Study of correlation of antidepressive effects of ethanolic leaf extract of Ocimum sanctum and Imipramine in albino mice

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Abstract

Introduction: Depression is a common mental disorder that is characterized by loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration, insomnia or hypersomnia, and occasionally suicidal thoughts. Though a number of synthetic drugs are being used as the standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. These conditions create an opportunity for alternative treatment of depression by use of medicinal plants or by plant-based antidepressant formulations. Aims and objectives: To correlate the anti depressant activity of Ocimum Sanctum with a standard drug for depression – Imipramine in albino mice. Materials and Method: Swiss albino mice weighing 20 to 40gms of either sex were divided into 4 treatment groups, each group containing 6 animals and orally administered with 1% Gum acacia (control), Imipramine 15mg/kg (standard), Ocimum Sanctum 4mg/kg and 8mg/kg (test drug). A total of 48 animals were used. The duration of immobility was observed for 6minutes in Tail suspension test and Forced swimming test on a separate set of animals on 1st day, 8th day and 15th day. Results: The data was analyzed for correlating statistical significance by using ANOVA. The ethanolic extract of leaves of O. Sanctum in the above mentioned doses significantly reduced the immobility time (p < 0.05) in both Forced Swimming test model and Tail Suspension test models compared to control and the effect seen more significantly on 15th day. The finding in standard treatment group and test group were also comparable. Conclusion: The alcoholic extract of leaves of Ocimum Sanctum has significant antidepressant activity in acute animal models of depression and it is comparable with standard drug - Imipramine.

INTRODUCTION

Depression is a common mental disorder that is characterized by loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration, insomnia or hypersomnia, and occasionally suicidal thoughts. Depending on the number and severity of symptoms, a depressive episode can be categorized as mild, moderate or severe. An individual with a mild depressive episode will have some difficulty in continuing with ordinary work and social activities, but will probably not cease to function completely. During a severe depressive episode, on the other hand, it is very unlikely that the sufferer will be able to continue with social work or domestic activities, except to a very limited extent. Various drugs are available for the treatment of depression. They include monoamine oxidase inhibitors, selective and non selective monoamine reuptake inhibitors and selective serotonin reuptake inhibitors. These medications work by normalizing the levels of neurotransmitters, notably serotonin and nor-epinephrine. Approximately two-thirds of the depressed patients respond well to these medications, but various patients do not respond to these medications or experience intolerable side effects.

patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Though a number of synthetic drugs are being used as the standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. These common adverse effects include dry mouth, fatigue, anxiety, agitation, drowsiness and cardiac arrhythmias. Several drug-drug interactions can also occur. These conditions create an opportunity for alternative treatment of depression by use of medicinal plants or by plant-based antidepressant formulations. Indian physicians practicing Ayurveda recognized the contributions of rejuvenating herbs 5000 years ago. Adaptogenic herbs are considered phytomedicines or natural product remedies based on plants. An adaptogen has the ability to increase the body’s resistance to stress by stimulating a nonspecific self regulation response in adapting to stress. Adaptogens also produce an increase in the power of resistance against multiple (physical, chemical or environmental) stressors. Tulsi has been recognized for thousands of years to be one of India’s greatest healing herbs. It enhances general health and well being, having positive overall effects on the body and mind. Here an attempt has been made to explore its antidepressant activity. Ocimum Sanctum (Tulsi) has been used successfully in the treatment and prevention of many stress disorders.

AIMS AND OBJECTIVES
To correlate the antidepressant activity of Ocimum Sanctum with a standard drug for depression – Imipramine in albino mice.

MATERIALS AND METHODS
Study design
The present study was conducted at Department of Pharmacology, Mysore Medical College and Research Institute, Mysore with aim to evaluate and compare the antidepressant activity of Ocimum Sanctum with a standard drug for depression – Imipramine in albino mice.

Materials and Solutions
1. Chemicals:
   a. Imipramine hydrochloride: It is a potent tricyclic antidepressant drug. Imipramine is a dibenzazepine tricyclic antidepressant. It is white slightly yellow crystalline powder freely soluble in water and in alcohol. A 10% solution in water has a pH of 3.6 to 5.0. Used as standard in the dose of 15 mg/kg, administered by oral route.
   b. Ocimum sanctum (OS): Ethanolic extract of leaves of Ocimum Sanctum was procured from Himalaya Drug Company, Bangalore. Extraction is usually carried out by hot extraction.
   c. Gum acacia: Gum acacia is a dried exudate from Acacia Senegal (a small tree) and certain other species of Acacia. It comes as a white powder. It is a suspending agent. Used as control in the dose of 0.1 ml/10 g (1%), administered by oral route. Used as a vehicle, to suspend standard drug (imipramine) and test drug (OS extract).

