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Original Article

Pharmacology Section

The Anthelmintic Activity of *Eupatorium triplinerve* and *Alpinia galanga* in *Pheritima posthuma* and *Ascardia galli:* A Comparative Study

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ABSTRACT

Aim: The ethanolic extracts from the leaves of *Eupatorium triplinerve* and the rhizome of *Alpinia galanga* were compared for their anthelmintic activities, based on traditional claims.

Methods: *Pheritima posthuma* and *Ascardia galli* were used as the suitable in vitro models; the time which was taken for paralysis (P) and death (D) were used as the parameters to assess their anthelmintic activities, with Albendazole 50mg/ml as the standard.

Results: *Eupatorium triplinerve* exhibited a dose dependent anthelmintic activity in both the models at the concentrations of 50mg/ml and 100mg/ml, thus indicating a broad spectrum of action, whereas *Alpinia galanga,* as compared to *Eupatorium triplinerve,* failed to show any activity against *Pheritima posthuma,* but exhibited a potent activity in a dose dependent manner which was comparable to that of the standard, albendazole 23.00 $\pm$ 2.60(P), 63.33 $\pm$ 2.33(D) at a 100mg/ml concentration against *Ascardia galli* at minutes 32.83 $\pm$ 4.07(P) and 69.33 $\pm$ 3.93.

Key Words: Anthelmintic, *Eupotorium triplinerve, Alpinia galanga, Ascardia galli* and *Pheritima posthuma*

INTRODUCTION

The medical symbol which is used worldwide, denotes even today to the modern world, about the success of the first treatable parasitic infection which is known to the human race (*Dracunculus medinensis*) [1]. Helminthiasis is mostly seen in the children of the tropical inhabitants of a low socioeconomic status. The available drugs like Albendazole, though they are capable of a broad spectrum action against the intraluminal parasites and the tissue parasites, have limitations for use in pregnancy and in children who are younger than 2 years of age [2]. Because of the high prevalence rate of helminthiasis since ages, traditional and folklore medicines for it have been in use. The medicinal properties of the leaves of *Eupatorium triplinerve* [3] and the rhizome of *Alpinia galanga* are utilized to treat various ailments which include helminthiasis [4]. With available literature on the traditional claims on the anthelmintic activity of the two medicinal plants, this study was an attempt to prove or disprove the traditional claims which were made by well established, in vitro, scientific methods of assessing the anthelmintic activity. If the claims were proved then the anthelmintic activities of the ethanolic extracts from the rhizome of *Alpinia galanga* and the leaves of *Eupatorium triplinerve* were compared.

MATERIALS AND METHODS

The methodology which was adopted to evaluate the anthelmintic activities of the two plants viz *Eupatorium triplinerve* and *Alpinia galanga* is as follows:

Plant Material

The leaves of *Eupatorium triplinerve and* the rhizome of *Alpinia galanga* were collected from the local areas of Kollam (Kerala, India) and Coimbatore (Tamil nadu, India) respectively. The collected material was authenticated by the Tropical Botanical Garden and Research Institute, (TBGRI), Trivandrum, (Collection No:31691, 31692, Account No: 20391,20392) India by Dr Sasikala Ethirajulu, Assistant Director (Pharmacognosy), Siddha Central Research Institute, Chennai, India.

Worm Collection and Authentication

The Indian earthworm, *Pheritima posthuma* was collected from the ponds and the marshlands of Kanchipuram, Tamilnadu, India. *Ascardia galli* were obtained from the Government veterinary Hospital and Research Institute, Kanchipuram, India. The earth worm and the round worm were authenticated by Dr Sudarsanam, Veterinary Microbiologist and Chief Veterinary officer, Department of Pharmacology, Meenakshi Medical College and Research Institute, MAHER (Meenakshi University) Kanchipuram, India.

Extract Preparation

The collected leaves and the rhizome materials were thoroughly washed under running water, shade dried for a week at 35-40°C, pulverized in an electric grinder and exhaustively extracted successively in a Soxhlet apparatus by using the solvent, ethanol. The extracts were concentrated under reduced pressure and were then powdered.

Sl. No.	Plant	Parts Used	Extract (Solvent)	Worms Used
1.	*Eupatorium triplinerve*	Dried Leaves	Ethanolic	*Pheritima posthuma Ascardia galli*
2.	*Alpinia galanga*	Dried Rhizome	Ethanolic	*Pheritima posthuma Ascardia galli*

[Table/Fig-1]: Materials Used

Anthelmintic Activity

The use of the *Ascaridia galli* species as a suitable model for the screening of anthelmintic drugs had been advocated earlier [5,6]. As the Indian earthworm, *Pheritima posthuma* has an anatomical and physiological resemblance to the intestinal roundworm parasite of human beings, it was used for the initial evaluation of anthelmintic compounds in vitro [7,8]. The anthelmintic activities of *Eupatorium triplinerve* and *Alpinia galanga* against *Pheretima posthuma and Ascardia galli* were investigated at the concentrations of 50 and 100 mg/ml. The anthelmintic activities of both the medicinal plants were compared with Albendazole as the standard reference and with normal saline as the control. They were tested by a bioassay, which involved the determination of the time of paralysis (P) and time of death (D) of the worms. [9,10]. All the worms of the two different species, *Pheretima posthuma and Ascaridia galli* were washed with normal saline to remove all the faecal matter and they were randomly selected for the anthelmintic study. The earthworms which were 5-8 cm in length and 0.1-0.2 cm in width were used for all the experimental protocols.

