EVALUATION OF METHANOLIC EXTRACT OF CELTIS TIMORENSIS FOR ITS ANTIDEPRESSANT ACTIVITY

Rajaneekar Dasari*1, Anusha Kunchibhotla1, Sathyavathi D1, P.Jayachandra Reddy2

1Mallareddy Institute of Pharmaceutical sciences, Maisammaguda, Dhulapally(Via Hakimpet), Secunderabad
2Jawaharlal Nehru Technological University, Hyderabad.

Two classic animal behavior despair tests—the Forced Swimming Test (FST) and the Tail Suspension Test (TST) were used to evaluate the antidepressant activity of methanolic extract of C. timorensis mice. It was observed that of 200 and 400 mg/kg significantly reduced the immobility time in the FST and TST in mice 30 min after treatment. Immobility displayed in both of these behavioral despair models has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in human. The probable mechanism of action may be MECT ability to increase levels of Ach and serotonergic transmission however the exact mechanisms have to be investigated further. The standard antidepressant used here is fluoxetine.

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ABSTRACT

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INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioral disorder. By the year 2020, depression is expected to constitute the second largest source of global burden of disease after heart disease. Depression is whole body illness which involves not only mood or emotion but also the physical body and thought process. The symptoms of depression are intense feelings of sadness, hopelessness, and despair, as well as the inability to experience pleasure in usual activities, changes in sleep patterns and appetite, loss of energy, and suicidal thoughts.

Depression is the most common of the affective disorders; it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Depression constitutes the second most common chronic condition in clinical practice. The causes of depression vary. Psychosocial factors, such as adverse living conditions, can influence the onset and persistence of depressive episodes. Genetic and biological factors also play a part. It is estimated that 5.8% of men and 9.5% of women will experience a depressive episode in any given year.

Depression is commonly accepted to be a disorder due to disturbances in neurotransmitters function, particularly serotonin, noradrenalin and dopamine. Reduction in brain serotonin has been reported to be one of the most important etiological factors for genesis of depression and the most widely used antidepressants namely serotonin reuptake inhibitors (SSRIs) increase extracellular availability of serotonin. Further, noradrenergic and dopaminergic systems are reported to be involved and act in tandem with the serotonergic system.

The major goal of the present study is to evaluate the potential antidepressant activity of MAR in rodent models of depression. Further, behavioral experiments were performed to ascertain the role of neurotransmitters in the antidepressant effect of MAR.

MATERIALS AND METHODS

Plant material

The dried plant material was obtained in thirupathi, andhra pradesh. The plant material was identified and authenticated taxonomically at Dr. Madhavashetti, Department of Botany, Sri Venkateshwara University, Thirupati. A voucher specimen of collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

The dried material were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted with methanol (50% v/v) maceration method. The extracts were filtered and concentrated at high vacuum (yield 3.4% w/w)

Drug treatment

For the pharmacological tests, the obtained extract was suspended in double distilled water containing propylene glycol (5% v/v CMC) in doses of 200,400-mg/kg p.o. The doses were fixed based on acute toxicity studies on the 50% methanolic extract of MECT (Methanolic Extract Of Celtis Timorensis) were administered at up to 2 g/kg to individual mice in-group. There was no mortality to treatment up to end of the observation period. The MECT caused no abnormality or death during the course of treatment.
Animals

Swiss mice of either sex weighing 18-20g (younger ones, aged 8 weeks) or 22-25 g (older ones, aged 28 weeks) were used in present study. They had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. They were acclimatized to laboratory conditions for 5 days before behavioral studies. All the readings were taken during the same time of the day i.e. between 8 a.m. and 11 a.m. The Institution Animals Ethics Committee (IAEC) had approved the experimental protocol, and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India.

Acute oral toxicity test:

The procedure was followed by using OECD guide lines (Organization of Economic Cooperation and Development) 423 (Acute Toxic class method). the acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality and /or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimum number of animals while allowing for acceptable data-band scientific conclusion.

The method used defined doses (5, 50, 500, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of the chemical, which cause acute toxicity.

Six male mice weighing 20-25 gms were selected for acute oral toxic studies. The starting dose of methanolic extract of *celtis timorensis* is 2000mg/kg body weight as most of the crude extracts possess LD50 value more than 2000mg/kg in b.w.p.o so 2000 mg/kg was used as starting dose. Dose was administered to the mice, which were fasted over night with water *ad libitum* food were with held for a further 3-4 hrs after administration of drugs & observed for another 14 days.

