"Pharmacological screening of *Trichosanthes Dioica Roxb* against helminthes"

[1]Swati J. Tembhurne, [2]Dr. Chakresh Patley, [3]Dr. Rajesh Mujariya, [4]Dr. Manjeet Singh, [5]Punam Bihone

[1,2,3,4,5]Institute of Pharmaceutical Science and Research (IPSR), Balaghat (MP), 481331, India
Corresponding Author: tembhurneswati20@gmail.com

Abstract:

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. The bark is used in leprosy and jaundice. Leaves of T. dioica have been investigated for their antioxidant3 and anti-inflammatory activities in the past. phytochemical study of methanol extract of seeds indicated existence of flavonoids, alkaloids, terpenoids and saponins. Antioxidant potential was measured through DPPH and H2O2 free radical scavenging action. Outcome confirmed that maximum scavenging effect of extract on DPPH radical was 70.83% which was compared with scavenging effect of ascorbic acid (81.25%) at 150 µg/ml. Scavenging action effect of extract on H2O2 radical was 67.97% was analogous to ascorbic acid (72.68%) at 150 µg/ml concentration.

Keywords: Anthelmintic activity, Trichosanthes, Soxhlet's apparatus, Earthworm.

Introduction

Anthelmintic are drugs used to either kill (vermicide) or expel (vermifuge) the parasitic worms or helminths that inhabit GI tract and other tissues and organs of the body.Vermicide: When Anthelmintic kill the parasitic worms. Vermifuge[1] When Anthelmintic remove the parasitic worms from GI tract by temporarily paralysing them they are called vermifuses. Widely used in veterinary medicine both prophylactically and to treat chronic and acute infections.[2] They help to reduce worm burdens and control worm larvae on the pasture or eggs in the environment. Helminths are internal parasites of animals and birds Phylum Platyhelminthes The phylum Platyhelminthes consists of two important classes of animal parasites Cestoda (tapeworms) & Trematoda (flukes). Cestoda (Tapeworms): The cestodes or tapeworms are elongated endoparasites with flat body.[3] The body consists of a head or scolek, a neck, and a strobila consisting of a number of segments or proglottids. Each proglottid usually contains one or two sets of reproductive organs.[4] The tapeworms are hermaphrodite. They require two or more hosts for completion of their life cycle.[5] Almost all tapeworms of veterinary importance belong to the order Cyclophyllidea, with two exceptions being in the order of Pseudophyllidea.

Ideal Anthelmintic

1. An ideal anthelmintic should have high level of anthelmintic activity.

2. The efficacy is said to be good if it removes 95% of a gastro-intestinal nematodes from ruminant spp.

3. If it removes only 70% of the worm burden it is considered as a poor anthelmintic.

4. It should have effect on both adult and larval stages of worms.

5. If it is effective only against adult worms it is repeated to eliminate adult worms that were unaffected during the first dose.

6. 100% removal of worm load also eliminates the source of antigenic stimulation and animal loses the acquired resistance to parasite.[6]

Helminth Control in domestic animals is widely based on the use of anthelmintic drugs in combination with grazing management. In the Sudan, the method of controlling internal parasites

which relies on rotational grazing is limited and so it is difficult to be practiced in this country.[7] The livestock are mostly raised under nomadic conditions with traditional methods of management and natural grazing.[8] Therefore, the control of helminth infections in animals to alleviate production losses relies totally on the use of anthelmintics; in the absence of sustainable control programs that can reduce the dependence on chemical control agents.[9]

Collection of Plant material and Authentication

The plant "Trichosanthes Dioica" was collected in December 2022 from the Botanical Garden Balaghat, and it was harvested at post flowering stage. The plant material was identified and authenticated taxonomically at Nirmal Institute of Agriculture Technology Gondia 441614 and the earthworm is collected from the mud of pangoli river Gondia.

