

**SYNERGISTIC ANHELMINTIC ACTIVITY OF DIFFERENT COMPOSITION OF VOLATILE OIL OF EUCALYPTUS AND NEEM OIL BY *PHERETIMA POSTHUMA***Amrita Asthana<sup>1\*</sup>, Somendra Kumar Maurya<sup>2</sup>, Satya Prakash Maurya<sup>3</sup>, Anita Maurya<sup>4</sup> and Pooja Maurya<sup>5</sup><sup>1</sup>Department of Pharmacy, Assistant Professor, Prasad Institute of Technology, Jaunpur U.P 222001.<sup>2,4,5</sup>Department of Pharmacy, Assistant Professor, Prasad Institute of Technology, Jaunpur U.P 222001.<sup>3</sup>Satya Prakash Maurya Academic head of R.D.S College of Pharmacy, Jaunpur 222136.**\*Corresponding Author: Amrita Asthana**

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**ABSTRACT**

The research work deals with the screening of synergistic anthelmintic activity of different composition of Eucalyptus (*Eucalyptus globulus*) and Neem (*Azadirachta indica*) oils. The *Pheretima posthuma* were divided into 9 groups and were administered orally in different ratios of Eucalyptus and Neem 1:1, 1:2, 1:3, 2:1 and 3:1, Control (normal saline DMSO and Tween 80) & Standard (Albendazol) for 5 hrs and to investigate the anti-helmintic activity of eucalyptus and neem oils may used in 1:1, 1:2, 1:3, 2:1 and 3:1 ratios and dissolved in DMSO and Tween80 were investigated for activity on Indian earthworms (*Pheretima posthuma*). Both ratios show a dose dependant reduction in paralysis and death time of the worm, were found to be more effective. Results were expressed as mean  $\pm$  SE. ANOVA followed Dunnet's multiple "t" test. P values < 0.05 are considered statistically significant using software Graph Pad Prism 5 and found to produce paralysis than death time recorded in experimental animals and the efficacy of the different combination of oil was found to be comparable with that of standard drugs used Albendazol.

**KEYWORDS:** Anthelmintic activity, DMSO, Tween 80, Albendazol, *Pheretima posthuma*.**INTRODUCTION**

According to the World Health Organization infectious diseases are the main cause of death and the key agents of the afflicting worldwide.<sup>[1]</sup> Anti-helmintic drugs target the helminth parasitic worms (helminths) and expel them from the body, either by stunning or by killing them. Moreover as helminthes are increasingly becoming resistant to classical drugs<sup>[2]</sup>, there is an urgent need for search and development of new anti-helmintic agent, preferably with novel mode of action. Even the most common drugs like albendazol have been shown to have side effects like nausea, intestinal disturbance and giddiness and the high cost of modern anthelmintics has limited the effective control of these parasites. This leads to renewed interest in screening of medicinal plants for their anthelmintic activity.<sup>[3,4]</sup>

Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections. However, apart from these uses, there are several reports on the biological activities and pharmacological actions based on modern scientific investigations, such as antiviral, antibacterial, antifungal and Chaurasia, anti-inflammatory and antipyretic, antiseptic, antiparalitic,

antioxidant, etc. The present study has been undertaken to evaluate the in-vitro anthelmintic activity.<sup>[5-11]</sup>

**MATERIAL AND METHOD****Volatile oil and Drug**

Volatile oils of eucalyptus oil (*Eucalyptus globules*) and neem oil are used in this study. All the oils are collected by cleverger's apparatus and their assessable tests are carried out. Albendazol are purchased from Azamgarh. All the other solvents and chemicals used during experimental protocol are of analytical grade.

**Animals**

Indian earthworms (*Pheretima posthuma*) are obtained from Azamgarh and washed with normal saline to remove all the adhering matter. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width are used in all the experimental protocol.

**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 Albendazol

All the *Pheretima posthuma* are treated with normal saline than Eucalyptus and Neem oils are kept in for 30 min. after 1hr. to 5hr. of treatment the evaluation of activities is performed.