2. Animals: Swiss albino mice weighing around 20 to 40 g of either sex were randomly selected from central animal facility, MMC and RI, Mysore with following inclusion and exclusion criterion.

Inclusion Criteria
1. Albino mice weighing 20 to 40 g of either sex.
2. Age 3-4 months.
3. Animals acclimatized to the experimental conditions for 2 days.
4. Healthy with normal behaviour and activity.

Exclusion Criteria
1. Mice <20 g and >40 g and age <3 months and > 4 months.
2. Pregnant animals.
3. Diseased animals.
4. Animals previously used in other experiments.

The experiment was conducted in central animal facility, MMC and RI, Mysore, between 9:00 A.M. to 3:00 P.M. The experiment room was equipped with standard fluorescent lighting. The food and water was removed for the duration of test. Animals were weighed and appropriate dose of drug was administered to different groups. The experiment was conducted 1 hour after administrating the drug. A total of 48 animals (n=48) were used. They were divided into 8 groups of 6 animals each.

Methods
The methods employed here to study the antidepressant activity in albino mice are:
   a) Forced swimming test (FST)

Group I: Received 0.1 ml/10 g of gum acacia orally (Control).
Group II: Received 15 mg/kg imipramine orally (Standard).
Group III: Received 4 mg/kg of ethanolic extract of leaves of OS orally.
Group IV: Received 8 mg/kg of ethanolic extract of leaves of OS orally.

b) Tail suspension test (TST)

Group V: Received 0.1 ml/10 g of gum acacia orally (Control).

Group VI: Received 15 mg/kg imipramine orally (Standard).

Group VII: Received 4 mg/kg of ethanolic extract of leaves of OS orally.

Group VIII: Received 8 mg/kg of ethanolic extract of leaves of OS orally.

Data was collected analyzed by calculating mean, standard deviation and unpaired t test.

RESULTS

Table 1A: Mean duration of immobility during Forced Swimming Test (FST) of group

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I 1% Gum Acacia</th>
<th>Group II Imipramine (15mg/kg)</th>
<th>Group III OS (4mg/kg)</th>
<th>Group IV OS (8mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>151.16 ± 17.16</td>
<td>134 ± 13.44</td>
<td>136 ± 6.19</td>
<td>132.66 ± 11.82</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>129 ± 13.43</td>
<td>108.33 ± 15.04</td>
<td>116.16 ± 13.36</td>
<td>111.66 ± 9.24</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>143.16 ± 8.95</td>
<td>83.66 ± 13.17</td>
<td>92.5 ± 12.98</td>
<td>83.83 ± 8.47</td>
</tr>
</tbody>
</table>

Table 1B: correlation between intergroups (test, control an standard) of Forced swimming test by ANOVA.

<table>
<thead>
<tr>
<th>Day</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Between Groups</td>
<td>1,325.239</td>
<td>3</td>
<td>441.746</td>
<td>2.705</td>
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<td></td>
<td>Within Groups</td>
<td>3,265.639</td>
<td>20</td>
<td>163.282</td>
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<td></td>
<td>Total</td>
<td>4,590.877</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td>Between Groups</td>
<td>1,478.157</td>
<td>3</td>
<td>492.719</td>
<td>2.940</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>3,352.169</td>
<td>20</td>
<td>167.608</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>4,830.325</td>
<td>23</td>
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<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>Between Groups</td>
<td>14,670.117</td>
<td>3</td>
<td>4,890.039</td>
<td>39.614</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>2,468.863</td>
<td>20</td>
<td>123.443</td>
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<tr>
<td></td>
<td>Total</td>
<td>17,138.980</td>
<td>23</td>
<td></td>
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</table>

*Significant Imipramine 15mg/kg (Group II), OS 4 mg/kg (Group III) and OS 8 mg/kg (Group IV) showed significant antidepressant activity compared to control (1% Gum acacia, Group I).

ON DAY 1
There were no significant differences in durations of immobility among different groups.

ON DAY 8
The inter group comparison (Table 1A and 1B) between Group I and II, III, IV showed that Imipramine 15mg/kg and OS 8 mg/kg showed significant reduction in duration of immobility than control group. There was no significant difference in reduction of immobility between OS 4mg/kg and control. When compared to day 1 there was reduction of durations of immobility in groups II, III and IV, but there were no significant differences in durations of immobility among different groups.