Preparation of extract of ingredient of Trichosanthes dioica

All the ingredients of were powdered by an electric grinder and sieved by Sieve no. 80 to get fine powder. The 50% hydro alcoholic extract (water: ethanol, 50:50 v/v) of the test drugs were obtained by using Soxhlet's apparatus in different drug and solvent ratios i.e. 1:5, 1:10 and 1:15. The extraction was done for 8 h at temperature 80ºC and filtered to obtain a clear filtrate then it was dried on water bath to obtain a solid residue of extract. The maximum yield percentage of aqueous extract was found to be 22.26% with 1:15 drug and solvent ratio while hydro alcoholic extract yielded 24.77% in 1:10 drug and solvent ratio. Further, standardization of the extract was carried out by the qualitative phytochemical analysis. Extracts of above ratio showed the maximum presence of phytoconstituent that is why; the same extracts were used for anthelmintic study. The extracts were stored in air tight container in refrigerator for further experiment.[10]

Preparation of Trichosanthes dioica

All the ingredients of were first washed with water to remove dirt and debris and then subjected to drying at 60ºC in an oven for 4 hours. The drugs were finely powdered by an electric grinder (Sieve no. 80) and stored. were soaked in water and allowed to stand for 24 hrs so that all the gum dissolved in water, then squeezed from a muslin cloth and filtrate was dried in an oven at a temperature less than 50˚C, collected, and the dried powder was passed through a sieve no. 80

and stored carefully in a desiccator until required.[11] All the ingredients were mixed together in appropriate ratio (drug and honey 1:3). After then, the powdered drugs were mixed thoroughly and stirred till all the ingredients were completely homogenized following that, vessel was removed from the fire and allowed to cool. No preservative was added in the formulation.[12]

Experimental animal

Most of the screening on anthelmintics reported are in vitro studies using Indian earthworm such as Phretimaposthuma, Eisenia fetida, Ascardia galli, Ascaris lumbricoidesetc. It has been demonstrated that all anthelmintic which are toxic to earthworms are creditable to study as an anthelmintic. They have anatomical and physiological resemblance with the intestinal roundworm parasite found in human beings.[13] Because of its easy availability; earthworms have been used extensively for the preliminary in vitro evaluation of anthelmintic compounds. Therefore, in the present study earthworm is taken as an experimental model to evaluate the anthelmintic activity of Trichosanthes dioica . Adult earthworms, Eisenia fetida were obtained from the moist soil of Pangoli river Gondia. They were kept in the required media at room temperature and maintained their environmental condition throughout the study.

Chemicals, solvents and reagents

All the chemicals and reagents used in this study were of analytical grade and procured from authentic sources. Ethanol was obtained from chemical store SPU Balaghat. The chemicals used in phytochemical analysis were obtained from Central Drug House (P) Ltd. New Delhi, India. While Tincture alkane was taken from chemical store SPU Balaghat India. Filter paper (Whatman no.40) and Millipore filter paper purchased from GE Healthcare UK Limited, UK. Albendazole,(Zental) of Glaxo Smith Kline Pharmaceuticals Ltd.

Experimental design

Indian adult earthworms (Eisenia fetida) of 3-5cm in length, 0.1-0.2cm in width and 0.7-1.2 g in weight were used for the study. They divided in to 4 groups and each group having 6 earthworms.

Plain control (Normal saline).

Test group A [Hydro alcoholic extract of the ingredients of T. dioica (HAETD)]

Test group B Aqueous extract of the ingredients of Trichosanthes dioica

Test group C [Powder of the ingredients of Trichosanthes dioica],

Standard group D [Albendazole] containing 6 earthworms in each group.

Since the aim of study was to determine the concentration dependent effect of each dosage form therefore, each group of particular dosage form was further subdivided in 6 groups containing 6 earthworms in each. Before the experiment, the earthworms were washed with normal saline to remove all faecal matter and waste surrounding their body.

