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EVALUATION OF ANTHELMINTIC ACTIVITY OF *TRICHOSANTHES DIOICA* (R.)

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ABSTRACT

Trichosanthes dioica seeds are mentioned in various traditional texts as a drug used for vermicidal anthelmintic, insecticidal, sedative, diuretic, demulcent, and expectorant purpose ethnopharmacologically. Objective of the present study was investigation of anthelmintic activity of different extracts of *Trichosanthes dioica* seeds. Annelids, *Pheretima posthuma* and nematodes, *Ascaridia galli* were used to carry out experiments for anthelmintic activity. Piperazine citrate was used as a standard. Time required for paralysis and death (lethal time) of worms were noted for each sample of *T. dioica* extracts and standard. The results demonstrated that treatment with *T. dioica* seeds extract significantly (P<0.05-P<0.01) paralyzed and killed both of the worms, *A. galli* and earthworms. The activity was found to be increased with dose. Ethanol and ethyl acetate extracts activity at 60 mg/ml concentration were comparable to the well known anthelmintic agent Piperazine citrate (10 mg/ml). In conclusion, the use of the seeds of *T. dioica* as an anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

KEYWORDS: ***Trichosanthes dioica,*** **Parwal, Anthelmintic activity,** ***Ascaridia galli,***

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INTRODUCTION

Recently, it has been observed that the use of anthelmintics produces toxicity in human beings. Hence the development and discovery of new substances acting as anthelmintics are being derived through plants which are considered to be the best source of bioactive substances. *Trichosanthes,* a genus of family Cucurbitaceae is an annual or perennial herb distributed in tropical Asia. Over 20 species are recorded in India out of which *T. anguina and T. dioica* are cultivated as vegitable. Other important species found in throught the world are *T. palmata, T. cordata, T. nervifolia, T. cucumerina, T. wallichiana & T. cuspida*.[1] Seeds paste is used to kill worms in wounds and fungal infections.[2] The bark is used in leprosy and jaundice. Leaves of *T. dioica* have been investigated for their antioxidant[3] and anti-inflammatory activities in the past.[4] *Trichosanthes dioica* (Parwal) screened for the treatment of Alzheimer's disease.[5] Fruits of *T. dioica* reported for the hypoglycemic effect for Prevention of Type-2 Diabetes. [6] The seeds of *T. dioica* were used in treatment of helminthes ethnopharmacologically (Charaka samhita & Ayurveda)[7] but no scientific data is available yet. Therefore, it was thought to investigate anthelmintic potential of seeds of *T. dioica.* Now a day, a lot of studies regarding anthelmintic property of many herbal drugs against *Pheretima posthuma* (annelids) and *Ascaridia galli* (nematods) have been done.[8-9] Hence the present study was thought to be investigate anthelmintic potential of one more promising herb which is mention in traditional texts.

MATERIALS AND METHODS

Plant material

The seeds of *Trichosanthes dioica* (Parwal) were collected in the month of May from a place near Meerut district and were authenticated by Department of Botany, Meerut College Meerut, Uttar Pradesh. (India).

Experimental worms

Indian earthworms (*Pheretima posthuma)* were collected from the waterlogged areas of Meerut. Adult Indian earthworms (*Pheretima posthuma*) have anatomical and physiological similarity with intestinal parasites of human being.[10-13] *Ascaridia galli* Schrank (Nematoda) were collected from the department of veterinary science, Pantnagar.

Preparation of Extracts

The seeds of *T. dioica* were dried under shade and crushed in an electric blender to form powder (200 g) and subjected to Soxhlet extraction by using petroleum ether, ethyl acetate and ethanol as solvents. The extracts were concentrated by rotary evaporator and used for testing anthelmintic activity.