ON DAY 15
The inter group comparison showed similar results (Table 1A and 1B). Comparison between Group I and II, III, IV indicated that Imipramine 15mg/kg, OS 4 mg/kg and OS 8 mg/kg showed significant reduction in duration of immobility compared to control. Comparison between Group II and III, IV showed that Imipramine 15mg/kg, reduced duration of immobility more than OS 4mg/kg and OS 8 mg/kg. Comparison between Groups III and IV showed OS 4mg/kg was inferior in reducing duration of immobility to OS 8mg/kg. The reduction in the duration of immobility with OS 8 mg/kg and Imipramine 15 mg/kg was almost similar showing that their antidepressant effects were comparable. When compared to day 1 and day 8 there was reduction in reduction of immobility in groups II, III and IV. ANOVA shows that there was significance between the groups with ‘P’ value < 0.05 on Day 15 only.
Table 2A: Mean duration of immobility during Tail suspension test (TST) of group

<table>
<thead>
<tr>
<th>Day</th>
<th>Group V 1% Gum Acacia Mean ± SD</th>
<th>Group VI Imipramine (15mg/kg) Mean ± SD</th>
<th>Group VII OS (4mg/kg) Mean ± SD</th>
<th>Group VIII OS (8mg/kg) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>175.33 ± 11.63</td>
<td>176.16 ± 12.54</td>
<td>171.33 ± 10.17</td>
<td>164.33 ± 9.22</td>
</tr>
<tr>
<td>8th day</td>
<td>171.83 ± 15.51</td>
<td>162 ± 15.03</td>
<td>144.83 ± 13.1</td>
<td>143.33 ± 12.83</td>
</tr>
<tr>
<td>15th day</td>
<td>159.16 ± 15.67</td>
<td>119.5 ± 16.94</td>
<td>114.16 ± 11.75</td>
<td>120.5 ± 10.36</td>
</tr>
</tbody>
</table>

Table 2B: correlation between intergroups (test, control and standard) of Tail suspension test by ANOVA

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Day 1 Between Groups</td>
<td>524.950</td>
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<td>174.983</td>
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<td>Day 1 Within Groups</td>
<td>2,417.319</td>
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<td>Day 1 Total</td>
<td>2,942.269</td>
<td>23</td>
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<tr>
<td>Day 8 Between Groups</td>
<td>3,425.260</td>
<td>3</td>
<td>1,141.753</td>
<td>5.690</td>
</tr>
<tr>
<td>Day 8 Within Groups</td>
<td>4,013.400</td>
<td>20</td>
<td>200.670</td>
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<tr>
<td>Day 8 Total</td>
<td>7,438.660</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 15 Between Groups</td>
<td>7,743.334</td>
<td>3</td>
<td>2,581.111</td>
<td>13.272</td>
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<td>Day 15 Within Groups</td>
<td>3,889.523</td>
<td>20</td>
<td>194.476</td>
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<tr>
<td>Day 15 Total</td>
<td>11,632.857</td>
<td>23</td>
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</table>

*Significant Imipramine 15mg/kg (Group VI), OS 4 mg/kg (Group VII) and OS 8 mg/kg (Group VIII) resulted in significant decrease in the duration of immobility compared to control (1 % Gum acacia, Group V).

ON DAY 1
There were no significant differences in durations of immobility among different groups.

ON DAY 8
The inter group comparison between Group V and VI, VII, VIII showed that OS 4mg/kg and OS 8 mg/kg showed highly significant reduction in duration of immobility than control group. Comparison between Group VI and VII, VIII showed that Imipramine 15mg/kg was inferior in reducing the duration of immobility more than OS 4mg/kg and OS 8 mg/kg. Comparison between Group VII and VIII showed OS 4mg/kg was inferior to combination and same response in reducing the durations of immobility to OS 8mg/kg. Thus the intergroup comparison shows that OS at doses 4 mg/kg and 8 mg/kg has antidepressant activity. However antidepressant activity of 8 mg/kg was more than 4 mg/kg. When compared to day 1 and day 8 there was reduction of immobility in groups VI, VII and VIII. When compared to day 1 there was reduction of durations of immobility in groups VI, VII and VIII but there were no significant differences in durations of immobility among different groups. ANOVA showed that there was significance between the groups with ‘P’ value < 0.05 on Day 8.

