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Synergistic Analgesic Activity of Different Composition of Volatile Oil of Eucalyptus and Neem Oil by Tail Immersion Model

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ABSTRACT

The research work deals with the synergistic analgesic activity of different composition of Eucalyptus (*Eucalyptus globulus)* and Neem (*Azadirachta indica)* oils. The albino rats were divided into 9 groups and were administered orally different ratios of Eucalyptus and Neem 1:1, 1:2, 1:3, 2:1 and 3:1, Control (normal saline was given orally) & Standard (Tramadol HCl) for 9 hrs. Results were expressed as mean ± SE. ANOVA followed Dunnet's multiple "t" test. P values < 0.05 (95% confidence limit) are considered statistically significant using software Graph Pad Prism 6 and found to produce analgesic effect in experimental animals and the efficacy of the different combination of oil was found to be comparable with that of standard drugs used Tramadol HCl.

INTRODUCTION

In modern competitive life strain in work like enhancing burden in acquirements, compressing and pain is responsible for the surge in incidence of variety of mental disorders. The volatile oils which are produced by different plants confirm analgesic and anti-inflammatory activities which affect by different ways. For instance, hot water extracts of dried leaves of *Eucalyptus* are traditionally used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections such as cold, flu and sinus congestion. Essential oils from *Eucalyptus* species are also widely used in modern cosmetics, food and pharmaceutical industries [1]. In this regard, monoterpenoid components of the aromatic constituents of the oils are commercially available for the treatment of the common cold and other symptoms of respiratory infections [2].

In this relation, a recovery of benefit in medicine from natural sources (mainly plant products) is detected and there is exceptional hope that drugs of plant origin will have significantly lesser side effects than that recorded with synthetic drugs while having comparable efficacy. There are a number of traditional herbal drugs used in combination as polyherbal formulation to get synergistic and desirable effects, therefore, the evaluation of synergistic analgesic activity of eucalyptus and neem oil in different ratios was carried out[3].

MATERIALS 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 (20–25g) in an 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 = 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 Tramadol HCl

All the animals are treated with volatile oils by oral administration. Animals were kept for 30min. and after 1hr. to 9hr of treatment for the evaluation of activities were performed.

Tail Immersion test:

Mice (70-120g body weight) were used. They are placed in plastic cages for testing in experiment and in a 12h 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 1h before testing. Groups are divided into control, test and standard and each may contain 6 animals. Drugs are given by oral route by garage and mice tail 5cm dip in hot water (50±55^{o}C) then calculate the tail flicking and recorded by stopwatch. The cut off time or Latency to Flick Tail is 15 s. The departure time of untreated animals were 1 and 5.5 s. A withdrawal time of more than 6 s, therefore, is regarded as a positive response [4].

Effect of Volatile oils on Tail immersion test:

The results of Tail immersion test of selected Volatile oils are given in Table and illustrated in Fig. The treatment with eucalyptus and neem oil showed decreased in duration of Tail flick was significant ($p<0.05$) whereas eucalyptus oil treated animals showed significant ($p<0.001$) decreased in Latency to Flick Tail.

Animals:

Mice are used with a body weight (70-120g) 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^{o}C. The animals were conforming to the laboratory condition before experiments. The animals were fasting overnight before drug administration,

Tail immersion test 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 [5].

Table: 1 Synergistic Analgesic activity of Eucalyptus and Neem oils on Tail Immersion Test (TMT)

