Screening for Antidepressant-Like Effect of Methanolic Seed Extract of *Avena Sativa* using Animal Models

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ABSTRACT

Depression affects about up to 20% of the population across the globe. The present study was designed to screen antidepressant activity of methanolic seed extract of *Avena sativa* (MSEAS). An in-vivo experimental methods were designed such as behavioral models like Forced swim test (FST), Tail suspension test (TST) and based on mechanism of action i.e., Antagonism of Apomorphine induced hypothermia on Swiss male albino mice. MSEAS 100 and 200 mg/kg, p.o were administered daily for 7 days. Fluoxetine 25mg/kg p.o was standard antidepressant drug in behavioral models and Desipramine 20mg/kg p.o in Apomorphine induced hypothermia. The methanolic extract produces a significant antidepressant effect in both FST and TST as they reduce the immobility. It was also found, effective in antagonizing or reversing hypothermia produced by apomorphine. The Anti-depressant activity of methanolic seed extract of *Avena sativa* was found to be significant at low doses (100mg/kg, po). The present study clearly demonstrated that *Avena sativa* exerts an antidepressant effect in these two behavioral models. The flavonoid components of MSEAS might be interacting with adrenergic system in mediating the anti depressant effect of *Avena sativa*.

Keywords: *Avena sativa*, forced swim test, tail suspension test, Apomorphine induced hypothermia

INTRODUCTION

Depression is a mental disorder characterized by a pessimistic sense of inadequacy and a despondent lack of activity with sad feelings of gloom, inadequacy and is present with depressive mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. It is mainly caused by decreased brain levels of monoamines like nor adrenaline, dopamine and serotonin. It is about twice as common in women as in men.

Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Thus, it is worthwhile to look for antidepressants from plants with proven advantage and favorable benefit-to-risk ratio. A number of medicinal plants and medicines derived from these plants have shown antidepressant properties by virtue of their medicinal constituents.

*Avena sativa* Linn. commonly known as oats belongs to the family of Gramineae; Poaceae. The primary chemical constituents includes saponins (*Avenacosides A and B*), flavonoids, starch, (trigonelline, avenine, gramine), steroids, calcium, B-vitamins, lysine, methionine and alkaloids such as gramine, they also contain iron, manganese, zinc. The plant used for depression, exhaustion, stress reduction, nervous system tonic, sexual performance, detoxification, nicotine cravings, fibroids, candidiasis, attention deficit disorder, respiratory and immune support and migraine headaches.

There is no significant work has been carried out on the antidepressant effect of this plant extract till date. Hence, the present study was designed to screen the antidepressant activity of *Avena sativa* using different animal models in mice.
MATERIALS AND METHODS

Materials

The plant material of *Avena sativa* seeds used for the investigation was collected from a local distributor in Tirupathi in the month of February 2012. The plant was identified and authenticated by Dr. K MadhavaChetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chittoor district, Andhra Pradesh. Apomorphine (Sigma life sciences, Bangalore), Fluoxetine (Fludac, Cadila), Desipramine (Sigma life sciences, Bangalore) and UgoBasile Rectal Thermometer. All other solvents and chemicals used were of analytical grade.

Preparation of extract

About 1kg of seeds of *Avena sativa* were shade dried at room temperature and ground coarsely (40 mesh size) before extraction. The seeds were extracted by Soxhlet apparatus by using solvent methanol. The resulting extract was concentrated in vacuum under reduced pressure using rotary evaporator and dried in desiccator and the percentage yield was found to be 42%. Further, extract was subjected to preliminary phytochemical analysis.[8]

Animals

The albino mice (20-30g) of either sex were used throughout the experimentation. After randomization into various groups, animals were acclimatized for a period of 10 days under standard husbandry conditions i.e. room temperature of 27±3°C, relative humidity of 65±10%, 12 h light/dark cycle. All the animals were fed with standard rodent pellet diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad and water *ad libitum*. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethic Committee (Reg. No. 1236/c/08/CPCSEA).

Acute oral toxicity study

*Determinations of Maximum Tolerance Dose (MTD)*

Acute toxicity studies were performed for selected plant methanolic extracts according to the toxic classic method as per guidelines 423 prescribed by OECD.[9] 2001 using female albino mice. The extracts showed neither visible sign of toxicity nor mortality.

Functional Observational Battery (FOB)

The Functional Observational Battery is a non-invasive procedure designed to detect gross functional deficits resulting from exposure to chemicals and to better quantify neurotoxic effects. A group of 6 mice of either sex were used to study the effect on general behavioral pattern. The mice were fasted for 3hrs prior to the oral administration of compound. Observations were taken at ½, 1, 2 and 4hrs intervals. The various parameters, their corresponding scores as per the method of Irwin[10–13] were recorded.

Experimental protocol

Mice were randomly divided into four groups and each group having six animals. Group I received distilled water 10ml/kg, p.o and served as a control; group II received standard antidepressant drug Fluoxetine(25mg/kg)/Desipramine(20 mg/kg) for 7&14 successive days; group III and IV received methanolic seed extract of *Avena sativa* (MSEA) 100 and 200 mg/kg, p.o respectively.