ON DAY 15
The inter group comparison (Table 2A and 2B) between Groups V and VI, VII, VIII indicates that Imipramine 15 mg/kg, OS 4 mg/kg and OS 8 mg/kg were highly significant better than control in reducing the duration of immobility (p < 0.05). Comparison between Group VI and VII, VIII showed that Imipramine 15mg/kg reduced duration of immobility more than OS 4mg/kg and OS 8 mg/kg. Comparison between Group VII and VIII showed OS 4mg/kg reduced the duration of immobility in compare to OS 8mg/kg. Also the reduction in the duration of immobility in OS 8 mg/kg and Imipramine 15 mg/kg was closer showing that their antidepressant effects were comparable. Thus the intergroup comparison showed that OS at doses 4 mg/kg and 8 mg/kg has antidepressant activity. However antidepressant activity of 4 mg/kg was more than 8 mg/kg. When compared to day 1 and day 8 there were reductions in immobility in groups VI, VII and VIII. ANOVA showed that there was significance between the groups with ‘P’ value < 0.05 on Day 15 also.
DISCUSSION
The present study evaluated the antidepressant activity of ethanolic extract of leaves of Ocimum Sanctum (tulsi) in two different animal models of depression, Tail suspension test and Forced swim test. Both these methods are widely used for screening antidepressant drugs. There is a significant correlation between the potency of antidepressants in both Forced-swim and Tail-suspension tests and clinical potency of the drugs. These tests are quite sensitive and relatively specific to all major classes of antidepressants like tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase (MAO) inhibitors and atypical antidepressants. It has been argued that Tail suspension is less stressful than Forced swim test and has greater pharmacological sensitivity. Comparison of reduction of immobility between 1st day, 8th day and 15th day showed that the reduction of immobility in different groups was more significant on 15th day compared to 8th and very less on 1st day. This showed that the ethanolic extract of leaves of Ocimum Sanctum on chronic administration of higher doses has antidepressant effect almost similar to standard drug like imipramine. To sum up, the present study has shown that the ethanolic extract of Ocimum Sanctum at a dose of 4 mg/kg, 8 mg/kg and combination significantly reduced the duration (time) of immobility of animals as compared to the control in both Forced swim test and Tail suspension test of depression, showing that in both the doses, it has significant antidepressant activity. Both the tests showed consistent results in terms of reduction in the duration of immobility. The initial development of the TCAs resulted from psychopharmacological characterization of a series of structural analogs that had been developed as potential antihistamines, sedatives, analgesics and antiparkinson drugs. One of the compounds, imipramine, which has a phenothiazine-like structure, modified behavior in animal models. Unlike the phenothiazines, imipramine had limited efficacy in schizophrenia patients, but improved symptoms of depression. Imipramine and related TCAs became the mainstream of drug treatment of depression until the later development of the SSRIs. It increases the synaptic concentration of serotonin and/or norepinephrine in the central nervous system by inhibition of their reuptake by the pre-synaptic neuronal membrane. However, additional receptor effects have been found including desensitization of adenyl cyclase, down regulation of beta-adrenergic receptors and down regulation of serotonin receptors.

Ocimum sanctum Linn. (botanical name) and commonly known as Tulsi, is the sacred plant of India and is also known by various names as Tulassi, Manjari, Krisna Tulsi, Trittavu, Tulshi, Thulsi. The plant is known in English as Holy Basil. Medicinal properties attributed to the plant are not only mentioned in Ayurveda and Siddha but also in Greek, Roman and Unani system of Medicine. O. sanctum has been shown to possess cortisol sparing immunostimulant and antioxidant activities. This cortisol sparing immunomodulatory activity of O. sanctum may also contribute to the behavioural disinhibitory activity. The precise mechanisms by which O. sanctum produced antidepressant-like effects are not completely understood. Various antidepressant drugs, either by inhibiting MAO enzyme or by inhibiting reuptake mechanism, increase the central monoamine levels or reverse the stress-induced depressive-like behaviour. Previous studies have shown that administration of O. sanctum had a normalizing action on noise stress-induced alteration in brain monoamine neurotransmitters (norepinephrine, epinephrine, dopamine and serotonin) and controlled the alteration in neurotransmitter levels due to stress. Therefore, the antidepressant activity of O. sanctum may be correlated with these studies. Thus the present work though preliminary in nature suggests that the Ocimum Sanctum extract has good antidepressant activity. Further elaborate research work involving more number of animals and different experimental models of antidepressant activity are needed to elucidate the exact molecular and biochemical mechanism of action to develop a more effective compound.

CONCLUSION
Thus in the end we conclude that the alcoholic extract of leaves of Ocimum Sanctum has significant antidepressant activity in acute animal models of depression and it is comparable with standard drug - imipramine.

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