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# Synergistic Muscle Relaxant Activity of Different Composition of Volatile Oil of Eucalyptus and Neem Oil by Rota Rod Model

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**ABSTRACT**

The research work deals with the screening of synergistic muscle relaxant activity of different composition of Eucalyptus (*Eucalyptus globulus)* and Neem (*Azadirachta indica)* oils. The CNS depressant action was studied in the muscle relaxant activity by rota rod test. The albino rats were divided into 9 groups and were administered orally different ratios 1:1, 1:2, 1:3, 2:1 and 3:1. Control (normal saline was given orally); Standard (Diazepam), Eucalyptus and Neem oil. Results were expressed as mean ± SE. ANOVA followed Dunnet's multiple "t" test. P values < 0.05 are considered statistically significant, using software Graph Pad Prism 6. Eucalyptus and Neem oils were administered singly for 9hr produced muscle relaxant effect in experimental animals and the efficacy of the different combination of oil was found to be comparable to that of standard drugs used Diazepam.

## INTRODUCTION

It has been for more than 5000 years now that herbal extracts are being used for the treatment of diseases in the Indian subcontinent [1]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine [2].

The oil of the plant is used therapeutically as a tonic, purgative, emetic and expectorant [3]. It also possesses anti-inflammatory and analgesic actions. It is also used as spermicidal, in treatment of piles, hysteria, epilepsy and anti-implantation [4]. Skeletal muscle relaxants are used to treat muscle spasm and spasticity. Muscle spasm is defined as a sudden involuntary contraction of one or more muscle groups and is usually an acute condition associated with muscle strain (partial tear of a muscle) or sprain (partial or complete rupture of a ligament). Skeletal muscle relaxants consist of both antispasticity and antispasmodic agents, distinction prescribers often overlook [5]. The antispasticity agents-baclofen, tizanidine, dantrolene, and diazepam in improving muscle hypertonicity and involuntary jerks. Antispasmodic agents, such as cyclobenzaprine, are primarily used to treat musculoskeletal conditions [6].

Traditionally most of the people take its juice as a tonic to increase appetite and use it in fever or to remove intestinal worms. Eucalyptus and neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, respiratory disorders, constipation and skin infections. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. 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. [7].

## 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 (70-120g) in experiment. Animals are kept under standard conditions at 23-25°C 12hr 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 Diazepam

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

### Rota Rod Model:

Mice are used with a body weight (70-120g). 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 2 animals. Drugs are given by oral route by garage and the apparatus consists of a horizontal wooden rod or metal rod coated with rubber with 3 cm diameter attached and rod may rotate at 5 rpm for 5 min with the speed adjusted to 2 rotations per minute. The rod is at a height of about 50cm above the

animals from jumping off the roller. The rat is placed for 1min on the rotating rod. The number of animals falling from the roller during this time is counted with stopwatch. Moreover, testing at various time intervals, time response curves can be obtained then calculated the ANOVA [8].

**Effect of Volatile oils on Muscle relaxant test:**

Results of Muscle relaxant test of selected volatile oils are given in Table:1 and illustrated in Fig. The treatment with eucalyptus and neem oil showed decreased in fall of time was significant ($p<0.05$). Where eucalyptus oil treated animals showed significant ($p<0.001$) decreased in fall of time.

**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 providing 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 suspension test Model was performed during day time between 7 a.m. and 5 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 [9].


**Fig: 1 Rot-Rod Model**

**Table: 1 Synergistic Muscle relaxant activity of Eucalyptus and Neem oils on Rota Rod Model**

| Group | Fall of time (in sec) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30 min | 1hr | 2hr | 3hr | 4hr | 5hr | 6hr | 7hr | 8hr | 9hr |
| Control | 48±0 | 95±0.5 | 91.0±1.0 | 93.0±1.0 | 120.0±0.0 | 92.0±0.0 | 91.0±0.5 | 92.0±.05 | 92.0±1.0 | 95.0±1.5* |
| Standard | 40±1 | 90.0±1.0 | 81.0±1.5 | 86.0±0.5 | 76.0±0.5 | 73.0±0.5 | 71.0±0.5 | 67.0±1.0 | 69.0±1.0 | 69.0±0.0* |
| Eucalyptus | 113±0.0** | 168.0±1.5** | 151.0±1.25** | 153.0±1.5* | 158.0±0.25* | 149.0±0.0* | 145.0±0.0* | 142.0±1.5** | 147.0±0.5* | 149.0±0.0* |
| Neem | 141±0.0** | 149.0±1.0** | 155.0±2.0* | 159.0±0.5* | 155.0±0.5* | 154.0±2.0* | 151.0±1.0* | 156.0±1.0* | 151.0±0.0* | 155.0±0.5* |
| 1:1 | 108±1.5* | 101.0±2.0* | 87.0±1.0* | 91.0±0.5* | 85.0±1.0* | 83.5±1.0* | 81.0±0.0* | 83.5±0.0* | 82.0±0.0* | 84.5±0.5* |
| 1:2 | 90±1.0* | 146.5±2.0* | 123.0±1.0* | 115.0±1.0* | 111.5±0.0* | 108.0±1.5* | 106.0±0.0* | 107.0±0.5* | 107.0±0.5* | 108.0±1.0* |
| 1:3 | 62±1.0* | 117.5±2.0* | 98.0±0.5* | 93.0±0.5* | 82.5±0.0* | 83.5±0.5* | 74.0±0.0* | 73.0±0.5* | 74.5±0.5* | 77.5±1.5* |
| 2:1 | 82±0.0* | 101.0±2.0* | 103.0±0.5* | 199.0±0.5* | 97.0±0.0* | 95.0±0.5* | 85.5±0.5* | 84.0±0.0* | 90.0±1.0* | 90.5±2.5* |
| 3:1 | 94±0.5* | 102±1.5* | 86.0±1.5* | 81.0±0.5* | 79.0±0.0* | 76.0±0.0* | 76.0±2.0* | 73.0±1.0* | 76.0±1.0* | 78.0±1.0* |

