

## Study of anxiolytic activity of some essential oils used by inhalational exposure in mice

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**Abstract** – At present competitive life due to stress and strain in work like enhancing burden in learning, pressure of doing well, worry about settlement or jobs are one of the major cause of human anxiety and affects one-eighth population worldwide. Aromatherapy is getting popularity as alternative therapy for treatment and management of CNS disorders. This present study was taken to study the affect of Sandalwood Oil, Eucalyptus Oil, Lemon Oil, Jasmine Oil, and agarwood on Behavioral anxiolytic animal models by exposing as inhalational route. Anxiolytic activity was studied in mice using Hole Board Test and Elevated Plus Maze after exposure of essential oils in inhalational chamber for 15 min. The no. of head dipping and line crossing in Hole board test and no. of entries and time spent in open arms are taken as index for measuring anxiolytic activity. From this behavioral study it can be concluded that by inhalational exposure of sandalwood oil, jasmine oil and agarwood oil produces anxiolytic activity but Eucalyptus oil and lemon oil may be anxiogenic. Although as observed, eucalyptus oil significantly decreased mobility in animals and that may be reason of opposite result in these behavioural studies. By further research, we can frame the pharmacological basis of aromatic oil for the treatment oil CNS diseases.

**Keywords** – Anxiety, Essential oil, Agarwood oil, Jasmine oil, Elevated Plus Maze, Hole Board Test.

## INTRODUCTION

In modern competitive life due to stress and strain in work like enhancing burden in learning, pressure of doing well, worry about settlement or jobs are one of the major cause of human anxiety. Stress involves complex biochemical, neurological and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, impotency and cognitive dysfunctions [1].

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat. Now a day, anxiety affects one-eighth population worldwide and has become an important research area in the field of psychopharmacology [2]. Anxiety related disorders such as generalized anxiety, panic, obsessive-compulsion, phobias or post traumatic stress disorders are common and major cause of disability. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. But they shows side effects like rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction, anticholinergic, antihistaminic effects (TCA's) and these agents primarily relieve the symptoms and offer a palliative

relief of a temporary nature have limited their use in patients [3]. Due to this many pharmaceutical companies are conducting studies to find an alternative medicine or plant-derived medications with more specific anxiolytic effects[4].

In recent years use of alternative medicine in particular, derived from plant have been screened and coming in market as drugs[5]. 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 [6]. Although essential oils have been used reputedly 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.

## MATERIAL AND METHODS

### Animals

Mice of either sex weighing 20-25 gm of body weight were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25°C 12 hr light/dark cycle and given standard pellet diet and water. Before using in experiment animals got clearance from CPCSEA. Registration No. 177/99/CPCSEA

### Drugs and Chemicals

Essential oils of sandalwood oil (*Santalum album*), (Md.ALI Lucknow perfumers, Machlikamam, Hyderabad.) eucalyptus oil (*Eucalyptus globules*), (Kola products, Bhimavaram.) lemon oil (*Citrus limonum*), (Srikrishna sudama ashramam, Saalipet, Tenali.) jasmine oil (*Jasminum sambac*) (SFP Sons (India) pvt. Ltd, Chennai.) and agar wood oil (*Aquilaria agallocha*) (Ajmal Perfumes, Assam) were used in this study. All the oils were purchased and their qualitative tests were carried out.

### Experimental design

For all experiments the animals were randomly divided into six groups of six animals each.

- Group I: Control
- Group II: Treated With Sandalwood Oil.
- Group III: Treated With Eucalyptus Oil
- Group IV: Treated With Lemon Oil
- Group V: Treated With Jasmine Oil.
- Group VI: Treated With Agar wood Oil

All the animals were treated with essential oils as inhalation way. Animals are kept in inhalational chamber for 30 min. and after 15 min of treatment the evaluation of activities were performed.

### Method of inhalation

The animals are placed in a closed chamber of 1 cubic feet. Four pieces of cotton plugs are placed at the four corners of the closed chamber. 1 ml each of essential oils was spilled on to cotton plugs and kept the oils for inhalational for 30 min. A small amount of sodium bicarbonate is placed in the closed chamber in order to absorb the carbon dioxide released by the animals. A provision is made in the chamber for the entry of oxygen [7].

