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Anthelmintic activity of aqueous extract of Tagetes patula flowers in adult earth worms.

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Abstract

Background: Helminthiasis is a serious health problem in human and other animals around the world. Thus, the present study is aimed to evaluate the anthelmintic activity of aqueous extract of Tagetes patula flowers on Indian earthworms (Pheretima posthuma).

Methods: Earthworms were divided into five groups with six worms in each group. Group1 (control) was treated with 2% gum acacia. Group 2 (standard) was treated with Albendazole suspension 25 mg/ml. Group 3, group 4 and group 5 were treated with aqueous extract of Tagetes patula (AETP) 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. Drugs were dissolved in 2% gum acacia and 10 ml formulation containing different concentrations were poured in separate petri dish. The worms were placed into it one at a time and the time of paralysis (P) and time of death (D) was recorded.

Results: The plant extract revealed significant anthelmintic activity in a dose dependent manner but less potent than the standard drug.

Conclusion: Findings of the present study confirms the ethno-medicinal claim of anthelmintic activity of this plant.

Keywords: Aqueous extract of Tagetes patula, anthelmintic activity, albendazole, Pheretima posthuma.

Introduction

Parasitic infection like helminthiasis is a serious health problem in the tropical regions including the Asian countries and affects more than two billions people worldwide. Helminths produce serious health problem in human and other animals around the world specifically to the third world countries.[1] In developing countries they are the major threat to public health, key contributing factor to the prevalence of

malnutrition, anaemia, eosinophilia and pneumonia.[2] Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs, infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation[3]. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). In the tropical countries there is existence of simultaneous infections with more than one type of helminth.[4] Despite the prevalence of parasitic infections, the research on anthelmintic drug is poor. As per WHO, only few drugs are frequently used for the treatment of these parasite infections. Traditional medicines report the efficacy of several medicinal plants in eliminating worms.[5] For most of our past history for ages, plant parts or entire plant extracts have been used to combat parasitism and in many parts of the world such natural products are still in use for this purpose.

Globally herbal medicines are extensively used due to their therapeutic efficiency and minimum side effects. Tagetes patula, also known as french marigold is commonly used for preparing ethnobotanical remedies against rheumatism, stomach and intestinal problems, kidney and hepatic disorders, fever and pneumonia.[6] This plant is also reported to possess nematocidal, insecticidal and larvicidal property.[7] The juice of young leaves is used in the treatment of different diseases including different worm infestations according to Ayurveda and several other folk medicinal practices. Even though the herb has been used for the treatment of various ailments, there is still paucity of scientific data to support the various uses. Therefore, the present study was aimed to investigate the anthelmintic potential of aqueous extract of the Tagetes patula flowers in three different concentrations (25, 50, and 100 mg/ml) on Indian earth-worm (Pheretima posthuma).

Materials and methods

Approval of Institutional Animal Ethics Committee (IAEC)

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal after getting approval of the Institutional Animal Ethics committee, RIMS, Imphal (No.1596/GO/a/12/CPCSEA).

Set up

Department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur in January 2020.

Requirements

Soxhlet apparatus, Tagetes patula flowers, mixer grinder, evaporating dish, distilled water, earth worm (Pheretima posthuma), gum acacia, petri dish, albendazole suspension (Zentel suspension, gsk pharmaceuticals limited).

Preparation of aqueous extract

Tagetes patula flowers were collected from Lamphel, Imphal, Manipur in the month of October, 2019. The plant was identified and authenticated by Professor PK. Singh, Department of Life Sciences, Manipur University, (Voucher no. MUMP-003631). The flowers were cleansed with water, air dried under shade and made into coarse powder by mixer grinder. Aqueous extract of Tagetes patula (AETP) was prepared by soxhlet extraction method described by Verma SCL and Agrawal SL.[8] 50 grams of the coarse powder was extracted with distilled water. The dried extract was scraped out, weighed and stored in airtight container. The yield was 14%.

Phytochemical analysis

Qualitative phytochemical analysis of aqueous extract was done prior the experiment.

Experimental Model

Adult Indian earthworms (Pheretima posthuma) were used to carry out the experiment.[9,10] The earthworms were collected from the local muddy fields, Imphal west, Manipur. Worms were washed with normal saline to remove all faecal matter. The earthworms of 6±1 cm in length and 0.4-0.5 cm in width were used for the experiment. The earth worms were authenticated by Department of Life Sciences, Manipur University.

Experimental design

The anthelmintic assay was carried out following the method described by Ajayieoba et al. with minor modifications.[11] The earthworms were divided into five groups with six worms in each group. 2% gum acacia was used as control (group1). Albendazole suspension 25 mg/ml was considered as standard (group2). AETP 25 mg/ml, 50 mg/ml and 100 mg/ml were used as different doses of test drug and were considered as group 3, group 4 and group 5 respectively. Drugs were dissolved in 2% gum acacia and 10 ml formulation containing different concentrations were poured in separate petri dish and the worms were placed into it one at a time. Time of paralysis (P) was noted when no movement of any sort could be observed in the worms except when the worms were shaken vigorously. The time of death (D) of worms were concluded and recorded after ascertaining that the worms neither moved when shaken vigorously nor moved after dipping into warm water (50^0 C) followed by white secretions and fading away of their body colour.[12]

Statistical analysis[13]

The results were analyzed using one way ANOVA followed by bonferroni test using SPSS version 21. Results were expressed in mean±SEM and $p<0.05$ was considered significant.

Results and discussion

Phytochemical screening

The preliminary qualitative phytochemical analysis of aqueous extract of Tagetes patula revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, gums and proteins.