Group	Latency to Flick Tail (in sec)									
	30 min	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr
Control	1.0±0.5	1.5±0.5	2.0±0.5	2.0±0.0	1.0±0.5	2.0±0.5	2.0±0.0	1.0±0.5	1.0±0.0	0.5±0.0
Standard	2.0±0.5	2.0±0.5	2.5±0.0	2.0±0.0	2.0±0.5	3.0±0.0	2.5±0.0	1.0±0.5	1.0±0.0	1.0±0.0
Eucalyptus	2.5±0.5*	2.0±0.5*	2.0±0.5*	1.25±0.25*	1.5±0.5*	1.5±0.5***	2.0±0.0***	1.0±0.5*	1.0±0.0*	1.0±0.5*
Neem	2.0±0.5*	2.0±0.5*	2.0±0.5*	2.0±0.5*	1.0±0.0*	1.0±0.0*	1.5±0.5***	1.0±0.5*	1.0±0.5*	1.0±0.0*
1:1	2.0±0.5*	2.5±0.5*	2.0±0.5*	2.25±0.75*	2.0±0.0*	2.0±0.0*	1.5±1.0*	1.5±0.0*	1.0±0.5*	1.0±0.0*
1:2	3.0±1.0*	2.5±0.5*	2.0±0.5*	1.0±0.0*	2.25±0.5*	2.0±0.5*	1.75±0.5*	1.5±1.0*	1.0±0.0*	1.0±0.5*
1:3	3.5±1.5*	2.5±0.5*	2.25±0.25*	1.0±0.0*	2.0±0.5*	1.5±0.5*	1.5±0.5*	1.25±1.0*	1.0±0.0*	1.0±0.25*
2:1	2.5±0.5*	2.0±0.0*	1.75±0.25*	1.0±0.0*	2.75±1.5*	2.5±0.5*	1.25±0.5*	1.0±0.5*	1.0±0.5*	1.0±0.5*
3:1	0.5±0*	1.5±0.5*	1.75±0.25*	1.5±0.5*	2.5±1.5*	2.0±0.0***	1.0±0.5*	1.0±0.5*	1.0±0.0*	2.0±0.5*

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

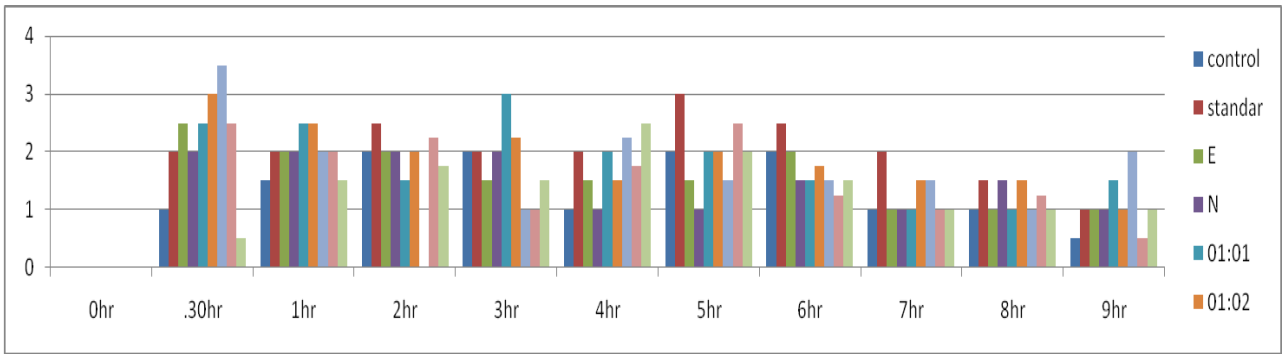

Fig: 1 Latency to Flick Tail (in sec)

Statistical Analysis

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

RESULTS AND DISCUSSION

The results of analgesic activity on Tail immersion test of selected essential oils are given in Table. Treatment with eucalyptus, neem and combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratio showed decreased in Latency to Flick Tail and the mixture of oils are given at dose of 100mg/kg body weight along with standard Tramadol HCl 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 2hr and significantly reduced stress even till 7hr than 9hr after drug admission as compared to control.

The analgesic activity using Tail immersion 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 comparison between different ratios, 1:1, 1:3 ratio was most effective one and be suitable for further herbal formulation.

Analgesic activities are evaluated using Tail immersion test. Duration of latency to tail flick is taken as analgesic activity and eucalyptus and neem essential oil decreased the latency to flick tail which indicates about their analgesic activity. Moreover, Eucalyptus oil increased duration of tail flick tests.

CONCLUSION

In analgesic activity, on 9hr treatment, animals treated with eucalyptus, neem show significant effect while those treated with and combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratios showed decreased in latency to flick tail showed more significant effect and the standard drug Tramadol HCl drug showed more significant effect in experiment (tail immersion model) indicating the analgesic activity. Tail flick tests are most sensitive to centrally acting analgesics combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratio also reduced the time spent in hot water by animal in the tail immersion test. This represented that eucalyptus and neem in 1:1, 1:2, 1:3, 2:1 and 3:1 ratio may show better analgesic activity. The analgesic effect of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1 and 3:1 ratio could be due to the interaction of flavonoids chemical constituent of eucalyptus and neem oil.

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