SCREENING FOR ANTIDEPRESSANT ACTIVITY

Behavioral Tests

*Forced Swim Test (FST)*

The FST is the most widely used *in vivo* screening model for assessing antidepressant activity. Experiment was carried out in narrow glass cylinder (15 cm in diameter × 24 cm high) containing water (25°C) to a depth of 10 cm, from which they cannot escape. All the animals were fasted for 3hrs prior to the oral administration of vehicle/standard/test drugs. Thirty minutes later, the animals were subjected to swim for 6 minutes; the first two minutes the animal is allowed to adjust to the new conditions; the next four minutes the immobilization time was measured with a stopwatch at 30, 60,120 and 240 minutes. Immobility time was the time during which the animals will be necessary to keep afloat.[14–17] Here the standard drug is Fluoxetine 25mg/kg p.o.3

*Tail Suspension Test (TST)*

The control, test and standard drugs were administered p.o., 60 minutes prior to testing. The mice were suspended on the edge of a shelf 58cm above the table top by adhesive tape placed approx. 1cm from the tip of tail. The duration of immobility was recorded for the period of 6 minutes by using stopwatch. After the initial period...
of vigorous motor activity, the mice became still. Mice were considered immobile when they hanged passively and completely motionless. The duration of immobility time was recorded before the treatment and 60 minutes after the treatment.\textsuperscript{[18–19]} Here the standard drug is Fluoxetine 25mg/kg p.o.

**Based on Mechanism of Action**

**Apomorphine induced hypothermia**

All the animals were fasted for 3 hrs prior to oral administration of vehicle/standard/test drugs. One hour after oral administration of the test compounds or the vehicle, 16mg/kg apomorphine was injected s.c. to the animals. The rectal temperature of each mouse was measured by an electronic thermometer at 10, 20, 30, 60 and 120 minutes after apomorphine treatment and the degree of hypothermia was determined. During the entire experiment, animals were housed in groups in glass jars at room temperature.\textsuperscript{[20-21]} Here the Standard drug is Desipramine 20mg/kg p.o.

**Statistical analysis**

Results will be presented as mean±SEM. The data will be subjected for statistical analysis by One way analysis of variance (ANOVA) followed by Dunnet’s t test and \( P<0.05^*, 0.01^{**} \) and \( 0.001^{***} \) were considered as significant.

**RESULTS**

**Phytochemical screening**

The Percentage yield of extract was found to be 42.23\% w/w and the preliminary phytochemical analysis showed the presence of carbohydrates, alkaloids, flavonoids, steroids, glycosides, saponins, amino acids, gums and mucilage.

**ACUTE ORAL TOXICITY STUDY**

**Determination of Maximum Tolerance Dose (MTD)**

The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. From this 20\(^{th}\) and 10\(^{th}\) parts were selected as dose for the experimental study. Hence \( \text{LD}_{50} \) determination was not possible and all the extracts tested are considered safe and nontoxic according to OECD guidelines 423.

**Functional Observational Battery (FOB)**

The methanolic seed extract of *Avena sativa* was subjected to FOB, which is a non-invasive procedure to detect gross functional deficits basing upon various behavioral parameters. The scoring of various parameters was shown in (Table1). The results showed an increase in spontaneous motor activity, ataxia and stereotypic behaviors like rearing and grooming.

**SCREENING FOR ANTIDEPRESSANT ACTIVITY**

**Forced Swim Test (FST)**

The result of the effect of methanolic seed extract of *Avena sativa* on the duration of immobility was shown in figure 1. The animals treated with 100mg/kg, p.o of MSEAS and standard antidepressant Fluoxetine 25mg/kg, p.o showed significant decrease in immobility time but not 200mg/kg, p.oof MSEAS when compared with control group.

**Tail Suspension Test (TST)**

The results were presented in (Figure 1), revealed that the immobility time was significantly decreased in animals treated with 100mg/kg, p.o of MSEAS and standard antidepressant Fluoxetine 25mg/kg, p.o but not 200mg/kg, p.o of MSEAS when compared with control group.

**Apomorphine induced hypothermia**

In this test, animals treated with two doses of MSEAS (100 & 200mg/kg, p.o) and standard antidepressant Desipramine (20mg/kg, p.o) showed significant antagonism of hypothermia induced by Apomorphine (16mg/kg, sc) when compared with control group and the results are shown in (Figure 2).

The percentage inhibition of immobility time in Forced Swim Test, Tail Suspension Test and temperature in Apomorphine induced hypothermia were calculated according to the following formula and results were shown in (Table 2).

\[
\%\text{ inhibition of immobility time} = \frac{\text{control standard/text}}{\text{control}} \times 100
\]
Table 1. Effect of MSEAS in Functional Observation Battery of Control group, Test dose (100mg/kg, p.o), Test dose (200mg/kg, p.o)

<table>
<thead>
<tr>
<th>Behavioral parameters</th>
<th>Normal score</th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>120 minutes</th>
<th>240 minutes</th>
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<tr>
<td></td>
<td>C</td>
<td>T₁</td>
<td>T₂</td>
<td>C</td>
<td>T₁</td>
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<tr>
<td>Spontaneous Motor Activity</td>
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<td>4</td>
<td>4</td>
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<td>38.5</td>
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<td>(0.25)</td>
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C= Control group, T₁= Test dose of 100 mg/kg , T₂= Test dose of 200 mg/kg

Figure 1. Histograms of Mean ± SEM of immobility time in Forced Swim Test and Tail Suspension Test.
Figure 2. Histograms of Mean ± SEM of temperature in antagonism of Apomorphine induced hypothermia.

