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Original Article

IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *PHYLLANTHUS FRATERNUS*WEB. IN ADULT EARTHWORMS

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ABSTRACT

Objective: The present study aimed to evaluate the anthelmintic activity of ethanolic extract of aerial part of *Phyllanthus fraternus* Web. leaves (EEPF) using adult earthworms (*Pheretimaposthuma*).

Methods: Adult earthworms (24in number) were divided into 4 groups with 6 worms in each group (n=6). The anthelmintic activity of EEPF at two different doses (25 mg/ml and 50 mg/ml) were evaluated by assessing the time of paralysis (min) and time of death (min) of the earthworms. Albendazole (25 mg/ml) was used as standard and 2% gum acacia as control.

Results: Data were analyzed statistically by ANOVA followed by Bonferroni test. P<0.05 was considered as significant. The result showed that EEPF had significant anthelmintic activity (p<0.001) but less potent than the standard drug albendazole.

Conclusion: EEPF demonstrated significant anthelmintic activity but less potent than the standard drug albendazole. However, further studies with higher doses are required to evaluate the dose-dependent activity and to evaluate the exact mechanism responsible for anthelmintic activity.

Keywords: Helminthiasis, *Phyllanthus fraternus* Web, *Pheretimaposthuma*, Albendazole, Anthelmintic

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INTRODUCTION

Intestinal parasitic nematode infect approximately two billion people worldwide and is considered the most common and persistent parasitic infection in humans, especially in developing countries affecting the poorest and most deprived communities [1]. It is the infestation with one of the parasitic intestinal worms that include the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). About 1.45 billion people are estimated to be infected with soil-transmitted helminthiasis worldwide, with children being the most affected group [2]. It causes detrimental effects on human growth and development affecting nutrition, cognition, school performance, work productivity, immune system and even pregnancy, severely impairing the quality of life [3, 4]. In addition it causesannual economic losses of billions of dollars worldwide by parasitic nematodes infection in livestock [5].

Anthelmintics have revolutionized the parasitic nematode infection in men providing global improvement in health and economic development. Commonly used anthelmintics are benzimidazoles (Albendazole), imidazothiazoles (levamisole), tetrahydropyrimidines, macrocyclic lactones (ivermectin) which are synthetic. Resistance to anthelmintics is a complex problem seen in livestock. The rate in which resistance develops is often within a few years of the introduction of the anthelmintic [6].

Wide variety of secondary metabolites encountered in plantssuch as alkaloids, flavonoids, chalcones, coumarins and terpenoids and tannins showed potential anthelmintic properties [7]. These properties can be harnessed and plants can be used to overcome the resistance of anthelmintics and its toxicity. The present study was to evaluate the anthelmintic activity of ethanolic extract of aerial parts of *Phyllanthus fraternus* Web. (EEPF) on adult Indian earthworms (*Pheretimaposthuma*).

MATERIALS AND METHODS

Collection of plants

The plant *Phyllanthus fraternus* web was collected from the fields in and around Imphal area was authenticated by Professor P. K. Singh, department of Life Sciences, Manipur University, Imphal, Manipur with Voucher no. 000874.

Preparation of plant extraction

Shade-dried arial parts of the plant was coarsely powdered with a mixer grinder. The ethanolic extract of the plant was prepared by Soxhlet extraction method. Prior to the extraction with ethanol, defat was done by using petroleum ether. The yield of the extract was about 10% and was used for the anthelmintic study.

Worm collection and authentication

Due to the resemblance of intestinal roundworm and adult Indian earthworms in anatomical and physiological features, adult earthworm was used for the study [8, 9]. The worms were collected from moist soil and were authenticated by Department of Life sciences, Manipur University. Worms of about 5-7 cm in length and 4–5 mm in diameter were washed to remove dirt with normal saline (0.9%) prior to the study.

Albendazole (Glaxo Smithkline Pvt. Ltd) was used for the study.

Phytochemical analysis

Using the standard methods [10], various components such as alkaloids, flavonoids, chalcones, coumarins and terpenoids and tannins were evaluated by preliminary phytochemical analysis.

Experimental design

Earthworms were divided into 4 groups, with 6 worms in each group as mentioned in table 1.

Table 1: **Different groups of earthworms and their treatment**

Groups	Drugs given
Group 1 (Control)	2 % gum acacia solution
Group 2 (Standard)	Albendazole: 25 mg/ml
Group 3 (Test Dose-A)	EEPF: 25 mg/ml
Group 4 (Test Dose-B)	EEPF: 50 mg/ml

Standard and test drugs were dissolved in 2 % gum acacia solution and 10 ml of desired formulation was poured in separate petri dish and the worms were placed in it one at a time.

Evaluation of anthelmintic activity

- Time of paralysis and time of death (in a minute) of worms were recorded individually.
- *Time of paralysis* was noted when any sort of movement could not be observed except when the worms were shaken vigorously.
- *Time of death* of individual worm was recorded when worms showed no movement even after vigorous shaking and then dipping in the warm water (50 °C) followed by fading of their body.

