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Short Communication

Evaluation of anti-helminthic activity of ethanolic extract of *withania coagulans* dunal (flower buds)

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Abstract

Withania coagulans is a short shrub with central stem. It has milk coagulating property and various parts of this plant were traditionally used as Emetic, Diuretic and Anti diabetic agent. It is also used in chronic liver failure. Berries of this plant contain amino acids, fatty and essential oil and alkaloids and also show anthelmintic activity. Hence the flower Buds of this plant were examined for anti-helminthic activity using earthworms (Pheretima posthuma). Numerous concentrations (25mgml^{-1}, 50mgml^{-1}, 75mgml^{-1} and 100mgml^{-1}) of ethanolic extract of Withania coagulans (EEWC) were studied. Albendazole (20mgml^{-1}) was used as standard drug for reference whereas normal saline (NS) as control. The time of paralysis and death of worms were determined and recorded. Extract exhibit significant anti-helminthic activity at the concentration of 75 and 100mgml^{-1}. The result shows that ethanolic extract of Withania coagulans possesses vermicidal activity against earthworms (Pheretima posthuma) and determined to be effective as anti-helminthics. Therefore, the anti-helminthic activity of the ethanolic extract of Withania coagulans dunal (Flower Buds) has been reported.

Keywords: *Withania coagulans*, vermicidal, anthelmintic, *pheretima posthuma*.

Introduction

Helminthic infections are called as neglected tropical diseases (NTDs). It is also a major cause of morbidity and mortality[1]. Eradication of helminthiasis is very difficult due to torrid, impoverishment, dearth of education, insufficient sanitary facilities and impure water supply. In humans there are some species that infect people predominantly are the whipworm (Trichuris trichiura), the roundworm (Ascaris lumbricoides) and the hookworms (Ancylostoma duodenale and Necator americanus)[2].

Wide range of symptoms are produced by the intestinal worms are that intestinal manifestations such as diarrhoea, abdominal pain, general malaise and weakness. Hookworms produce chronic intestinal blood loss, which lead to the anaemia[2]. Round worms have been implicated in the pathogenisis of bronchial spasm in endemic areas. Peripheral blood eosinophilia occurs in all nematode infestations (except enterobiasis). A careful examination of stool may often spare the unnecessary removal of teeth and tonsils, usually blamed for 'spectic foci'. In cases with 'resistant urticaria'.

The helminths are parasites. Clinically it is classified based on their general shape and the host organ they inhabit. They possess bisexual characters and also hermaphroditic in nature. Helminths are classified as Fluke worms (Trematodes), Roundworms (Nematodes) and Tapeworms (Cestodes) based on the external and internal morphology of egg, larval, and adult stages[3].

Anthelmintics are drug used in the treatment of helminthiasis. The anthelmintic drug which kills the worm is called vermicidal, while that which affects the worm in such a way that it is easily expelled is known as vermifuge[4,5].

The helminthiasis is tremendously widespread notably in third world countries due to substandard control measures. However, development of resistance in helminthes against anti-helminthics has led to finding out the natural plants for their anti-helminthic activity. The natural plants are rich source of botanical anti-helminthics[4,6].

Drugs which derived from the plants serve as a prototype to develop more effective and less toxic medicines. *Withania coagulans* is short shrub with central stem, lanceolate-oblong leaves; flowers are dioecious, in axillary clusters. It has milk coagulating property. It is traditionally used as Emetic, Diuretics and Anti Diabetic agent, Riped fruits of this plant contains sedative, CNS depressant and anti-inflammatory activity. It is also employed in the management of chronic liver trouble. Dried fruits had certain therapeutic activity such as carminative, dyspepsia and flatulence. Leaves of this plant were used as alterative and febrifuge[7-9]. Hence the objective of this work was to evaluate the potency of *Withania coagulans* dunal (flower buds) in the management of helminthiasis.

Materials and methods

Plant material: The whole plant of *Withania coagulans* was purchased from chennai, Tamil Nadu, India. It was recognized and certified by Prof. Dr. V. Nandagopalan, Dean of Science and Department Head, Botany, National College of Arts and Science (Autonomous), Trichy, Tamil Nadu, India.

Preparation of plant extract: The Flower buds of *Withania coagulans* were collected then dried in the absence of direct sunlight and coarsely powdered. About 500g of the dry powder was defatted with petroleum ether and extracted constantly in soxhlet extractor with ethanol for 3 days (72hrs), after 3 days, the ethanol was vaporized to get the unrefined extract then it was allowed to dry under vacuum[10,11].

Experimental worms: An adult indian earthworms (*Pheretima posthuma*) were used in this experiment because of its analogy to the intestinal roundworm parasites which is present in human beings. The worms were collected from dewy soil and all fecal matters were removed by dousing with water.

Anthelmintic activity: Six groups of nearly same size of worms were used. Each group contains six earthworms which were left in to the petridish consists of desired dose of standard drug and extracts. First group was control group added with NS (10ml). Second Group was standard group added with the albendazole (20mgml^{-1}). Group 3rd, 4th, 5th, 6th were test group added with ethanolic extract of *Withania coagulans* in various concentrations (25, 50, 75, 100mgml^{-1})[12-17].

Preparation of albendazole: Normal saline was used as a vehicle to prepare Albendazole (20mgml^{-1}).