Experimental Design

Six worms were released into 50 ml of the solutions (which were reconstituted with sterile water) of Albendazole (40mg/ml), the ethanol extracts of the leaves of *Eupatorium triplinerve,* the ethanol extracts of the rhizome of *Alpinia galanga* (25, 50 and 100 mg/mL) and normal saline (100mg/ml). Similar experiments were done for the *Ascardia galli* species also. The end point of the study was the time which was taken for the paralysis and/or the death of the worms. The worms are randomly divided into 8 groups (with six worms in each group).

Group – 1 Albendazole 50 mg/ml in 50ml solution
Group – 2 *Alpinia galanga* 50 mg/ml in 50ml solution
Group – 3 *Alpinia galanga* 100 mg/ml in 50ml solution
Group – 4 *Eupatorium triplinerve* 50 mg/ml in 50ml solution
Group – 5 *Eupatorium triplinerve* 100 mg/ml in 50ml solution
Group – 6 Normal saline 100 mg/ml in 50ml solution

Assessment of the Anthelmintic Activities

The time which was taken for the death of the worms was observed by two different persons and it was recorded after ascertaining that the worms neither moved when they were shaken vigorously nor when they were dipped in warm water (at approximately 50^{0}C). The treatment with normal saline served as the control. The experiments were carried out in triplicates to avoid an observational bias and to minimize other sources of errors. Paralysis was said to occur when the worms do not receive even in normal saline. Death was said to occur when the worms lost their motility, followed by the fading away of their body colour.

This study was conducted in the Experimental Pharmacology Lab, Department of Pharmacology, Meenakshi Medical College and Research Institute during May 2012.

Statistical Analysis

The data were analyzed by using the one way analysis of variance (Anova) with the Graphpad instat demo version and a p value of < 0.05 was considered as statistically significant. The mean and the standard deviation were calculated for each parameter in each group. All the experiments were done in triplicates by three different observers to avoid observational bias and any sources of error.

RESULTS

The Anthelmintic Activity against *Pheretima posthuma*

The ethanolic leaf extract of *Eupatorium triplinerve,* at the concentrations of 50mg/ml and 100mg mg/ml, produced an anthelmintic activity in a dose dependent manner [54.83min (P-paralysis), 129.83min (D-death) and 50.66 min (P), 152.33 (D) respectively] [Table/Fig-1]. At the dose of 100 mg/ml concentration giving shortest time of paralysis (P) and death (D) which is significant with results of control group and comparable with the Albendazole treatment group 41.83min (P) and 83 min(D). The ethanolic rhizome extract of *Alpinia galanga* failed to cause both paralysis and death in both the 50mg as well as the 100mg concentrations.

The Anthelmintic Activity against *Ascaridia galli*

The anthelmintic activity of *Alpinia galanga* against *Ascardia galli* was 33.83min (P), 86.16min (D) in 50mg/ml Conc. and 32.83min (P) 69.33min (D) in the 100mg/ml conc., which was much better and almost comparable to that of the albendazole treatment group [23.00min (P) 63.33min (D)]. *Eupatorium triplinerve* exhibited paralysis by 54.33min and 39.00min in the 50mg/ml and the 100mg/ml concentrations respectively. The death of the worms was observed at 150.50min and 119.83 min in the 50mg/ml and the 100mg/ml conc. respectively.

Group	Conc. (mg/ml)	Time taken for paralysis (min)	Time taken for death of worms (min)
Albendazole	50	41.83 ± 3.54^x	83 ± 6.38^x
Eupatorium triplinerve	50	54.83 ± 4.70^x	129.83 ± 8.40^x
	100	50.66± 4.59^x	152.33 ± 5.16^x
Alpinia galanga	50	>180	>180^{NS}
	100	>180	>180^{NS}
Distilled Water	–	>180	>180

[Table/Fig-2]: Anthelmintic activity of *Eupatorium triplinerve* and *Alpinia galanga* against Pheretima posthuma
± SD, > More than, ^{x}- P < 0.001, NS- Not Significant.