Table 2. Effect of MSEAS on % inhibition of immobility time in Forced Swim Test, Tail Suspension Test and temperature in Apomorphine induced hypothermia

<table>
<thead>
<tr>
<th>Time (Min.)</th>
<th>% inhibition of immobility time in Forced Swim Test</th>
<th>% inhibition of immobility time in Tail Suspension Test</th>
<th>% Inhibition of temperature in Apomorphine induced hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluoxetine (25mg/kg)</td>
<td>MSEAS (100mg/kg)</td>
<td>MSEAS (200mg/kg)</td>
</tr>
<tr>
<td>30</td>
<td>33.16</td>
<td>8.69</td>
<td>13.78</td>
</tr>
<tr>
<td>60</td>
<td>36.52</td>
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<tr>
<td>120</td>
<td>48.2</td>
<td>17.95</td>
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<td>140</td>
<td>35.52</td>
<td>4.62</td>
<td>13.74</td>
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MSEAS: methanolic seed extract of *Avena sativa*

DISCUSSION

Since the introduction of the herbal medicines, many people were impelled to consider the importance of many herbs for treating several forms of disorders. Modern day lifestyle leads to numerous stress conditions, among which depression is a widely prevalent senile neurological disorder. It is mainly caused by decreased brain levels of monoamines like Noradrenaline, Dopamine and Serotonin. Depression is a mental disorder associated with lot of morbidity due to its high incidence in the community. Hence it is necessary to look for Anti depressants with proven advantage and favorable benefit-to-risk ratio. Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Therefore, there is an immense requirement for alternative remedies for depression. The present work was subjected to investigation for the evaluation of the Anti depressant activity of methanolic seed extract of *Avena sativa* in animal models.

In Acute Oral Toxicity study, MSEAS did not show any lethal effect even up to the doses of 2000mg/kg, po and complete absorption of drug through GIT was observed.
and thus the test doses of 100 & 200mg/kg, po were used. The Functional Observational Battery is a non-invasive procedure designed to quantify neurotoxic effects. It is used for assessing the behavioral parameters in the mice when exposed to chemicals. The behavioral parameters observed in MSEAS extract were increase in spontaneous motor activity; ataxia and stereotypic behavior like rearing and grooming were observed.

For the purpose of investigation of antidepressant activity, two animal models viz., the forced swim test and tail suspension test were used. These tests were quite sensitive and relatively specific to all major classes of Antidepressants. The immobility displayed by rodents when subjected to unavoidable stress such as FST & TST are thought to reflect a state of despair or lowered mood, which are thought to reflect depressive disorders. In addition, immobility time has been shown to be reduced by treatment with antidepressant drugs.

Results showed that the administration of the MSEAS produced a diminution of duration of immobility time of mice exposed to the both FST & TST. In the present study, the MSEAS (100mg/kg, po) administered to mice produced significant antidepressant effect in both FST & TST models and their efficacies were found to be comparable to standard drug Fluoxetine (25mg/kg, po).

For the assessment of mechanism of action of MSEAS, antagonism of Apomorphine induced hypothermia model was used to know MSEAS acting through Nor-adrenaline. Antagonism against Apomorphine induced hypothermia can be regarded as a hint for antidepressant activity through Nor-adrenaline uptake. Compounds with a marked Nor-adrenaline or Dopaminergic components are active against Apomorphine induced Hypothermia but not through serotonergic system. In this model, two doses of MSEAS (100 & 200mg/kg, po) and Desipramine (20mg/kg, po) significantly antagonized the Apomorphine induced hypothermia (16mg/kg, sc) when compared with control, representing that MSEAS may be acting through Adrenergic system but not through serotonergic system.

From all the above, the antidepressant activity of methanolic seed extract of Avena sativa was found to be significant at low doses (100mg/kg, po). The flavonoid components of MSEAS might be interacting with adrenergic system in mediating the anti depressant effect of Avena sativa.

CONCLUSION

The MSEAS contained carbohydrates, alkaloids, flavonoids, steroids, glycosides, saponins, amino acids, gums and mucilage. It has not produced any lethal effect even up to the dose level of 2000mg/kg, p.o during acute oral toxicity study. The findings of the present investigation suggests that the Anti-depressant activity of MSEAS was significant at lower dose of 100mg/kg, p.o in Forced swim test, Tail suspension test and also Antagonism of Apomorphine induced Hypothermia indicated that MSEAS is showing the Antidepressant activity by acting through Adrenergic system.

However, more extensive pharmacological studies of this plant are required for complete understanding of the Antidepressant activity of methanolic seed extract of Avena sativa.

REFERENCES