Test samples

Test samples were prepared freshly. Varying concentrations of three test extracts petroleum ether ethyl acetate and ethanol viz. 10, 20, 40, 60 mg/ml for each were prepared by dissolving or suspending in distilled water for annelids. Similar dilutions were made in phosphate buffered saline (pH 7.2, 0.15 M), supplemented with 2 % dimethyl sulfoxide (DMSO) for nematodes.

Experimental Design

The fresh worms of nearly equal size were selected for the study. Each type of worms were divided into 14 groups of six worms in each.The first group acted as positive control and kept in 9 cm Petri dishes containing 20 ml of Piperazine citrate (10mg/ml) in distilled water (for annelids) and in 2 % DMSO (dimethyl sulphoxide) in phosphate buffered saline (PBS, pH 7.2, 0.15 M) (for nematodes). Piperazine citrate (10mg/ml) was served as reference vermicide drug in the positive control group.The second group was served as negative control and kept in distilled water for annelids & 2 % DMSO in PBS (pH 7.2, 0.15 M) for nematodes.Twelve groups were placed in Petri dishes containing 20 ml of each extracts at four different concentrations (10, 20, 40 & 60 mg/ml). Paralysis time has been noted when no movement was observed except when the worms were shaken. Time for death (lethal time) of worms had been recorded after confirming that worms were neither moved when shaken nor when dipped in warm water (50^0C). The worms were considered dead when they lost their body colour. [14-16]

Statistical analysis

The software “Graph pad prism” (version 5.01) was used for stastical analysis.The result were express as Mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnett's Multiple Comparison Test. ** ($P<0.01$), * ($P<0.05$) were considered statistically significant.

RESULTS & DISCUSSION

The results of *in vitro* evaluation of different test extracts from *T. dioica* in *P. posthuma* and *A. galli* are summarized in Table 1 and 2 respectively. All the test extracts (60 mg/ml and 40 mg/ml) exhibited significant ** ($P<0.01$), *($P<0.05$) paralytic and lethal actions against *P. posthuma* in a concentration dependent manner (Table 1). The ethanolic extract was the most potent showing the shortest paralysis and lethal time, followed by ethyl acetate and petroleum ether extract which were least active (Figure 1). The lower concentrations i.e. 10 mg/ml and 20 mg/ml of petroleum ether extract exhibited very long duration of period for paralysis & lethal time. In case of *A. galli* also all the test extracts exhibited concentration dependent paralysis and lethal effects (Table 2). Ethanol extracts of *T. dioica* had significant anthelmintic activity and it was found that the ethanol extract activity was higher than other both extracts at all the concentrations against *A. galli* (Figure 2).

Table 1
Effects of T. dioica leaves against P. posthuma

Treatment	Concentration (mg/ml)	Mean paralysis time (min) ± SEM	Mean lethal time (min) ± SEM
Positive control(Piperazine citrate)	10	18.66 ± 0.33**	27.83 ± 0.47**
Negative control(Vehicle only)	NIL	NIL	NIL
Ethanol extract	10	139.66 ± 0.61	182.16 ± 0.54
	20	73.500 ± 0.56	101.66 ± 0.21
	40	31.16 ± 0.30	56.50 ± 0.61
	60	25.66 ± 0.33**	37.33 ± 0.42**
Ethyl acetate extract	10	181.50 ± 0.71	201.83±0.60
	20	113.66 ± 0.61	126.50 ± 0.67
	40	91.66 ± 0.42	97.50 ± 0.67
	60	69.83 ± 0.47*	76.00 ± 0.41*
Petroleum ether extract	10	254.00 ±1.0	273.33±0.80
	20	236.00 ±0.12	248.33±0.05
	40	236.83 ± 0.87	241.33 ± 0.80
	60	202.33 ± 0.42*	212.50 ± 0.56*

Number of worms per group (n) = 6, SEM = Standard Error of Mean, Vehicle: Distilled water
The activity was found to be increased with dose. Ethanol and ethyl acetate extracts activity at 60 mg/ml concentration were comparable to the well known anthelmintic agent Piperazine citrate (10 mg/ml).

