EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 6
177-180
Year: 2015

SYNERGISTIC ANTI-ANXIETY ACTIVITY EVALUATION OF DIFFERENT COMPOSITION OF VOLATILE OIL OF EUCALYPTUS AND NEEM OIL BY ELEVATED PLUS MAZE MODEL

Amrita Asthana*, Vandana R. Nair and Kishu Tripathi

Pharmacy College, Itaura, Chandeshwar, Azamgarh, U.P., India.

***Author for Correspondence: Dr. Kishu Tripathi**
Pharmacy College, Itaura, Chandeshwar, Azamgarh, U.P., India.

Article Received on 04/09/2015 **Article Revised on 24/09/2015** **Article Accepted on 14/10/2015**

ABSTRACT
To evaluate anti-anxiety activity of eucalyptus and neem oils in Swiss albino Mice. In this study six groups (n=5) of mice were used. Standard, control and test drugs were given to each animal orally; behaviour testing was performed in animal models after 30 min time spent in open arm was observed for 5 min duration (300 s). Significant increase in percentage of time spend in open arm of EPM method indicate anxiolytic effect respectively. Significant decrease in above parameters indicates anxiogenic effect. Eucalyptus and neem oil in 1:1, 1:2, 1:3, 2:1 and 3:1 ratios significantly increased percentage of time spend in open arm of EPM method as compared to vehicle treated mice in both EPM behavioural models.

KEYWORDS: Anxiety, Eucalyptus and Neem oil, Elevated plus Maze Model, Dizepam.

INTRODUCTION
In modern competitive life due to stress and strain in work like enhancing burden in acquirements, compressing of doing well, worry about establishment or business are one of the major cause of human anxiety and affects one-eighth population worldwide. Fragrance is receiving popularity as alternative therapy for treatment and management of CNS disorders. Stress involved complex biochemical, neurological and immunological mechanisms and plays a role in the origin and regression of a variety of disease states covering from mentally illness disorders like depression and anxiety, immunosuppressant, endocrine disorders including diabetes mellitus, impotency and cognitive dysfunctions, etc.[1] In experience regular anxiety is a normal part of life. However, people with anxiety disorders regularly have inadequacy, insufficiency and fear about everyday situations.[2] Anxiety is common mental disorders that share extreme or pathological fear as the primary disturbance in mood or emotional tone. All anxiety disorders are a state of increased fear and enhance version of the acute stress response.[3]

Common anxiety are feeling nervous, feeling powerless, having a sense of impending danger, panic or doom, increase heart rate, breathing rapidly, sweating, trembling and feeling weak or tired.

MATERIAL 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 assessable tests are carried out. Male or female rats are used with a body weight (20–25 g) in experiment. Animals are kept under standard conditions at 23-25°C 12 hr light/dark cycle and given standard pellet diet and water.

Animals
Male or female rats are used with a body weight (200–250 g in experiment. Animals were procured and were feeding normal diet and water ad libitum and were provide 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 were fasting over night before drug administration, Elevated plus Maze Model was performed during day time between 7 a.m. and 7 p.m. Experimental protocol are 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]

Drugs and chemicals
Thymol, Diazepam, Ethanol and Sodium chloride were used in this study. Thymol was procured from Central Drug House (CDH) Ltd. India. Diazepam (Calmpose);

Ranbaxy Laboratories, Ltd., Gurgaon, India. Normal saline (0.9% NaCl) was used as vehicle for Diazepam while absolute ethanol solution (0.01%) was used as vehicle. Volume of injection for mouse was 10 mL/kg.

