Synergistic Anti-Depression Activity Evaluation of Different Composition of Volatile Oil of Eucalyptus and Neem Oil by Tail Suspension Model

Keywords: Depression, Eucalyptus and Neem oil, Tail Suspension Test Model, Imipramine

ABSTRACT

Depression is one the major CNS disorder in modern life and it affects around 5% world population. With respect of aggressive lifestyle currency of depression and depression spark suicide is increasing day by day. Different therapeutic kinds are used to treat depression. Aromatherapies were getting popular as an alternative therapy for treatment and management of CNS disorders. The present studies were taken to evaluate the effect of Eucalyptus oil and Neem oil and ratios of eucalyptus and neem oils (1:1, 1:2, 1:3, and 2:1and 3:1). The activity of depression was studied in mice using Tail Suspension Test (TST). Duration of immobility time was noted as index of depressive activity. All the oils except eucalyptus and neem oil decreased of duration of immobility and eucalyptus and neem oil was statistically significant. Eucalyptus and neem oil produced significantly increased of duration immobility. These discoveries suggest that essential oils can be used to treat the CNS disorders and gives pharmacological evidence of aromatherapy.
INTRODUCTION

Depression is a mood disorder that caused by a persistent feeling of sadness and loss of interest. Also called as major depression, major depressive disorder or clinical depression, it affects how you ambience, think and perform and can lead to a variety of senses and physical problems. Depression is an affective disorder characterized by change in disposition, lack of interest in the surroundings, psychomotor retardation and disconsolation. The prevalence of depression in the general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. Suicide is one of the most common outcomes of melancholia [1].

Aromatherapy is gaining popularity in a log phase and is currently used worldwide in the management of chronic pain, depression, anxiety, some cognitive disorders, insomnia and stress-related disorders. Although essential oils have been used reputedly and effectively for centuries as a traditional medicine, there is very little verified science behind this use. Therefore, the pharmacology of the essential oils and their chemical constituents remains largely undiscovered till date [2].

MATERIALS AND METHODS

Essential oils and animals:

Essential oils of eucalyptus oil (Eucalyptus globules) and neem oil are used in this study. All the oils are collected by Clevenger’s apparatus and their assessment tests are carried out. Male or female rats are used with a body weight (20–25 g) in the experiment. Animals are kept under standard conditions at 23-25°C 12 hr light/dark cycle and given standard pellet diet and water.

Experimental design

For all experiments, the animals are randomly divided into nine groups of (n = 2) animals each.

- **Group I:** Control
- **Group II:** Treated With Eucalyptus oil.
- **Group III:** Treated With Neem oil
- **Group IV:** Treated With Eucalyptus and Neem oil ratio 1:1
- **Group V:** Treated With Eucalyptus and Neem oil ratio 1:2

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Group VI: Treated With Eucalyptus and Neem oil ratio 1:3
Group VII: Treated With Eucalyptus and Neem oil ratio 2:1
Group VIII: Treated With Eucalyptus and Neem oil ratio 3:1
Group IX: Standard Treated With Imipramine

All the animals are treated with volatile oils as oral administration. Animals were kept for 30 min. and after 1 hr. to 7 hr. then after 24 hr. of treatment the evaluation of activities is performed.

**Tail Suspension:**

Male or female rats were used with a body weight (20–25 g). They are placed in plastic cages for testing in the experiment and in a 12 h light and dark cycle and fasting overnight. Animals are transported from the housing room to the testing area and allowed to adapt to the new environment for 1 h before testing. Groups are divided into control, test and standard and each may contain 2 animals. Drugs were given by oral route by the garage. For the test, the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for periods of 5 min. Mice were considered immobile time recorded by stopwatch when they hang passively and completely motionless for at least 1 min [3].

