



In Vitro Evaluation of Anthelmintic Activity of Ethanolic Extract of Leaves of *Enhydra fluctuans*

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ABSTRACT: The aim of the present study was to in vitro evaluation of the anthelmintic activity of ethanolic extract of leaves of *Enhydra fluctuans* Linn using *Pheretima posthuma* and *Tubifex tubifex* as test worms. The time of paralysis and time of death were studied and the activity was compared with Albendazole as reference standard. The ethanolic of the leaves of *Enhydra fluctuans* exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time of test worms. The results thus support the use of *Enhydra fluctuans* as an anthelmintic agent, which have less side effect compare to synthetic drugs like albendazole.

KEYWORDS: Anthelmintic activity, *Pheretima posthuma*, albendazole, *Enhydra fluctuans*, *Tubifex tubifex*

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I. INTRODUCTION:

Helminth infections are the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a major threat to public health and contribute to the prevalence of nutrient deficiency disease, anemia, eosinophilia, and pneumonia. Anthelmintic are medicines which either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. They damage the host by depriving him of food, inflicting blood loss, injury to organs, enteric or humor obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity.¹

Enhydra fluctuans is a tree belonging to the family Asteraceae whose different parts are used as traditional medicine as analgesic, anti-inflammatory, diuretic, febrifuge, and hepatoprotective, antifungal and in gastrointestinal problems. Some locality of India, juice of helencha use as laxative and skin protective for cure of skin disease. In this study we investigate the anthelmintic activity of the ethanolic extract of the leaves of *Enhydra fluctuans* in comparison to standard drug Albendazole.²

The present study was undertaken to evaluate in vitro, the anthelmintic effect of ethanolic extract of leaves of *Enhydra fluctuans* on Indian earthworms (*Pheretima posthuma*) and Aquarium worms (*Tubifex tubifex*).

II. MATERIALS AND METHODS:

A. Plant materials

The present study was conducted in the Department of Pharmacology, Bharat Technology, Uluberia, Howrah, West Bengal, India. The plant was collected from the Field of Uluberia, Howrah, West Bengal, India and identified at the Botanical Survey of India, Shibpur, Howrah.

B. Preparation of plant extract³

The leaves of the plant were shade dried and powdered using a mixer grinder and ethanolic extract was obtained by the extraction procedure using soxhlet apparatus as described by Santanu Sannigrahi et al. The percentage yield was 28 % and the extract thus obtained was used for the anthelmintic study.

C. Experimental worms^{4,5}

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil of the village Uluberia, the average size of earthworm being 7-8 cm. They were washed with tap water for the removal of the adhering dirt. Aquarium worms *Tubifex tubifex* (Annelida) were collected from the local fish food market of Uluberia. The average sizes of the worms were 1-2 cm.

D. Evaluation of anthelmintic activity^{6, 7, 8}

Two type of experimental worms were divided into 3 groups of each type with six worms in each group. 1.5 % gum acacia was used as control (group 1), Albendazole 20 mg/ml as standard (group 2) and EEEF 60 mg/ml as test drugs in group 3 respectively. Standard and test drugs were dissolved in 1.5 % gum acacia and 10 ml of desired formulation was poured in separate Petridis and the two different type worms were placed in it one at a time. Time taken for paralysis and death of various worms were observed individually. Time for paralysis was noted when no movement of any sort by worms could be observed except when the worms were shaken vigorously with Petri dish. Time for death of individual worm was recorded when worms showed no movement even after vigorous shaking of Petridis or dipping in the warm water (55° C) followed by fading of their body colour.

E. Statistical analysis

Data were analysed using One way ANOVA followed Dunnett’s multiple comparisons test using InStat III trial. Result were expressed in mean+ SEM where P<0.05, P<0.01 will be considered as statistical significant.

III. RESULT

Table 1:-In vitro anthelmintic activity of EEEF on earthworms

Group	Drug	<i>Pheretima posthuma</i>		<i>Tubifex tubifex</i>	
		Time of paralysis(in min.)	Time of death(in min.)	Time of paralysis(in min.)	Time of death(in min.)
1	Control(1.5% gum acacia)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	Standard(Albendazole 20mg/ml)	11.58±0.49*	16.21±0.30**	11.03±0.47*	15.32±0.21**
3	EEEF (60mg/ml)	11.13±0.43**	16.02±0.27**	10.17±0.45*	15.07±0.17*

Values are mean± SEM (n=6), ##P<0.01 considered statistically significant as compare to normal control group; *P<0.05, ** p<0.01 considered statistically significant when compared to control group

Fig 1&2:-Anthelmintic activity of EEEF when compared to standard drug albendazole on time of paralysis and death of *Pheretima posthuma*.