Experimental design

For all experiments the animals are randomly divided into nine groups of (n =6) 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
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 Dizepam

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

ELEVATED PLUS MAZE MODEL

Elevated plus maze (EPM) is best models of anxiety. It comes under ethologically based animal model of fear and anxiety and involves the animal's spontaneous or natural reactions (e.g. flight, avoidance and freezing) to stress stimuli that do not explicitly involve pain or discomfort. Mice were used with a body weight (70-150 g). The animals was starved overnight and divided into three groups- control, standard and test each group may contains six animals The plus-maze consists of two open arms 50 × 10 × 40 cm and two enclosed arms 50 × 10 × 40 cm. The mice was kept on elevated plus maze of 50 cm of these mice. During the experiment each rats are placed in the central compartment face towards either of open arm, observed for 5 min to record the time spent in open arms, the total observation of experiment are 5 min (300 s).[5]

Percent of time spent by mice in open arms are calculated as follows:

$$\% = \frac{\text{Number of seconds spent in open arms}}{\text{300 total sec (5 min observation times)}} \times 100$$

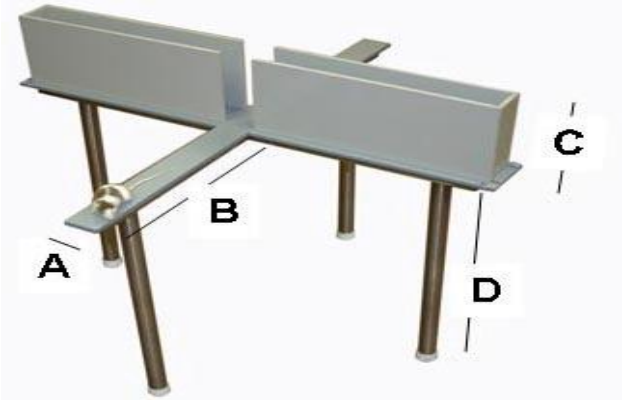

Fig: 1 Elevated Plus Maze Model.

Table: 1. Synergistic Anti- anxiety activity of Eucalyptus and Neem oils on Elevated Plus Maze Model.

Group	Avg. no. of entry in the open arms									Avg. time spent in the open arms								
	I	II	III	IV	V	VI	VII	VII	IX	I	II	III	IV	V	VI	VII	VII	IX
Control	0.0 ±0.0	1.0 ±0.0	0.0 ±0.0	1.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.5 ±0.0	0.0 ±0.0	0.2 ±0.0	0.0 ±0.0	0.0 ±.00	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Standard	0.0 ±0.0	1.5 ±0.5	0.5 ±0.5	0.5 ±0.5	0.5 ±0.5	0.0 ±0.0	1.0 ±1.0	1.5 ±0.5	1.0 ±1.0	0.0 ±0.0	18.5±0.5	0.0 5±5.0	6.0 ±2.0	6.0 ±2.0	0.0 ±0.0	9.0 ±1.0	8.5 ±1.5	4.0 ±1.0
Eucalyptus	0.0 ±0.0	1.0 ±0.0*	2.0 ±0.0*	1.0 ±0.0*	1.0 ±0.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	10.0±0.0*	0.1 6±0.0*	0.5 ±0.0*	0.6 ±0.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Neem	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0*	1.0 ±0.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 2±0.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1:1	0.0 ±0.0	1.0 ±1.5*	0.5 ±0.5*	0.5 ±0.5*	1.0 ±0.0*	0.5 ±0.5*	1.0 ±1.0*	0.0 ±0.0	0.5 ±0.5*	0.0 ±0.0	0.0 ±0.0	4.0 ±4.0*	15.0±5.0*	20.5± 1.5	9.0 ±6.0*	7.0 ±3.0*	0.0 ±0.0	8.0 ±4.0*
1:2	0.0 ±0.0	1.0 ±1.5*	0.5 ±0.5*	2.5 ±0.5*	2.5 ±0.5*	1.0 ±0.0*	0.5 ±0.5*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	37.5±0.5*	3.0 ±1.0*	0.0 ±0.0	7.5 ±2.5*	0.0 ±0.0	0.0 ±0.0
1:3	1.5 ±0.5*	1.5 ±0.5*	1.5 ±0.5*	2.5 ±0.5*	1.5 ±0.5*	0.5 ±0.5*	0.0 ±0.0	0.0 ±0.0	1.5 ±0.5*	11.0±1.0**	32.5±0.5*	27.5±2.5**	49.5± 2.5*	9.0 ±4.0*	6.0 ±2.0*	0.0 ±0.0	0.0 ±0.0	12.0±4.0*
2:1	0.0 ±0.0	0.5 ±0.5*	0.5 ±0.5*	1.0 ±1.0*	0.0 ±0.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.0 ±0.0*	0.0 ±0.0	3.5 ±1.5*	6.7 ±2.5*	2.0 ±0.0*	9.0 ±2.0*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	7.0 ±3.0*
3:1	0.0 ±0.0	1.0 ±1.0*	1.0 ±1.0*	0.5 ±0.5*	0.5 ±0.5*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.5 ±1.5*	4.0 ±1.0*	4.5 ±1.5*	0.1 ±0.0*	1.5 ±0.5*	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Values are in Mean ± S.E.M (n=6) Data were 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.

