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SYNERGISTIC ANTI-ANXIETY ACTIVITY OF DIFFERENT COMPOSITION OF VOLATILE OIL OF EUCALYPTUS AND NEEM OIL BY LIGHT AND DARK MODEL

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ABSTRACT

The research work deals with the screening of synergistic anti-anxiety activity of different composition of Eucalyptus (*Eucalyptus globulus)* and Neem (*Azadirachta indica)* oils in Swiss albino Mice. In this study were used 9 group (n=5) of mice. Standard, control and test drugs were given to each animal orally; behaviour testing was performed in animal models after 30 min time spent in dark arm was observed for 5 min duration (300 s). Significant increases in percentage of time spend in dark arm of Light and Dark arms indicate anxiety 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 dark arm of Light and Dark method as compared to vehicle treated mice in both Light and Dark method behavioural models.

KEYWORDS: Anxiety, Eucalyptus and Neem oil, Light and Dark Model, Diazepam.

INTRODUCTION

At present 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. Anxiety disorders are considered to be a major cause of disability worldwide and comprise generalized anxiety disorder and other commonly associated conditions, such as phobias, postmenopausal stress, post-traumatic syndrome, somatisation and cognitive dysfunction, among others. Patients diagnosed with generalized anxiety disorder exhibit functional impairment as well as a tendency to develop comorbid psychiatric disorders.[1] Anxiety may also be referred to an elaborate form of fear and causes an increase in the ability of an individual to adapt and plan for the future fear and anxiety are difficult to distinguish.[2] Sometimes, despite a thoughtful evaluation of a patient, no treatable primary illness is found, or if one is found and treated, it may be desirable to deal directly with the anxiety at the same time. In such situations anti-anxiety medications are frequently and appropriately used.[3]

For example, excessive or unreasonable anxiety about life circumstances (generalized anxiety disorder), panic disorders and agoraphobia are amenable to drug therapy, usually in conjunction with psychotherapy. In many cases, anxiety is a symptom of psychiatric problems that may warrant the use of antidepressant or antipsychotic drugs.[4]

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 appreciate version of the acute stress response .Anxiety related disorders such as generalized anxiety, fear, obsessive compulsion, dislike 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

Common anxiety-Feeling nervous, Feeling powerless, Having a sense of impending danger, panic or doom, Increase heart rate, Breathing rapidly, Sweating, Trembling, Feeling weak or tired.[5]

MATERIAL AND METHODS

Volatile oils and animals

Volatile 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, Light and Dark 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 [6].

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.

