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IN-VITRO ANTHELMINTIC ACTIVITY OF *ENICOSTEMMA LITTORALE* BLUME

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ABSTRACT

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on antihelmentic drug is poor. As per WHO, only few drugs are frequently used in the treatment of helminthes in human beings. Antihelmentic from the natural sources may play a key role in the treatment of these parasitic infections. In view of this petroleum ether (60-80º C) and ethanolic extracts of aerial parts of *Enicostemma littorale* Blume were evaluated separately for the activity on adult Indian earthworms, *Pheretima posthuma*, using albendazole as reference standards**.** Five concentrations (2.5, 5, 10, 25 & 50 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. The results indicated that the ethanolic extract was more potent than the petroleum ether extract.

INTRODUCTION: *Enicostemma littorale* Blume (Gentianaceae), also known as chota chirayata, is an erect and procumbent herb. Leaves are numerous, opposite, Flowers small, in axillary clusters. The plant is very bitter and is much used as a stomachic in Madras. In addition to its tonic properties it is also somewhat laxative. The plant is crushed and applied locally in snake-bite (Blatter). The plant is pungent and very bitter, anthelmintic, cures fevers and "vata" diseases (Ayurveda) [1]. Objective of the present study has to prove traditional anthelmintic use of this plant.

MATERIAL AND METHODS:

Plant Material: The plant *Enicostemma littorale* Blume was collected from Kanpur (U.P.), India. It was authenticated by the Dr. Pramod Khare, Department of Botany, Dr. Harsingh Gaur University, Sagar (M.P.) India.

Preparation of Extract: The aerial plant part was selected for the activity. The aerial part was allowed to get shade dried. The shade dried plant parts were powdered to get a coarse powder.

The powdered plant material was treated for successive extraction with petroleum ether (60^{o}-80^{o}C) and ethanol using Soxhlet apparatus.

The dried extract were suspended in 1% gum acacia in normal saline (vehicle) and used for antihelmentic activity.

Experimental Model: Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the antihelmentic activity. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [2, 3, 4]. Because of easy availability, earthworms have been widely used for the initial evaluation of antihelmentic compounds in vitro [5, 6, 7]. Thirteen groups of approximately equal size earthworms consisting of six earthworms in each group were used for the present study.

Standard Drug: Piperazine citrate and Albendazole are taken as standard drug and the concentration of the standard drug was prepared in 1% gum acacia in normal saline to give 15 mg/ml and 10 mg/ml respectively [8, 9, 10].

Antihelmentic Investigation: The antihelmentic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [11]. The method of Mathew *et al,* [12] and Dash *et al,* [13] was followed for antihelmentic screening. Thirteen groups, each consisting of six earthworms of approximately equal size were released in to 50 ml of desired formulation.

Each group was treated with one of the following: vehicle (1% gum acacia in normal saline), piperazine citrate (15 mg/ml), albendazole (10 mg/ml) or extracts (2.5, 5, 10, 25 or 50 ml) in normal saline containing 1% gum acacia. Observations were made for the time taken to paralyze and/or death of individual worms up to four hours of test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body color [14].

Statistical Analysis: All the data obtained was presented as Mean± SEM (**Table 1**) and were analyzed with student-t test.

RESULTS AND DISCUSSION: Preliminary phytochemical screening of petroleum ether extract revealed the presence of phenolic compounds, tannins, and steroids. On the other hand ethanolic extract showed presence of glycosides, alkaloids, phenolic compounds. It can be concluded from the **table 1**, the predominant effect of piperazine citrate on the worm is to cause a

flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper-polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.

The perusal of the data reveals that the petroleum ether extract did not show antihelmentic activity at a concentration of 2.5 mg/ml, whereas the ethanolic extract showed only paralysis but no mortality at similar concentration. The other test concentrations of both the extracts showed marked degree of antihelmentic activity. The antihelmentic effect of petroleum ether extract at 50 mg/ml concentration is comparable with that of the effect produced by the reference standards albendazole and piperazine citrate. However, the ethanolic extract showed the effect beyond 25 mg/ml concentration that is comparable with the reference standards.

The present study, therefore reveals that the ethanolic extract was more potent than the petroleum ether extract, even though both the extracts were endowed with antihelmentic property. The activity reveals concentration dependent nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/ death of the worms.

Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show antihelmentic activity [15]. Some synthetic phenolic antihelmentics e.g., niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [16]. It is possible that phenolic content in the extract of *Enicostemma littorale* Blume produced similar effects.

The above findings justify the antihelmentic properties of the aerial parts which augments its use in the Ayurveda. Further studies regarding the isolation and characterization of the active principle responsible for antihelmentic activity are currently under process.

TABLE 1: *IN-VITRO* ANTHELMINTIC ACTIVITY OF *ENICOSTEMMA LITTORALE* BLUME EXTRACTS

Treatment	Time taken for paralysis (min)	Time taken for death (min)
Vehicle	-	-
Albendazole (10 mg/ml)	32.66±0.72	62.13±0.72
Pet. Ether extract		
2.5 mg/ml	-	-
5.0 mg/ml	119.50±2.10	158.4±3.42
10 mg/ml	69.42±0.75	116.17±0.81
25 mg/ml	33.66±0.52	54.83±0.54
50 mg/ml	28.0±0.20	45.62±0.51
Ethanolic extract		
2.5 mg/ml	136.44±2.32	-
5.0 mg/ml	94.83±2.0	162.62±4.02
10 mg/ml	49.33±0.32	86.72±0.06
25 mg/ml	17.51±0.23	43.66±0.22
50 mg/ml	8.33±0.42	17.12±0.21

Results expressed as Mean± SEM from six observations

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