### Test for Anxiolytic activity

#### Hole Board Test in mice

Exploratory behavior was evaluated in Hole Board paradigm [8-9]. The open field was made up of plywood and comprises of 40 X 50 X 60 cm dimension. The entire apparatus was painted black and was divided into 16 squares with white lines on the floor. Each animal was placed at one corner of the apparatus and for the next 5 minutes they were observed for their ambulation such as line crossings and head dipping. After treatment of the drug in inhalational chamber for 30 min and after 15 min of exposure each animal was placed on a corner of the hole board and observed for line crossing and nose poking for 5min and noted

#### Elevated Plus maze test in mice

The apparatus consists of two open arms (35 X 6 cm) and two enclosed arms (35 X 6 X 15 cm). The arm was connected together with a central square of 5 X 5 cm. The maze was elevated to a height of 60 cm. The maze was placed inside a light and sound attenuated room. Mice were placed individually at the end of an open

arm of elevated plus maze (EPM) facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms Transfer Latency (TL) was recorded [10, 11]. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. After treatment of the drug in inhalational chamber for 30 min and after 15 min each animal was placed on EPM and the number of entries and the time spent in the open and closed arms were recorded during a 5- min test period.

### Statistical Analysis

The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t" test. P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

## RESULTS AND DISCUSSION

### Effect of essential oils on mice in Hole- board

All the animals were observed after inhalation treatment of essential oils on hole board test. The no. of head dipping and line crossing for 5 min was observed for each animal and the result is given in **Table-1**. All the animals treated with essential oil decreased the head dipping but lemon oil and sandalwood oil increases line crossing non significantly and Eucalyptus oil significantly reduced the no. of line crossings.

Hole-board model indicated that head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behavior [12]. Except sandalwood and lemon oil, other not increased line crossing as this does not give a clear conclusion about anxiolytic activity based on hole board test. It has been suggested that some animal models based on spontaneous behaviour or ethologically based models [13] like the light-dark test, may be more sensitive to the behavioural responses than conditioned paradigms [14].

### Effect of essential oils on mice in Elevated Plus Maze

All the animals were observed after inhalation treatment of essential oils on Elevated Plus Maze. The no. of entries in open arm and time spent in open arm were noted for 5 for each animal and the result is given in **Table-2**. The animals treated with essential oil like sandalwood oil, agarwood oil and jasmine significantly increased no of entries and time spent in open arm while eucalyptus oil and lemon significantly decrease the no entries and time spent in open arm.

The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than evoked by the exposure to an enclosed arms. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed

by the increased time spent and entries in to the open arm. The primary index is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds.[15].

**Table-1: Effect of essential oils for anxiolytic activity on Hole Board test in mice.**

Groups	Treatment	Head Dipping; (Mean ± SEM)	Line Crossings; (Mean ± SEM)
I	Control	8.83± 0.83	46.00±3.75
II	Sandal wood oil	4.00±0.81***	56.83±4.40 ns
III	Eucalyptus oil	4.16±0.54**	26.17±1.66***
IV	Lemon oil	3.83±0.54***	52.33±3.54 ns
V	Jasmine oil	5.83±1.19*	40.50±2.84 ns
VI	Agar wood oil	4.33±0.61**	42.83±2.54 ns

(Values are in Mean ± S.E.M (n=6); <sup>ns</sup> -Non Significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Control using One way ANOVA followed by Dunnet’s “t” test.)

**Table-2: Effect of Essential oils for anxiolytic activity on Elevated Plus maze**

Groups	Treatment	No of entries; (Mean ± SEM)		Time spent (sec); (Mean ± SEM)	
		Open arm	Closed arm	Open arm	Closed arm
I	Control	4.50±.076	11.00±1.18	69.83±6.93	212.7±5.33
II	Sandal wood oil	8.16±0.94**	9.00±0.73ns	116.00±5.50***	175.8±6.34**
III	Eucalyptus oil	4.00±0.51ns	6.16±.087*	39.17±5.75**	250.0±7.78**
IV	Lemon oil	3.50±.056ns	9.33±1.62ns	36.50±4.08**	253.0±6.67***
V	Jasmine oil	6.16±0.65ns	8.33±1.30ns	126.2±8.11***	140.0±6.17***
VI	Agar wood oil	6.66±1.05ns	6.16±0.94*	121.2±5.40***	165.3±7.39***

(Values are in Mean ± S.E.M (n=6); <sup>ns</sup> -Non Significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Control using One way ANOVA followed by Dunnet’s “t” test.)

**CONCLUSION**

From this behavioral study it can be concluded that by inhalational exposure of sandalwood oil, jasmine oil and agarwood oil produces anxiolytic activity but Eucalyptus oil and lemon oil may be anxiogenic. Although as observed, eucalyptus oil significantly decreased mobility in animals and that may be reason of opposite result in these behavioural studies. By further research, we can frame the pharmacological basis of aromatic oil for the treatment oil CNS diseases.

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