Preparation of extract: The suspension of Ethanolic extract of *Withania coagulans* at numerous concentration (25mgml^{-1}, 50 mgml^{-1}, 75mgml^{-1} and 100mgml^{-1}) were prepared by using normal saline as vehicle up to 10ml.

Experimental design: An adult Indian earthworms were laid in petridish having numerous doses (25mgml^{-1}, 50mgml^{-1}, 75mgml^{-1} and 100mgml^{-1}) of Ethanolic extract of *Withania coagulans*, Albendazole 20mgml^{-1} and Normal saline (10ml). Six worms were laid into the each petridish and detected for immobility or fatality. Mean time for the paralysis of worms were recorded when there is no movement of worms, not including when the worm get strenuous shaking; the fatality of worm (min) was noted when that worms neither moved during shaking the petridish nor when the external stimuli was given. The study results were equated with standard drug Albendazole (20mgml^{-1}) treated samples. The time period of study is 3 hours.

Statistical analysis: The results are expressed as Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** P<0.001, ** P<0.01 and * P<0.05 were considered as statistically significant[15,16].

Results and discussion

The data reveals that of Ethanolic extract of *Withania coagulans* showed paralysis and death at various concentrations (25mgml^{-1}, 50mgml^{-1}, 75mgml^{-1} and 100mgml^{-1}). The effects produced by the EEWC were comparable with that of the effects exhibited by the albendazole (20mgml^{-1}) which is used as reference standard. This study shows that the nature of extract at various concentration. The anti-helminthic potency of the extracts was determined to be inversely proportional to the time taken for paralysis or death of the worms.

Table-1: Anti-helminthic activity of ethanolic extract of *Withania coagulans* against *Pheretima posthuma.*

Group	Dose	Pheretima posthuma	
		Paralysis (Min)	Death (min)
Control (1% CMC in 10ml of Normal Saline)	10 ml of 5% CMC	180.40 ± 0.24	244.50 ± 0.22
Standard (Albendazole + 1% CMC in 10ml of Normal Saline)	20 mgml^{-1}	8.00 ± 0.25***	22.66 ± 0.16***
Test (Ethanolic extract of Withania coagulans + 1% CMC in 10ml of Normal Saline)	25 mgml^{-1}	15.00 ± 0.25**	19.66 ± 0.21**
	50 mgml^{-1}	13.00 ± 0.25**	14.83 ± 0.21**
	75 mgml^{-1}	11.00 ± 0.25**	12.16 ± 0.16**
	100 mgml^{-1}	7.33 ± 0.21***	11.16 ± 0.16***

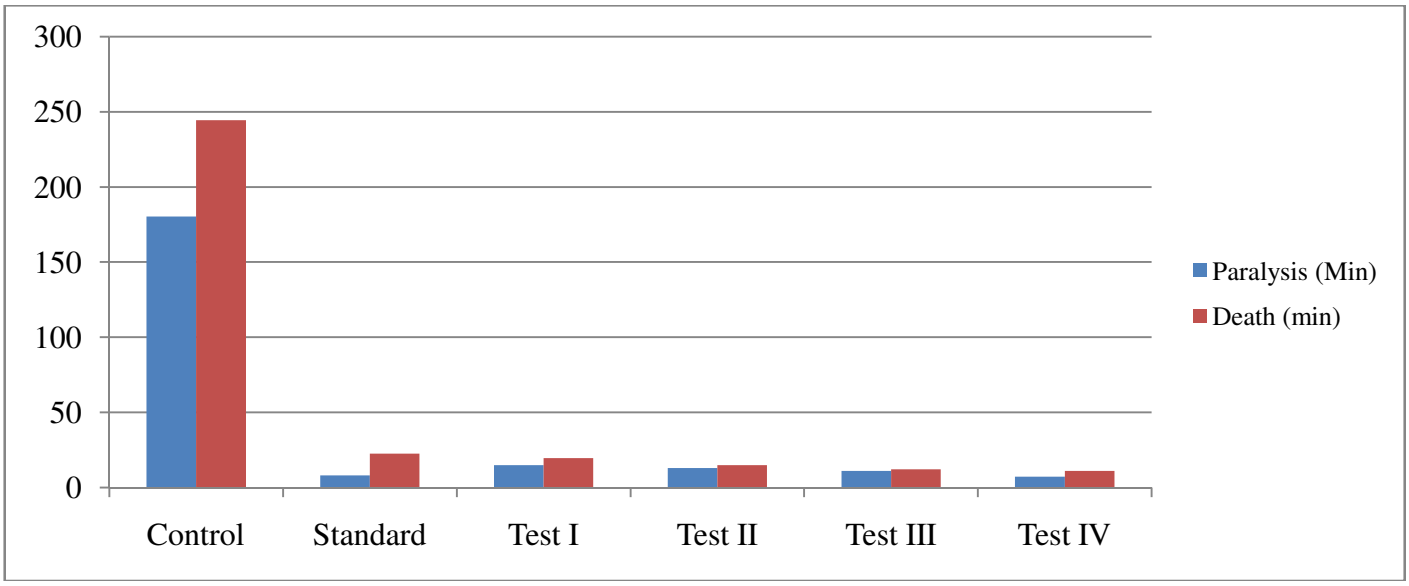

Figure-1: Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma.*

Conclusion

The ethanolic extract of Withania coagulans was subjected to the Anthelmintic activity against *Pheretima posthuma*, showed a significant inhibitory effect at higher doses 75mgml^{-1} in Normal Saline and 100mgml^{-1} in Normal Saline.

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