![Image of tail suspension test](image-url)

**Fig: 1 Synergistic Anti-depression of Eucalyptus and Neem oils on Tail**
Suspension Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre Treatment</th>
<th>Immobility Period (in sec)</th>
<th>Immobility Period (in sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control</td>
<td>171.0±1.0</td>
<td>93.0±1.0</td>
<td>125.5±0.5</td>
</tr>
<tr>
<td>Standard</td>
<td>92.0±1.0</td>
<td>130.0±2.0</td>
<td>71.5±2.0</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>50.0±0.0*</td>
<td>65.0±0.0*</td>
<td>139.0±0.0*</td>
</tr>
<tr>
<td>Neem</td>
<td>61.0±0.0*</td>
<td>103.0±0.0*</td>
<td>216.0±0.0*</td>
</tr>
<tr>
<td>1:1</td>
<td>83.0±2.0*</td>
<td>83.0±2.0*</td>
<td>163.5±2.0*</td>
</tr>
<tr>
<td>1:2</td>
<td>64.0±1.0**</td>
<td>119.2±2.0*</td>
<td>124.5±1.0*</td>
</tr>
<tr>
<td>1:3</td>
<td>60.0±1.0**</td>
<td>138.5±2.0*</td>
<td>106.0±1.0*</td>
</tr>
<tr>
<td>2:1</td>
<td>63.5±2.0**</td>
<td>113±1.0*</td>
<td>147.5±2.0*</td>
</tr>
<tr>
<td>3:1</td>
<td>48.0±1.0**</td>
<td>82.5±2.0*</td>
<td>115.5±2.0*</td>
</tr>
</tbody>
</table>

Values are in Mean ± S.E.M (n=6). Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett’s test, (n=6) animal in each group. ** (p<0.01), *(p<0.05), ns (non-significant) compared to control group.

**Effect of essential oils on Tail Suspension test (TST)**

The results of CNS depressant or Stimulant activity on Tail Suspension test of selected essential oils are given in individual and different ratios. The treatment with eucalyptus and neem oil show decreased duration of immobility, are significant (p<0.05). Where eucalyptus oil treat animals show significant (p<0.001) decreased duration of immobility.

**Animals**

Swiss albino mice are used with a body weight (100-150 g) in experiment. Animals were procured were feeding normal diet and water *ad libitum* and were provided to natural light and
dark cycle at controlled room temperature of 20-25°C. The animals were conforming to the laboratory condition before experiments. The animals fasted overnight before drug administration, Elevated plus Maze Model was performed during day time between 7 a.m. and 7 p.m. Experimental protocol is approved by Institutional Animal Ethics Committee (IAEC). Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India [4].

![Fig: 2 Immobility Periods (in sec)](image)

**STATISTICAL ANALYSIS**

The statistical analyses are carried by one way ANOVA followed by Dunnet’s multiple “t” tests. P values < 0.05 (95% confidence limit) are considered statistically significant, using software Graph Pad Prism6.

**RESULTS AND DISCUSSION**

The results for CNS depressant or Stimulant activity on Tail Suspension test of selected essential oils are given in Table. The treatment with eucalyptus, neem and combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1 and 3:1 ratio showed decreased duration of immobility and the mixture of oils are given at dose of 100 mg/kg body weight along with standard Imipramine given orally. It is found that eucalyptus and neem essential oil at different ratio (1:1, 1:2, 1:3, 2:1 and 3:1) exhibited maximum activity after 2 hr and significantly reduced stress even till 6 hr after drug administration as compared to control.
The anti-stress activity using Tail Suspension test in the present study showed that the eucalyptus and neem essential oil at different ratio have enough ability to control the stress might be due to various chemical constituents present in volatile oils. On the comparison between different ratios, 1:1, 1:3 ratios are most effective one and be suitable for further herbal formulation.

The CNS depressant and antidepressant activities are evaluated using Tail Suspension test. Duration of immobility is taken as anti-depressant activity and eucalyptus and neem essential oil decreased the duration of immobility which indicates about their antidepressant activity. Moreover, Eucalyptus oil increased the duration of immobility in both tests.

CONCLUSION

From this work, it becomes clear that aromas of essential oils have various pharmacological activities and give valuable assets for using in aromatherapy. Further studies, like Molecular Docking for active aroma components of each essential oils against different receptors like GABA, NMDA, Cholinergic and adrenergic receptors and different channels; neurochemical, and biochemical estimation of the various transmitters are needed to know the exact pharmacological mechanism of these oils.

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