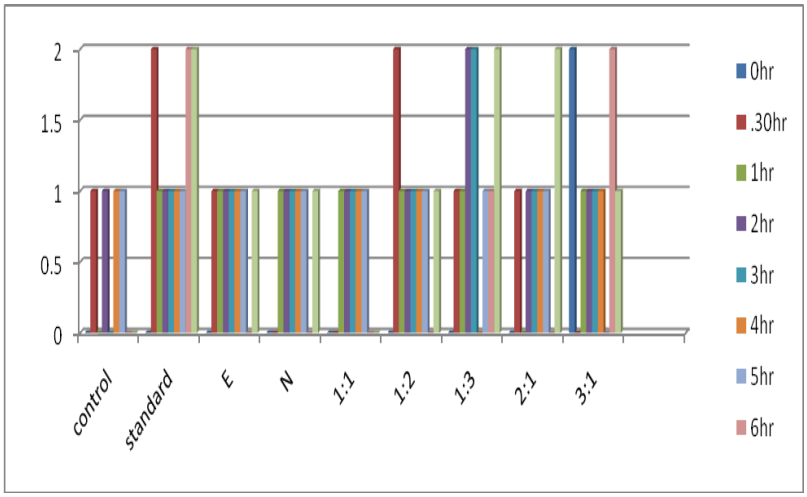
LIGHT-DARK MODEL

Light and dark model 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 are used with a body weight (20–25 g). They are placed in plastic cages for testing in experiment and in a 12 h light and dark cycle and fasting over night. 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. Drugs are given by oral route by gavage and the testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone. A polypropylene animal cage, 44 × 21 × 21 cm, is darkened with black spray over one-third of its surface. A partition containing a 13 cm long × 5 cm high opening separates the dark one third from the bright two thirds of the cage. Rats are placed into the cage. The animals are pre-treated 30 min before the experiment and are then observed for 5 min. Groups of seven group (n=5) animals are used for each dose. Time spend in dark arms during this time is counted with stopwatch. Moreover, testing at various time intervals, time response curves can be obtained than calculated the ANOVA.[7]

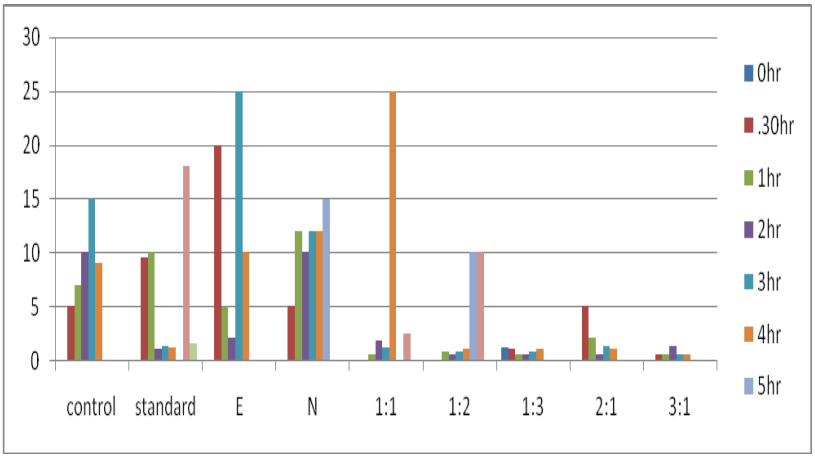
Table: 1 Synergistic Anti- anxiety activity of Eucalyptus and Neem oils on Light and Dark Model

Group	Avg. no. of entry in the Light arms									Avg. time spent in the Dark arms								
	I	II	III	IV	V	VI	VII	VIII	IX	I	II	III	IV	V	VI	VII	VIII	IX
Control	0.0± 0.0	1.0± 0.0	0.0± 0.0	1.0± 0.0	1.0± 0.0	0.5± 0.0	1.0± 0.0	0.0± 0.0	0.0± 0.0	0.0±0. 0	0.5±0. 0	7.0±0. 0	0.2±0. 0	10.0± 0.0	15.0± .00	9.0±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	1.0± 0.0	1.0± 1.0	1.5± 0.5	1.0± 1.5	0.0±0. 0	19.5± 0.5	10.0± 5.0	6.0±2. 0	6.0±2. 0	25.0± 1.0	7.0±1. 0	8.5± 1.5	10.0± 1.0
Eucalyptus	0.0± 0.0	1.0± 0.0*	2.0± 0.0*	1.0± 0.0*	1.0± 0.0*	1.0± 0.0	1.0± 0.0	1.0± 0.0	0.5± 0.0	0.0±0. 0	20.0± 0.5*	12.0± 0.0*	0.5±0. 0*	0.6±0. 0*	25.0± 0.0	10.0±0 .0	15.0 ±0.5	0.0±0. 0
Neem	0.0± 0.0	0.0± 0.0	1.0± 0.0*	1.0± 0.0*	0.5± 0.5	1.0± 0.0	1.0± 0.0	1.0± 0.0	0.1± 0.0	0.0±0. 0	5.0±0. 0	12.0± 0.5	0.12± 0.0*	10.0± 0.0	12.0± 0.0	12.0±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*	1.0± 0.0	0.5± 0.5*	0.0±0. 0	0.0±0. 0	45.0± 1.5*	15.0± 5.0*	56.5± 1.5*	20.0± 1.0*	25.0±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*	0.5± 0.0	1.0± 0.0	0.0±0. 0	0.0±0. 0	19.0± 1.0	37.5± 0.5*	20.0± 0.5*	12.0± 1.0	25.5±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*	1.0± 0.0	0.5± 0.0	1.5± 0.5*	12.0± 1.0**	30.0± 0.5*	10.5± 0.5**	49.5± 2.5*	39.0± 2.0*	21.0± 2.0*	25.0±1 .0	10.0 ±0.0	12.0± 4.0*
2:1	0.0± 0.0	0.5± 0.5*	0.5± 0.5*	1.0± 1.0*	0.5± 0.5*	2.0± 1.0	1.0± 0.0	0.5± 0.0	2.0± 0.0*	0.0±0. 0	3.5±1. 5*	6.7±2. 5*	2.0±0. 0*	15.0± 2.0*	2.0±0 .0	4.0±1. 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*	1.0± 0.0	1.0± 0.0	2.0± 1.0	1.0± 0.0	0.0±0. 0	3.5±1. 5*	21.0± 1.0*	4.5±1. 5*	40.0± 1.0*	15.5± 0.5*	25.0±2 .0	0.0± 0.0	0.0±0. 0