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.

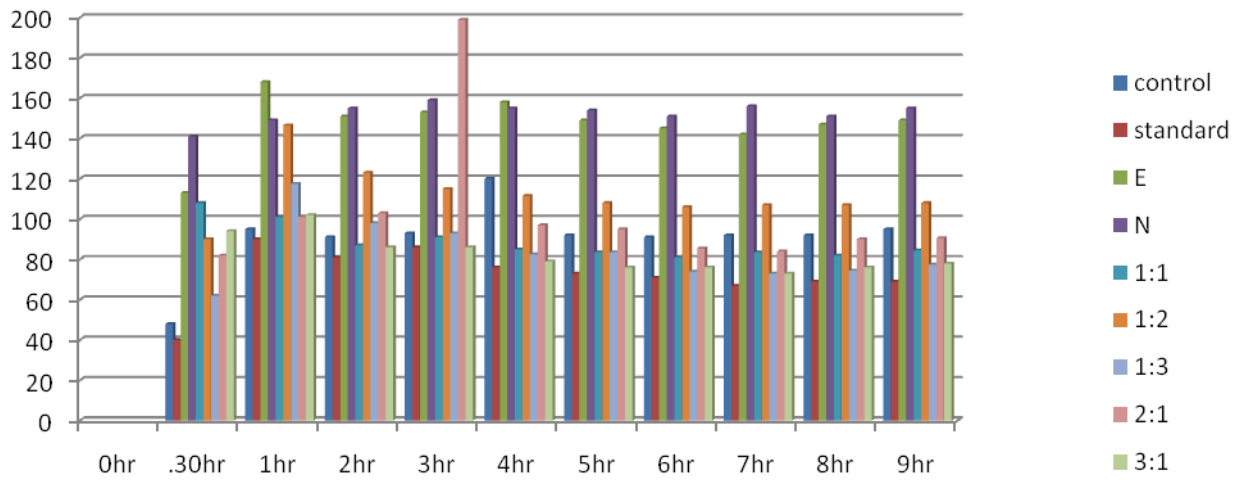


**Fig: 2 Fall of time (in sec)**

## STATISTICAL ANALYSIS

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

## RESULTS AND DISCUSSION

Results of Muscle relaxant test of selected volatile 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 Fall of time and the mixture of oils are given at dose of 100mg/kg body weight along with standard Diazepam given orally. It was found that eucalyptus and neem volatile oil at different ratio (1:1, 1:2, 1:3, 2:1 and 3:1) exhibited maximum activity after 2hr and significantly reduced Muscle relaxant even till 7hr than 9hr after drug admission as compared to control.

Muscle relaxant activity using Muscle relaxant in the present study showed that the eucalyptus and neem essential oil at different ratio have enough ability to control the Muscle relaxant might be due to various chemical constituents present in volatile oils. On comparison between different ratios, 1:3, 2:1, 1:3 ratios are most effective one and be suitable for further herbal formulation.

The muscle relaxant activities are evaluated using Muscle relaxant test. Duration of fall of time is taken as muscle Co-ordination activity and eucalyptus and neem essential oil decreased the fall

of time which indicates about their muscle Co-ordination activity. Moreover, Eucalyptus oil increased duration of tail flick in both tests.

## CONCLUSION

In muscle relaxant 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 ratio showed decreased in Fall of time showed more significant effect and the standard drug diazepam drug showed more significant effect in both the experiment (rota rod model) indicating the muscle relaxant activity. Testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants combination of eucalyptus and neem in 1:1, 1:2, 1:3, 2:1, 3:1 ratio also reduced the time spent on the revolving rod by animal in the rota rod test, a test mainly used to screen centrally acting muscle relaxants. This represented that eucalyptus and neem in 1:1, 1:2, 1:3, 2:1 and 3:1 ratio may show better muscle relaxant activity. The muscle relaxant 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 the brain.

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