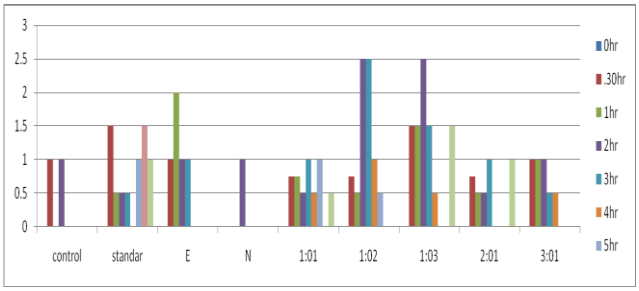

Fig: 2 Entries in the open arm.

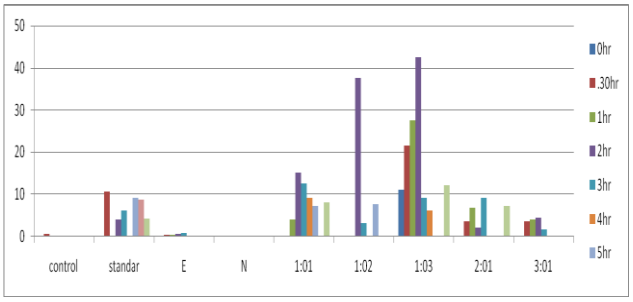

Fig: 3 Time spent in the open arms.

STATISTICAL ANALYSIS

Analyses are carried by one way ANOVA followed by Dunnet's multiple "t" tests. P values < 0.05 (95% confidence limit) are considered the statistical statistically significant, using software Graph Pad Prism6.

RESULTS AND DISCUSSION

The results for CNS depressant or anxiety activity on elevated plus maze method 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, and 2:1, 3:1 ratio (Table:1) showed significant increase in percentage of time spent in open arms (Fig:2) and the mixture of oils are given at dose of 100 mg/kg body weight along with standard Diazepam given orally. It may 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 2hr and significantly reduced stress even till 6hr after drug administration as compared to control.

Elevated plus maze method in the present study showed that the eucalyptus and neem essential oil at different ratio have enough ability to control the stress which might be due to various chemical constituents present in volatile oils. On comparison between different ratios, 1:2

and 1:3 ratios are most effective and suitable for further herbal formulation.

In elevated plus maze method significant increase in percentage of time spent in open arms indicate anxiolytic-like effect respectively. Significant decreased in above parameters indicates anxiogenic effect. Diazepam significantly increased percentage of time spent in open arms of elevated plus maze.

CONCLUSION

Diazepam binds to a specific subunit on the GABA A receptor at a site distinct from the binding site of the endogenous GABA molecule, known as an allosteric site. The GABA A receptor is an inhibitory channel which, when activated, decreases neuronal activity. Benzodiazepines cause an increased opening of the chloride ion channel when GABA binds to its site on the GABA A receptor, leading to more chloride ions entering the neuron, which in turn leads to enhanced central nervous system depressant effects. From this works 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; neuro-chemical and biochemical estimation of various transmitter are need to know the exact pharmacological mechanism of these oils.

ACKNOWLEDGEMENT

I would like to thank Director Prof. Dr. Kishu Tripathi and Prof Bajrang Tripathi, Chairman, Pharmacy College, Azamgarh for providing constant encouragement, valuable guidance and facilities at all stages of this work.

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