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.



ENTRY IN THE LIGHT ARMS



TIME SPENT IN THE DARK ARMS

STATISTICAL ANALYSIS

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

RESULTS AND DISCUSSION

The results for CNS depressant or anxiety activity on Light and Dark 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 showed significant increase in percentage of time spent in dark arms and the mixture of oils are given at dose of 100mg/kg body weight along with standard Diazepam given orally. It are found that eucalyptus and neem essential oil at different ratio (1:1, 1:2, and 1:3, 2:1 and 3:1) exhibited maximum activity after 2hr and significantly reduced stress even till 6hr than 24 hr after drug admission as compared to control.

Light and Dark method 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 comparison between different ratios, 1:2, 1:3 ratio are most effective one and be suitable for further herbal formulation.

In Light and Dark method significant increase in percentage of time spent in dark arms indicate anxiolytic effect respectively. Significant decreased in above parameters indicates anxiogenic effect. Diazepam significantly increased percentage of time spent in dark arms of Light and Dark Method.

CONCLUSION

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed, it is a universal human emotion, closely allied with appropriate fear and presumably serving psychobiological adaptive purposes.

Testing compounds for anxiety activity of different combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratio also reduced the time spent in dark arm, a test mainly used to screen anxiety behaviour . This represented that eucalyptus and neem in 1:1, 1:2, 1:3, 2:1 and 3:1 ratio may show anxiety activity. The anxiety effect of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratio could be due to the interaction of flavonoids

chemical constituent of eucalyptus and neem oil with the GABA/benzodiazepine receptor complex in brain.

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.

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REFERENCE

1. Wittchen HU, Zhao S, Kessler RC, Eaton WW. DSM III-R generalized anxiety disorder in the National Comorbidity Survey. *Arch Gen Psychiatry*, 1994; 51(5): 355–364.
2. Barlow DH, Unravelling the mysteries of anxiety and its disorders from the perspective of emotion theory. Am Psychol, 2000; 55: 1247-63.
3. Taylor CB, Treatment of anxiety disorders, American Psychiatric Textbook of Psychopharmacology, 2nd Ed., American Psychiatric Press.Washington DC, 1998; 775-789.
4. Stovdemirea A. Epidermology and psychopharmacology of anxiety in medical patients. J Clin Psychiatry, 1996; 57: 64.
5. Steimer T. The biology of fear and anxiety related behaviours. Dialogues Clin Neurosci, 2002; 4: 231-249.
6. Nishino T, Takeuchi T, Takechi K, Kamei C. Evaluation of anxiolytic-like effects of some short acting benzodiazepine hypnotics in mice. J Pharmacol Sci., 2008; 107: 349-354.
7. Vogal Gerhard.H, Drug Discovery and evaluation of pharmacological assays, 2002; 2: 434.