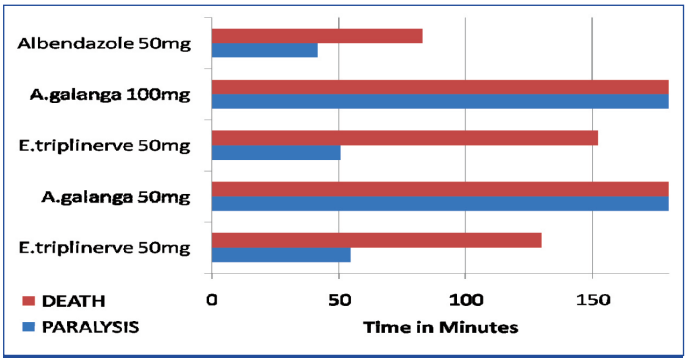
Group	Conc. (mg/ml)	Time taken for paralysis (min)	Time taken for death of worms (min)
Albendazole	50	23.00 ± 2.60	63.33 ± 2.33
Eupatorium triplinerve	50	54.33 ± 3.32^x	150.50 ± 3.83
	100	39.00 ± 2.19^x	119.83 ± 3.81^x
Alpinia galanga	50	33.83 ± 3.18^x	86.16 ± 3.65^x
	100	32.83 ± 4.07^{xx}	69.33 ± 3.93^{xx}
Normal saline	9	>180	>180

[Table/Fig-3]: Anthelmintic activity of *Eupatorium triplinerve* and *Alpinia galanga* against Ascardia galli
± SD, > More than, ^{x} P < 0.005, ^{xx} P < 0.001.

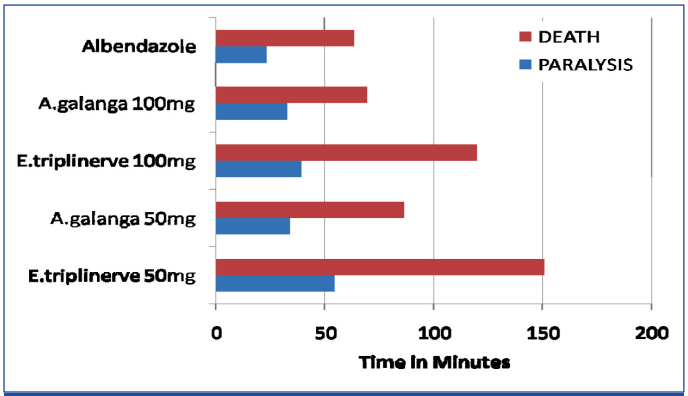
DISCUSSION

Most of the anthelmintic drugs act by either killing or expelling the infesting helminths without harming the host. Albendazole, the congener of mebendazole, acts by increasing the chloride ion conductance of the worm muscle membrane, leading to hyperpolarization, thus causing a flaccid paralysis that results in the expulsion of the worm by peristalsis of the host gastrointestinal tract [11].

[Table/Fig-4]: Specimens



[Table/Fig-5]: Anthelmintic activity of *Eupatorium triplinerve* and *Alpinia galanga* against Pheretima posthuma



[Table/Fig-6]: Anthelmintic activity of *Eupatorium Triplinerve* and *Alpinia galanga* against Ascardia galli

The standard Albendazole solution showed an enhanced anthelmintic activity. Among the tested medicinal plants, *Eupatorium triplinerve* had a significant antihelmintic activity on both the models, whereas *Alpinia galanga* exhibited an antihelminthic activity specifically only on *Ascardia galli*. Similar results had been observed by Kakeysa Raj et al in 1975 [12]. The rhizome of *Alpinia galanga* contains flavanoids such as kaemperol, kaempferide, galangin and alpinin. These flavanoids have multiple biological activities that may be responsible for its anthelmintic activity [13].

The phytochemical analysis of the crude extracts of E.triplinerve revealed the presence of Coumarins and phenolic compounds as the chemical constituents, whereas the phytochemical constituent analysis of *Alpinia galanga* revealed the absence of phenolic compounds. Phenolic compounds which have anthelmintic activities have been studied [14]. Similarly, the anthelmintic drugs like oxyclozanide, niclosamide and bithionol belong to the group of synthetic phenols which have been proved to interfere with the energy generation in the helminth parasites [15]. Similarly, it was possible that the phenolic contents in the extracts of *Eupatorium. Triplinerve* had produced similar and better effects than those in *Alpinia galanga*. The anthelmintic activity of *Alpinia galanga* on *Ascardia galli* was proven as a specific activity, whereas *Eupatorium triplinerve* was found to have a broad spectrum anthelmintic activity when it was used on lubricoides.

CONCLUSION

In conclusion, the traditional use of the leaves of *Eupatorium Triplinerve* and *Alpinia galanga* as anthelmintics has been confirmed. In comparison, the leaf extracts of *Eupatorium triplinerve* displayed an antihelminthic activity against both the worms which were used in the study as compared to the standard, albendazole. Extensive in vivo research is needed to determine the individual components which are responsible for the anthelmintic activities of these plant extracts and the molecular mechanisms which are responsible for the same.

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