The results of *in vitro* evaluation of different test extracts from *Trichosanthes dioica* seeds in *P. posthuma* and *A. galli* are summarized in Table 1 and 2 respectively. All the test extracts exhibited significant paralytic and lethal actions in a concentration dependent manner against *P. posthuma* (Table 1). The ethanolic extract was the most potent showing the shortest paralysis and lethal time, followed by ethyl acetate and petroleum ether extract which were least active, only at higher concentrations, exhibited most prolonged paralytic and lethal time (Figure 1). The lower concentrations i.e. 10 mg/ml and 20 mg/ml of petroleum ether extract exhibited very long duration of period for paralysis & lethal time (Figure 1).

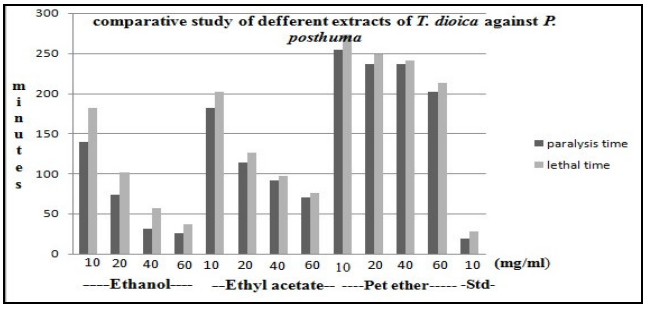

Figure 1
Graph showing effect of T. dioica seeds against P. posthuma

Table 2
Effects of T. dioica seeds against A. galli.

Treatment	Concentration (mg/ml)	Mean paralysis time (min) ± SEM	Mean lethal time (min) ± SEM
Positive control(Piperazine citrate)	10	11.16 ± 0.30**	14.33± 0.21**
Negative control(Vehicle only)	NIL	NIL	NIL
Ethanol extract	10	41.50± 0.34	53.16 ± 0.30
	20	31.83. ± 0.40	44.66 ± 0.42
	40	22.50 ± 0.56	36.33 ± 0.49
	60	15.83 ± 0.40**	19.83 ± 0.47**
Ethyl acetate extract	10	48.50 ± 0.76	61.50. ± 0.50
	20	35.33 ± 0.80	48.33 ± 0.55
	40	26.16 ± 0.40	41.16 ± 0.40
	60	18.50 ± 0.42*	24.50 ± 0.56*
Petroleum ether extract	10	175.83 ± 0.87	107.33 ± 0.80
	20	170.16 ± 3.26	101.16 ± 1.1
	40	146.83 ± 0.79	94.33 ± 0.95
	60	94.16 ± 0.94*	81.00 ± 0.89*

Number of worms per group (n) = 6, SEM = Standard Error of Mean,
Vehicle: 2 % DMSO in PBS (pH 7.2, 0.15 M)

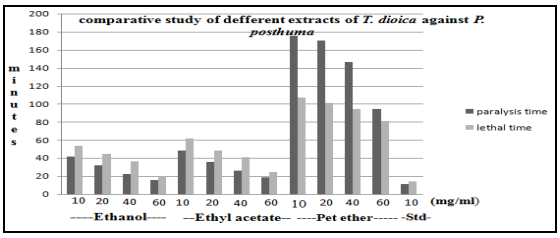

Figure 2
Graph showing effect of T. dioica seeds against A. galli

In case of *A. galli* also all the test extracts exhibited concentration dependent paralysis and lethal effects (Table 2). Ethanol extracts of *T. dioica* have significant anthelmintic activity and it was found that the ethanol extract activity was higher than other both extracts at all the concentrations against *A. galli* (Figure 2).The activity was found to be increased with dose. Ethanol and ethyl acetate extracts activity at 60 mg/ml concentration were comparable to the well known anthelmintic agent Piperazine citrate (10 mg/ml).