Anthelmintic activity

The anthelmintic activity was assessed by observing the time of paralysis (P) and time of death (D) in earth worms after applying different concentration of test and standard drugs.

Table 1: Anthelmintic activity of aqueous extract of Tagetes patula flowers on Pheretima posthuma.

Groups	Drug concentration	Time of paralysis (P) in min.	Time of death (D) In min.
Group 1	2% gum acacia	----------	----------
Group 2	Albendazole 25 mg/ml	8.20±0.44¤	12.76±0.77¤
Group 3	AETP 25 mg/ml	30.02±0.64	41.38±0.60
Group 4	AETP 50 mg/ml	18.70±0.51†	27.69±0.52†
Group 5	AETP 100 mg/ml	14.32±0.45†‡‡	21.51±0.50†‡

Values were expressed as Mean ± SEM; n=6 in each group, *p < 0.001 with respect to Group 3, 4 and 5; †p < 0.001 with respect to Group 3; ‡p < 0.001 with respect to Group 4; ‡‡p < 0.01 with respect to Group 4.The experiment was carried out in vitro utilization of adult Indian earthworm Pheretima posthuma owing to its anatomical and physiological resemblance with intestinal roundworm parasite of human beings for preliminary investigation of anthelmintic activity.[9,10] Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic activity. Albendazole is a drug belongs to the benzimidazole group of broad spectrum anthelmintic, effective against helminth, larvae and eggs. It is believed that albendazole disrupts final common metabolic pathway by inhibiting β-tubulin polymerase

causing ultimate disruption of cytoplasmic microtubule formation.[14] In the present study albendazole (25mg/ml) showed best anthelmintic activity with time of paralysis (P) and time of death (D) 8.20±0.44 mins and 12.76±0.77 mins respectively while AETP (25 mg/ml, 50 mg/ml, 100 mg/ml) revealed time of paralysis (P) and time of death (D) 30.02±0.64 mins and 41.38±0.60 mins, 18.70±0.51 mins and 27.69±0.52 mins, 14.32±0.45 mins and 21.51±0.50 mins respectively. AETP revealed significant anthelmintic activity in a dose dependent manner. However extract at any dose was found to be less effective than the standard drug. The phytochemical analysis of extract revealed the presence of tannins, which were shown to produce anthelmintic activities.[15] Tannins present in the AETP extract may interfere with energy generation in helminth by uncoupling oxidative phosphorylation and could be the probable mechanism behind anthelmintic activity.[16] Another probable mechanism could be binding of tannins to gastrointestinal tract free protein of host animal and causing death.[17]

Conclusion

The study permits us to conclude that aqueous extract of Tagetes patula flower possesses significant anthelmintic activity. However, further studies are needed to elucidate the exact mechanism of action of anthelmintic activity offered by its phytoconstituents and its clinical application to prevent or cure helminthiasis.

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References

1. Tripathi KD. Essentials of Medical Pharmacology. 7th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2013. p. 849-51.
2. Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of Tamarindus indica Linn. Indian J Pharm Sci 2011;73(1):104-7.
3. IK Idika, EA Okonkwo, DN Onah, IO Ezeh, CN Iheagwam, CO Nwosu. Efficacy of levamisole and ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. Parasitol Res 2012;111(4):1683-87.
4. Bundy DA. Immunoepidemiology of intestinal helminthic infections. 1. The global burden of intestinal nematode disease. Trans R Soc Trop Med Hyg. 1994;88(3):259-61.
5. Dwivedi A, Dwivedi S, Sitoke AK, Patel R, Jhade D. Anthelmintic Activity of a Polyherbal Preparation. Ethnobotanical Leaflets 2009;13(1):259-62.
6. P Vasudevan, S Kashyap, S Sharma. “Tagetes: a multipurpose plant,” Bioresource Technology. 1997;62(2):29–35.
7. Farzana Nikkon M, Rowshanul Habib, Zahangir Alam Saud, Rezaul Karim M. (Medicinal plants: conservation and sustainable use) Efficacy evaluation of Azadirachta indica, Calotropis procera, Datura stramonium and Tagetes erecta

against root-knot nematodes Meloidogyne incognita. Pakistan Journal of Botany 2011;43(1):197-204.

8. Verma SCL, Agarwal SL. Studies on Leptadenia reticulata: part II. Preliminary chemical investigations. Indian J Med Res 1962;50(3):439-50.
9. Chatterjee KD. Patasitology.13th ed. New Delhi: CBS Publishers and distributors Pvt. Ltd; 2015.
10. Vigar, Z. Atlas of Medical parasitology, 2nd ed. P.G. Publishing House. Singapore, 1984; 242.
11. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Bio 2001;39(2): 217- 20.
12. Goswami S, Pandey A, Tripathi P, Singh A, Rai A. An in vitro evaluation of the anthelmintic activity of Hedychiumspichatum rhizomes and Zingiber zerumbet rhizomes on Pheritima posthuma model: A comparative study. Pharmagnosy Res 2011;3(2):140-2.
13. Wayne Daniel W. Biostatistics, A foundation for the analysis in the health sciences. In (2004): 7th, John Wiley and Sons (Asia) Pvt. Ltd, pp. 312.
14. Lacey E. The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. International Journal of Parasitology 1988;18: 885–936.
15. Kaur S, Kumar B, Puri S, Tiwari P, Divakar K. Comparative study of anthelmintic activity of aqueous and ethanolic extract of bark of Holoptelea integrifolia. Int J Drug Dev Res 2010;2(4):758-63.
16. Martin RJ. Modes of action of anthelmintic drugs. The Veterinary Journal 1997;154(1): 11-34.
17. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Veterinary Parasitology 2001;99(3):205-19.