Body weight of the mice before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted.

It is found that the dose of 2000mg/kg did not produce any lethal effects and this dose was considered as safe for the animals. According to some toxic reactions produced like slight skin lesions two doses were fixed for the experiment 200mg/kg and 400mg/kg.

In the present study the antidepressant activity of MECT is evaluated using the following models:

1. Forced swimming test (FST).
2. Tail suspension test (TST).

**Forced swimming test:**

Behavior despair was proposed as a model to test for antidepressant activity by Porsolt *et al.*9,10 Mice were forced to swim individually in a glass jar (25 × 12 × 25 cm³sub) containing fresh water of 15 cm height and maintained at 25°C (± 3°C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water.
without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

Group I: Treated with vehicle (5%v/v propyleneglycol) administered orally.

Group II: It represented group treated with standard drug (fluoxetine 5mg/kg).

Group III: It represented group treated with MECT (200mg/kg).

Group IV: It represented group treated with higher dose of MECT (400 mg/kg).

In the above test vehicle and the test drug are administered orally and the test drug is administered intraperitoneally. All the drugs are administered 60 min before the commencement of the test. A time gap of 30 min is sufficient between the administration and test for drugs which are given intraperitoneally.

**Tail suspension test (TST)**\(^{11,12}\): The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al as a facile means of evaluating potential antidepressants. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively.

Group I: Treated with vehicle (5%v/v propyleneglycol) administered orally.

Group II: It represented group treated with standard drug (fluoxetine 5mg/kg).

Group III: It represented group treated with MECT (200mg/kg).

Group IV: It represented group treated with higher dose of MECT (400 mg/kg).

In the above test vehicle and the test drug are administered orally and the test drug is administered intraperitoneally. All the drugs are administered 60 min before the commencement of the test. A time gap of 30 min is sufficient between the administration and test for drugs which are given intraperitoneally.

**RESULTS:**
Effect on immobility periods in Forced swimming test and Tail suspension test:

As shown below in the table immobility in the FST was significantly reduced in the MECT treated mice when compared to the control group which are untreated and is similar to the positive control group which is fluoxetine treated.

The decrease in immobility time in the TST has showed similarity to that seen in the FST. The overall antidepressant activity of MECT is compared with that of standard drug fluoxetine and activity of MECT is evaluated.
FORCED SWIM TEST:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Immovable time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>2.66±0.45</td>
</tr>
<tr>
<td>2</td>
<td>Fluoxetine</td>
<td>39.50±1.66</td>
</tr>
<tr>
<td>3</td>
<td>MECT 200mg/kg</td>
<td>17.83±0.64</td>
</tr>
<tr>
<td>4</td>
<td>MECT 400mg/kg</td>
<td>21.50±0.76</td>
</tr>
</tbody>
</table>

** P <0.01 and *** P <0.001 Control Vs treated groups using one way ANOVA followed by Dunnett’s test.

TAIL SUSPENSION TEST:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Immovable time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>2.83±0.47</td>
</tr>
<tr>
<td>2</td>
<td>Fluoxetine</td>
<td>53.41±1.47</td>
</tr>
<tr>
<td>3</td>
<td>MECT 200mg/kg</td>
<td>13.66±0.49</td>
</tr>
<tr>
<td>4</td>
<td>MECT 400mg/kg</td>
<td>19.83±1.01</td>
</tr>
</tbody>
</table>

** P <0.01 and *** P <0.001 Control Vs treated groups using one way ANOVA followed by Dunnett’s test.

DISCUSSION:

Both forced swimming and tail suspension tests are the accepted stress models of depression. Immobility has been shown to reflect a state of ‘behavioral despair and variants’ or ‘failure to adapt to stress. Immobility displayed in both of these behavioral despair models has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in human. There was a significant correlation between clinical potency and the potency of antidepressants in both models. Thus, these two models are usually used to screen or evaluate antidepressants.¹³

In the present study MECT produced significant antidepressant-like activity in both the FST and TST in mice, which indicates that Celtis timorensis has some antidepressant effects. But the probable mechanisms of action must be verified further.

REFERENCES