CONCLUSION

Ethanopharmacological use of *T. dioica* as an anthelmintic has been confirmed. All the test extracts exhibited significant paralytic and lethal actions in a concentration dependent manner against *P. posthuma* and *A. galli.* The ethanolic extract was the most potent showing the shortest paralysis and lethal time, followed by ethyl acetate and petroleum ether extracts which were least active, only at higher concentrations, exhibited most prolonged paralytic and lethal time. Further studies are needed to be carried out to recognize the specific active constituent responsible for anthelmintic activity and mechanism of action.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Nadkarni A.K., Indian materia medica. 3rd ed. Mumbai Popular prakashan; 1996. p. 1236.
2. Kirtikar KR., Basu BD., Indian medicinal plant. 2nd ed. Dehradun: Oriental enterprises; 2001. p. 1533.
3. Ghaisas MM, Tanwar MB, Ninave PB, Navghare VV, Deshpande T. Hepatoprotective activity of aqueous and ethanolic extract of T.dioica in ferrous sulphate induced liver injury. Pharmacologyonline. 2008;3:127–35.
4. Shivhare Y, Singh P, Patil UK. Healing potential of Trichosanthes dioica Roxb. on burn wounds. Res J Pharmacol Pharmacodyn. 2010;2:168–71.
5. Suh SJ, Koo BS, Jin UH, Hwang MJ, Lee IS, Kim CH. Pharmacological characterization of orally active cholinesterase inhibitory activity of *Prunus persica* L. Batsch in rats. J Mol Neurosci. 2006;29(2):101-7.
6. Park S W, Survay N.S., Ko E. Y., Upadhyay C.P., Mi1 J. Hypoglycemic Effects of Fruits and Vegetables in Hyperglycemic Rats for Prevention of Type-2 Diabetes, Kor. J. Hort. Sci. Technol. 2010;28(5):121.
7. Kim Y, Koo B, Gong D, Lee Y, Ko J, Kim C. Comparative effect of Prunus persica L. BATSCH water extract and tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride) on concentration of extracellular acetylcholine in the rat hippo-campus. J Ethnopharmacol 2003; 87149–54.
8. Kumar N, Chaudhary A. Anthelmintic activity of *Prunus persica* (L.) Asian J Pharm Clin Res.2015;8(5):163.
9. Macedo ITF, Oliveira LMB, Ribeiro WLC, Santos JML, Silva KdC, Filho JVA, Vasconcelos ALF, Bevilaqua CML. Anthelmintic activity of Cymbopogon citrates against Haemonchus contortus. Braz. J Vet Parasitol, Jaboticabal. 2015; 24(3): 268-75.
10. Thorn GW, Adams RD, Brundwald E, Isselbacher KJ, Petersdorf RG. Harrison's Principles of Internal Medicine. New York: McGraw Hill Co.1977.p.1088.
11. Vigar Z. Atlas of Medical Parasitology. 2nd Ed. Singapore: P. G. Publishing House; 1984: 216.
12. Vidyarthi RD. A Text Book of Zoology. New Delhi: S. Chand & Co., 1967: 329-70.
13. Chatterjee KD. Parasitology, Protozoology and Helminthology. Calcutta: Guha Ray Sree Saraswati Press Ltd. 1967;168-69.
14. Ghosh T, Maity TK, Bose A, Dash GK. Anthelmintic activity of *Bracopa monnieri*. Indian J Nat Prod. 2005;21(2):16-19.
15. Lalchhandama K. Nematocidal effects of piperazine and the extract of *Acacia oxyphylla* stem bark on the poultry nematode, *Ascaridia galli*. Pharmacologyonline 2008;3:864-9.
16. Khan S, Afshan K, Mirza, B, Miller JE, Manan A, Irum S, Rizvi SS, Qayyum M. Anthelmintic properties of extracts from Artemisia plants against nematodes, Trop Biomed. 2015;32(2):257-68.