Evaluation of *in vitro* anthelmintic activity

The method was followed with some modification in the dose regime. Six earthworms were released into same size of Petri dishes containing normal saline, with varying concentration of each dosage forms such as *Trichosanthes dioica*, powder, aqueous and hydro alcoholic extracts of *Trichosanthes dioica* and standard drug Albendazole. The suspension of *Trichosanthes dioica*, powder, hydro alcoholic and aqueous extract of the ingredients of *Trichosanthes dioica* at different concentration (50, 100, 150, 200, 250, 300 mg/ml) were prepared by using 0.5% (w/v) of CMC as a suspending agent and final volume was made upto 10 ml for respective concentration by addition of normal saline. Test drug and the standard drug solution of respective concentration were freshly prepared before starting the experiments and poured in different petridishes. While plain control was treated only with 10 ml normal saline. Soon after releasing the earthworm in respective petridishes, they were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms continuously for 3 h. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death time was noted when the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) even after performing the prick test, (pricking the body of earthworm by a sharp needle followed by pouring of warm water drop by drop on them) followed by fading away of their body colors. For this purpose the worms along with the Petri dish content was poured in wash basin and allowing the worms to move freely. The paralysis time and death time were recorded in terms of minute.

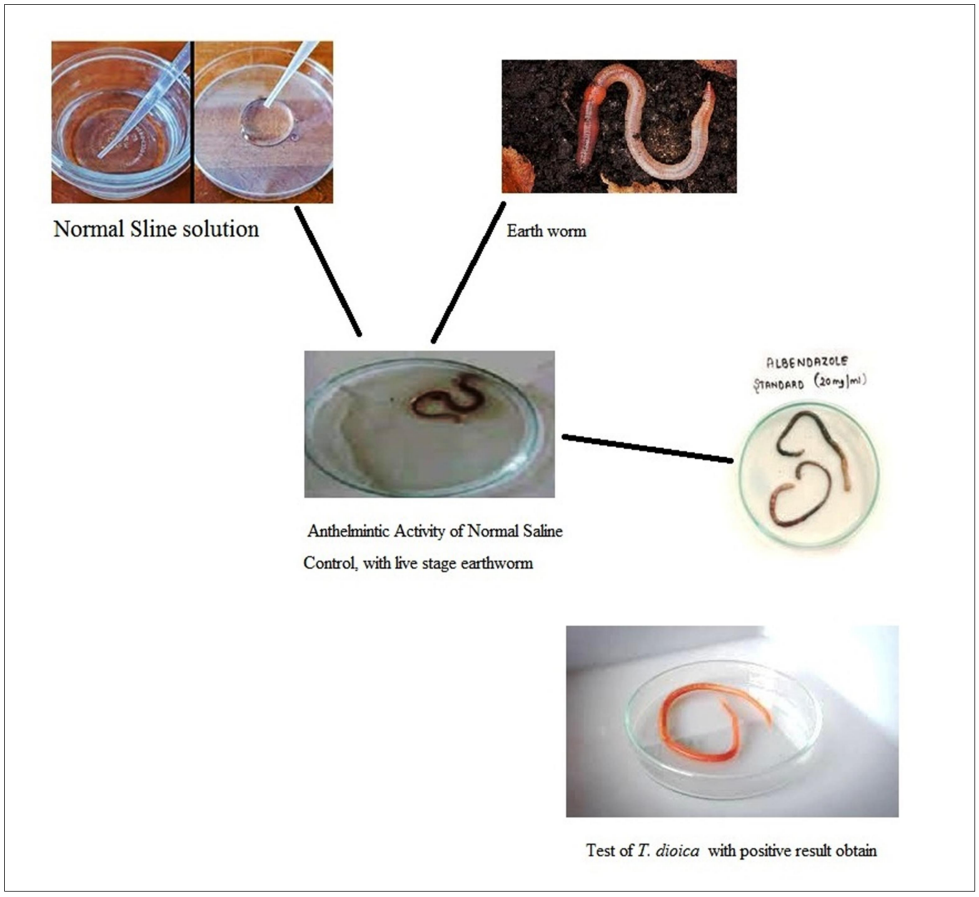

Fig.no.1. Flow chart of experiment profile

Results and discussion

In-vitro anthelmentic activity:

Clinical observations:

No clinical signs were observed by any of the uninfected control group during the experimental period. No evidence of toxicity of *Trichosanthes dioica* or was recorded on the experimental doses during or after treatment. Results showed that Trichosanthes dioica significantly acts as anthelmintic as compared to STD drug and ethanol extract of *Trichosanthes*. *dioca* showed more significant as compared to std drug.

