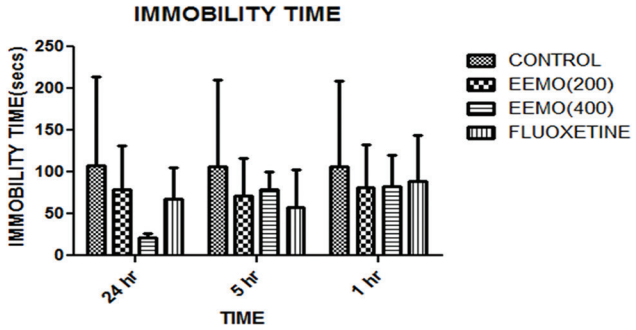
One-way ANOVA followed by Dunnett's multiple comparison test was used for statistical analysis. Significance level of <0.05 was considered as significant for the experiment.

**RESULTS AND OBSERVATION**

**FST**

| Groups | Immobility time At 24 h (s) | Immobility time At 5 h (s) | Immobility time At 1 h (s) |
|---|---|---|---|
| A - Control | 213.68±2.20 | 209.94±4.06 | 209.01±4.7 |
| B - EEMO (200) | 132.07±27.00[a] | 115.94±26.13[a] | 132.17±29.64 |
| C - EEMO (400) | 26.25±15.45[a] | 100.30±58.72[a] | 120.26±44.90 |
| D - Fluoxetine | 105.17±29.93[a] | 102.94±13.70[a] | 143.83±83.68 |
| ANOVA | | | |
| F | 12.80 | 6.92 | 1.552 |
| p | <0.05 | <0.05 | >0.05 |
| dF | 16, 3 | 16, 3 | 16, 3 |

[a]p<0.05 is considered significant. One way ANOVA followed by Dunnett's multiple comparison test. Values are expressed as mean±SEM.




**Tail suspension test (TST)**

TST was done according to described by Steru *et al.*

Mice were suspended on a string held by a metal stand, by an adhesive tape placed 1 cm from the tip of the tail. This string was 60 cm above the table top. The activity was recorded for a period of 5 min after three doses of drug. When mice will be completely motionless then only, they were considered immobile [12].

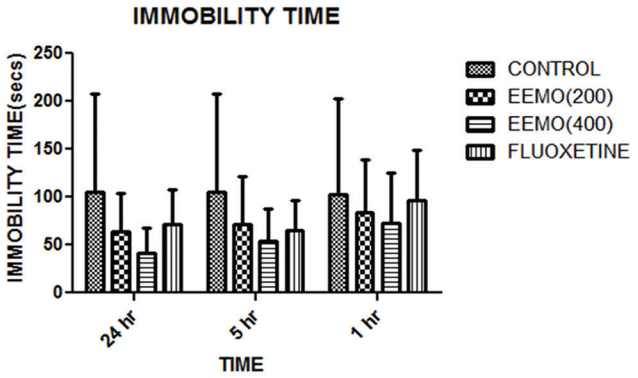
**DISCUSSION**

Many human diseases are produced from stress, which is caused by free radicals. Major damage in the body is caused by the free radicals. Modern lifestyles have enhanced the exposure of human beings to stressful conditions resulting in physical and psychological abnormalities. Synthetic drugs cannot be used at broad spectrum due to their higher

STRUGGLING IMMOBILITY

| Groups | Immobility time At 24 h (s) | Immobility time At 5 h (s) | Immobility time At 1 h (s) |
|---|---|---|---|
| A - Control | 207.5±2.318 | 207.2±3.68 | 203.3±2.759 |
| B - EEMO (200) | 104.1±23.46[b] | 121.2±22.72[b] | 138.9±29.17 |
| C - EEMO (400) | 67.59±14.35[b] | 87.17±19.72[b] | 125.8±20.78 |
| D - Fluoxetine | 107.9±35.66[b] | 96.08±34.71[b] | 149.4±44.30 |
| ANOVA | | | |
| F | 7.077 | 5.652 | 1.423 |
| p | <0.05 | <0.05 | >0.05 |
| dF | 16, 3 | 16, 3 | 16, 3 |

[b]p<0.05 is considered significant. One-way ANOVA followed by Dunnett's multiple comparison test. Values are expressed as mean±SEM.




cost and side effect associated with them, so the researchers looking for alternative methods [13].

In this study, the anti-stress activity of EEMO has been evaluated at given doses (200 and 400 mg/kg) using different acute stress behavioral tests such as FST and TST.

The FST is a broadly accepted model for evaluating anti-stress activity and it is based on monitoring that animals swim in the water, assumed a specific immobile posture, without of any activity. The appearance of immobility therefore shows a condition of decrease stamina, fatigue, and tiredness with the endpoint being the movement when the animal could not swim further and started drowning. The results of the swimming test demonstrated the significant increase in swimming time in mice, pre-treated for 7 days with test extract with increased physical performance and therefore assuring its anti-depressant property [14].

TST shows significant sensitivity to monoamine. In this study, EECP shows remarkable reduction in the immobility at the highest dose of 400 mg/kg which was compared to standard drug. Thus, the anti-stress activity showed probably by inhibiting Monoamine Oxidase-A and Monoamine Oxidase-B [15].

In the acute model of FST and TST with mice, all test groups, that is, EEMO (200) and EEMO (400) showed a significant reduction in immobility time as compared to control group ($p<0.05$) except at 1 h after their administration. The immobility time decreased with increase in the dose of EEMO. The reduction in immobility time was more than that of standard drug (fluoxetine) in the dose of 400 mg/kg EEMO but not with that of 200 mg/kg EEMO.

## CONCLUSION

This study was investigated the putative behavioral effects of EEMO in various acute anti-depressant models. Results of the behavioral model revealed that EEMO having higher anti-stress activity at the dose of 400 mg/kg per orally. The anti-depressant activity of EEMO was probably due to its anti-oxidant property due to the presence of alkaloids. This finding suggests that *M. oleifera* is worthy of further investigation as a potential antidepressant.

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## AUTHORS' CONTRIBUTION

All the authors have contributed equally in conduction of the experiment, compiling the result, and editing the manuscript.

## CONFLICTS OF INTEREST

The authors declare that there is no any conflicts of interest as the present work is solely done by the authors.

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