#### Anthelmintic activity

Earth worms (*Pheretima posthuma*) are collected Earth worms are thoroughly washed with normal saline to

remove the adhering material. Petridishes of equal size are collected and divided into control, test and standard. Eucalyptus and Neem oil, DMSO and tween 80 dissolved in emulsifying and placed in petridishes and divided into different ratios (1:1, 1:2, 1:3, 2:1 and 3:1) in control water DMSO and Tween 80 than standard drug Albendazol solution on petridishes, respectively. Then Eucalyptus and Neem are taken in 1:1, 1:2, 1:3, 2:1 and 3:1 ratios on petridishes, respectively. Placed earth worms of nearly equal size in each petridishes and time taken for the induction of paralysis (motion less) and complete death of earth worms is noted.<sup>[12]</sup>

#### Statistical Analysis

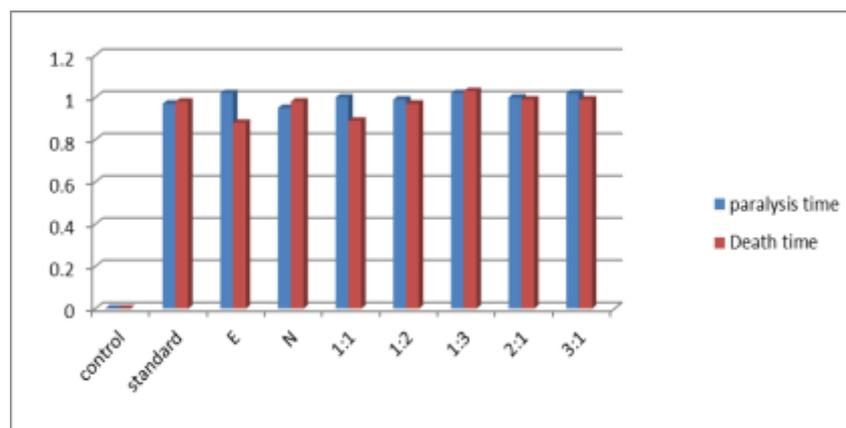
All the data are expressed as mean  $\pm$  S.E.M. (standard error of the mean). The significance level are determined using the Student's 't' test. A *p* value of  $<0.05$  are considered statistically significant.



Fig: 1.

Table 1: Synergistic Anthelmintic activity of Eucalyptus and Neem oils on *Pheretima posthuma*.

S.N.	Groups	Paralysis Time	Death Time
1.	Control	-	-
2.	Standard	0.98 $\pm$ 0.0	0.988 $\pm$ 1.0
3.	Eucalyptus	1.05 $\pm$ 0.0***	0.87 $\pm$ 2.0***
4.	Neem	0.97 $\pm$ 1.5****	0.97 $\pm$ 2.0****
5.	1:1	1.0 $\pm$ 0.0****	0.88 $\pm$ 2.5***
6.	1:2	0.99 $\pm$ 0.5****	0.98 $\pm$ 2.0****
7.	1:3	1.05 $\pm$ 0.0****	1.05 $\pm$ 1.5****
8.	2:1	1.0 $\pm$ 0.0****	0.99 $\pm$ 2.5****
9.	3:1	1.05 $\pm$ 1.5****	0.99 $\pm$ 2.0****



Anthelminthic Activity of *Pheretima Posthuma*

## RESULTS AND DISCUSSION

The results of the above studies demonstrated that, the Eucalyptus and Neem oil have showed dose dependent anthelmintic activity against earthworms tested in three different ratios. The Eucalyptus and Neem oil individual ingredients possess potent anthelmintic activity with varying magnitudes. But the Eucalyptus and Neem oil showed highest activity, which is almost equal in effectiveness to standard drug Albendazol. The difference in the time taken for induction of paralysis in both Albendazol and the Eucalyptus and Neem oil are insignificant or almost same. However, significant differences are observed when compared the induction of paralysis time of Albendazol. The mode of action for the Albendazol is generally by paralysing parasites, which allows the host body to easily remove or expel the invading organism (Table 1). The preliminary phytochemical observations of the Eucalyptus and Neem oil different test samples ratios such as 1:1, 1:2, 1:3, 2:1, 3:1 ratios of Eucalyptus and Neem oil ratios. Eucalyptus and Neem oil are a mixture all these phytoconstituents and interaction all these chemicals might be resulted in synergistically enhanced therapeutic efficacy of anthelmintic activity. However parasite survived up to (0.0±0.0) h in control. With Albendazol, both paralysis and time of mortality showed significantly longer time [(2.5±1.5) h and (3.5±2.0) h] compared to all three plant oil tested, at indivisible Eucalyptus and Neem oil and different ratios of eucalyptus and neem oil (1:1, 1:2, 1:3, 2:1, 3:1 ratios) consecutively.

## CONCLUSIONS

The anti-helminthic activity of eucalyptus and neem oil in different composition of eucalyptus and neem oil were comparable to standard Albendazol and could be more effective against parasitic helminths of humans and animals.

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