CONCLUSION

T. dioica is a popular plant used in Indian system of medicine. Various herbal tests prove it is promising herb, has been in use as folk remedies in different countries for treating various ailments and has wide range of therapeutic actions. Preface phytochemical study of methanol extract of seeds indicated existence of flavonoids, alkaloids, terpenoids and saponins. Antioxidant potential was measured through DPPH and H_2O_2 free radical scavenging action. Outcome confirmed that maximum scavenging effect of extract on DPPH radical was 70.83% which was compared with scavenging effect of ascorbic acid (81.25%) at 150 µg/ml. Scavenging action effect of extract on H_2O_2 radical was 67.97% was analogous to ascorbic acid (72.68%) at 150 µg/ml concentration.

Refference

1. Caroline Komen, Frederick, M. Wanjala, Paul C. Kiprono. (2005). Efficacy of Jasminum abyssinicum treatment against Haemonchus contortus in sheep. *Afr. J. Trad. CAM* . 2 (3): 264 – 268.
2. Chappell, L. H., (1988). The interactions between drugs and the parasite surface.
3. Charles I. Muleke, Ruofeng Yan, Yanming Sun, Guangwei Zhao, Lixin Xu, Xiangrui Li. (2007). Vaccination of goats against Haemonchus contortus with arecombinant cysteine protease. Small Ruminant Research. 73: 95–102
4. Charleston, W. A. G. (1965). Pathogenesis of experimental heamochosis in sheep, with special reference to the development of resistance. Journal of Comparative Pathology and Therapeutics. 75: 55-67.
5. Chartier, C.; Etter, E.; Hoste, H.; Pors, I.; Koch, C. and Dellac, B. (2000). Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. Veterinary Research Communications. 24: 389 - 399.
6. Chartier, C.; Pors, I.; Hubert, J.; Rocheteau, D.; Benoit, C. and Bernard, N. (1998). Prevalence of anthelmintic resistant nematodes in sheep and goats in

Western France. Small Rumin. Res. 29: 33 – 41.

7. Chartier, C.; Soubirac, F.; Pors, I.; Silvestre, A.; Hubert, J.; Couquet, and Cabaret, J. (2001). Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. J. Helminthol. 75: 325–330.
8. Chiejina, S. N.; Fake, B. B. and Eze, P. L. (1989). Development and survival of free living stages of gastrointestinal nematodes of sheep and goats on pasture in Nigerian derived savanna. Veterinary Research Communication. 13: 103-112.
9. Chitwood, M. B. (1969). Systematics and biology of some parasitic nematodes in: Chemical zoology. M. Florkin and B. T. Scheer (Editors), volume III. PP. 223- 244.
10. Choi, M. H., Park, I. C., Li, C., Hong, S.T. (2003). Excretory-secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA. Korean Journal Parasitology. 41: 5–9.
11. Chopra, R. N., S. L. Nayyar and I. C. Chopra. (1956). Glossary of Indian Medicinal Plants, p. 160. Council of Scientific and Industrial Research, New Delhi, India
12. Chrai,S.S.,Murari,R.&Ahmad,I.2002.Liposomes(AReview).PartTwo: DrugDeliverySystem.Cooper, G. M. & Hausman, R. E. 2009. The Cell: A Molecular Approach, Washington,Asm Press/ Sinauer Associates.
13. Clark, C. H.; Kiesel, G. K. and Goby, C. H. (1962). Measurement of blood loss caused by Haemonchus contortus infection in sheep. American Journal of Veterinary Research. 23: 977-980.