Statistical analysis

One-way ANOVA followed by Bonferroni test was used to analyse the data. Results were expressed in mean±SD. p value<0.05 was considered significant.

Table 2: **Anthelmintic activity of EEPF on Indian earthworm (*in vitro*)**

Group	Drug	Time of paralysis (min)	Time of death (min)
1	Control(2% gum acacia)	--	--
2	Standard (Albendazole: 25 mg/ml)	8.17±1.169*	11.83±.753*
3	EEPF (25 mg/ml)	24.50±1.049 *#	31.17±0.983 *#
4	EEPF (50 mg/ml)	13.83±0.753 *#	16.83±0.753 *#

Results were expressed in mean±SD. n=6. *p value<0.001 when compared to control, #p<0.05 when compared with standard to test groups. Data were statistically analysed by one-way ANOVA followed by Bonferroni test. P<0.05 was considered significant.

RESULTS

The standard drug albendazole at 25 mg/ml showed its best activity for time of paralysis and time of death at 8.17±1.169 and 11.83±0.753 min, respectively. The ethanolic extract of the plant *Phyllanthus fraternus* web. Demonstrated the paralysis as well death of the worms. Time of paralysis and death in EEPF at 25 mg/ml were 24.50±1.049 and 31.17±0.983 min, respectively, while EEPF at 50 mg/ml were 13.83±0.753 and 16.83±0.753 min, respectively (table 2). Both the doses of ethanolic extract of the plant showed significant anthelmintic activity as compared to control group. But compared to the standard drug, the time of paralysis and death is more in ethanolic extract of plant. The EEPF at higher doses (50 mg/ml), however showed less time of paralysis and death than the EEPF at a lower dose (25 mg/ml) as given in fig. 1.

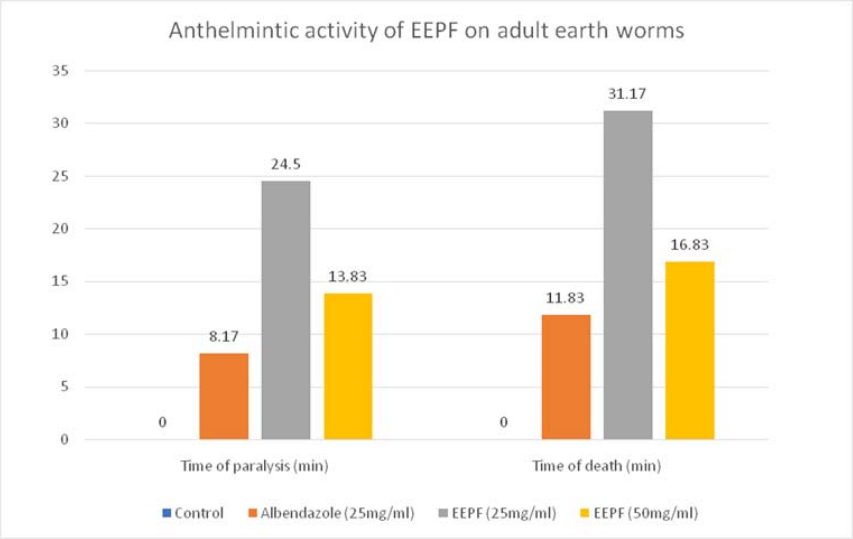

Fig. 1: Anthelmintic activity of EEPF (Time of paralysis and Time of death) when compared to standard drug albendazole

DISCUSSION

The conventional dose of albendazole is effective against nematodes; the ethanolic extract of *Phyllanthus fraternus* Web. also showed almost similar reaction at the higher concentration (50 mg/ml). Anthelmintic plants offer a traditional alternative to manufactured anthelmintics that is both sustainable and environmentally acceptable. The problem of anthelmintic resistance and toxicityhas led to a renewal of interest in the use of plant-based drugs. The presence of phytochemicals like tannins, saponins and alkaloids in EEPF as detected by preliminary phytochemical analysis, could be responsible for the anthelmintic activity**.** Natural compounds from plants can provide a unique opportunity in the search for new, effective and safe anthelmintics [11]. Though there is limited systematic, scientific evaluation of efficacy, mode of action and identity of the active component(s) of plants as anthelmintic, these natural medicines may be acting on pathways in worms that differ from targets of currently used anthelmintic drugs [12]. The phytochemicals present in plants can bring about changes in permeability of the membrane of the parasite, uncoupling of oxidative phosphorylation and thus interfere with energy generation in helminthes [13]. The selective toxicity of albendazole and its resistance against helminths could be outdone, if alternative plants derivatives could be used as anthelmintics. This can be the future prospect of new drug development from plant sources. Similar study was done by Datta S *et al.* by using the same standard drug Albendazole and extract of *Centella asiatica* linn. showed results which are comparable with the present study [14].

CONCLUSION

The study demonstrated that EEPF had significant anthelmintic activity but less potent than the standard drug albendazole. Plants with anthelmintic property can be a source of safe alternative to

standard drugs and remedy from plant origin an advancement in the treatment of helminth infections. However, further studies are required to evaluate the dose-dependent activity and to evaluate the exact mechanism responsible for anthelmintic activity.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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