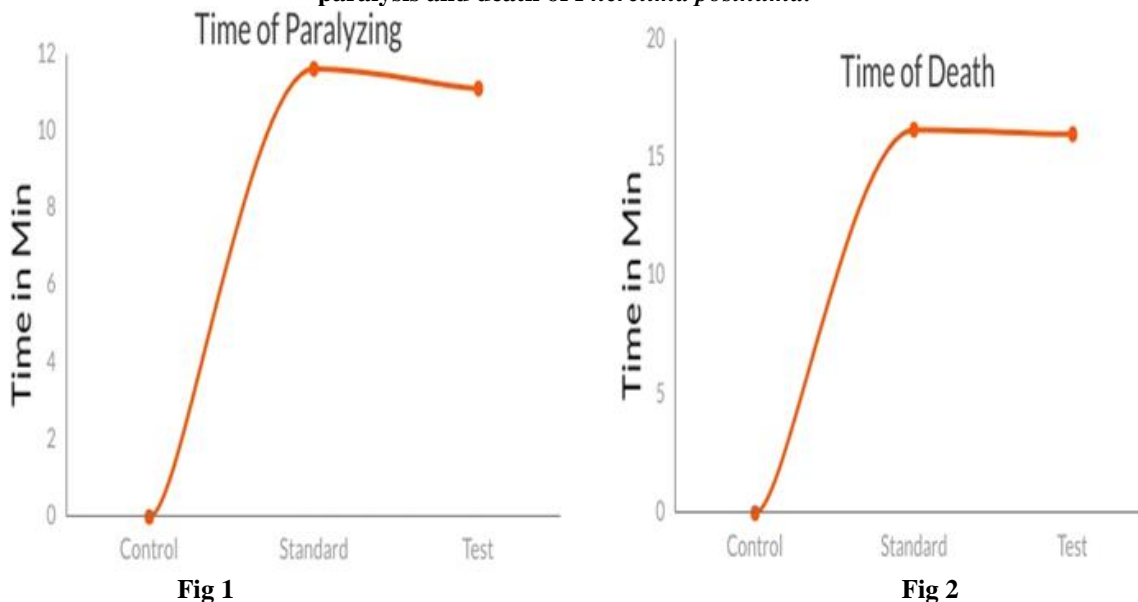


Fig 3&4:-Anthelmintic activity of EEEF when compared to standard drug albendazole on time of paralysis and death of *Tubifex tubifex*.

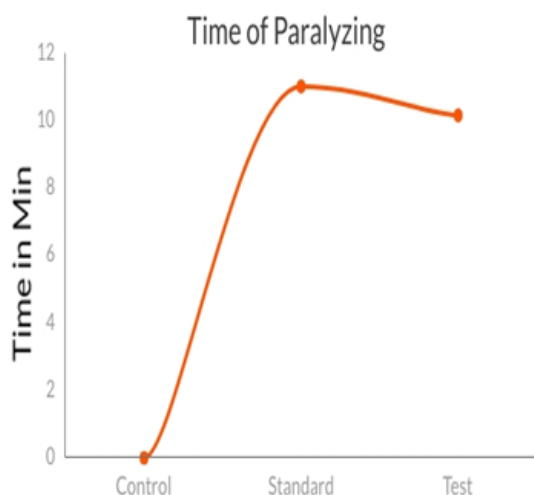


Fig 3

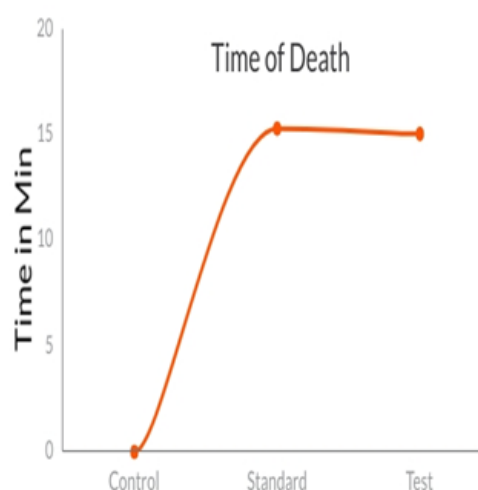


Fig 4

IV. DISCUSSION

From the observations made, paralytic effect much earlier and the time of death was observed. Although, extracts showed anthelmintic activity in a continuous manner but the ethanolic extract appeared to be few less effective for both types of worms. Evaluation of anthelmintic activity was compared with reference standard albendazole. The ethanolic extract of the leaves of *Enhydra fluctuans*, caused paralysis at 11.13 min and time of death at 16.02 min for *Pheretima posthuma* and 10.17 min as paralysis time and 15.07 min as death time for *Tubifex tubifex* worms respectively. The reference drug albendazole of earthworm *Pheretima posthuma* and worm *Tubifex tubifex* resulted in a paralysis time of 11.58 min respectively while time of death was 11.3 and 15.32 min, respectively. Control group don't showed any mortality of worms. Considering the ethanolic extract of leaves showed comparable activity, it would be important to identify the key phytoconstituents.

V. CONCLUSION

The present study enables us to conclude that ethanolic extract of leaves of *Enhydra fluctuans* (EEEEF) has significant anthelmintic activity but less potent than the standard drug, albendazole. There was also definite increased anthelmintic activity of EEEF at higher dose (60 mg/ml). Phytochemicals like tannins and polyphenols present in EEEF could be responsible for the anthelmintic activity. However further studies with higher doses are required to evaluate the dose dependent anthelmintic activity and also to determine lead compound to find out the exact mechanism responsible for anthelmintic activity.

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