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Evaluation of *in-vitro* anthelmintic activity of *Heliotropium indicum* Linn. leaves in Indian adult earthworm

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PEER REVIEW

Peer reviewer

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This paper is a good way of presenting the contribution in the field of helminthiasis. To broaden the studies, further *in vivo* models are necessary to prove the effectiveness for the use of leaf as anthelmintic drug. This paper on the whole is an excellent research in the field of microbial infections.

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ABSTRACT

Objective: To carry out an exhaustive study with a view to substantiate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* using mebendazole as a reference standard.

Methods: Forty five worms were collected and were divided into nine groups and were washed in normal saline before they were released into 10 mL of respective drug solutions using distilled water as vehicle. Different concentrations (25 mg/mL, 50 mg/mL and 100 mg/mL) of the test (MEHI and AEHI) and standard solution were prepared before the commencement of the experiment. Time for paralysis and time for death were recorded for each group.

Results: Crude methanolic extract with concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL produced dose-dependent paralysis. Time of paralysis and death increased with concentration of 25 mg/mL, 50 mg/mL as compared with that of standard drug mebendazole. Methanolic extract of *Heliotropium indicum* Linn. (*H. indicum*) gave shorter paralysis and death time at 100 mg/mL as compared to aqueous extract *H. indicum* Linn. Results are expressed as mean±SEM ($P < 0.05$) of 5 worms in each group.

Conclusions: From the investigation, conclusion can be drawn that the methanolic extract of *H. indicum* (Boraginaceae) showed better activity than aqueous extract of the same to treat intestinal worm infections. In comparison with the standard drug mebendazole, methanolic extract of *H. indicum* showed significant anthelmintic efficacy.

KEYWORDS

Heliotropium indicum Linn., Anthelmintic, Methanolic extract, Aqueous extract, *Pheretima posthuma*, Mebendazole

1. Introduction

In helminthiasis disease, a part of the body is infested with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs^[1]. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia^[2]. The parasitic worms are divided into three groups: cestodes

or tapeworms; nematodes or roundworms; and trematodes or flukes^[3]. Parasitic diseases may cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis^[4]. Most diseases caused by helminthes are of a chronic nature. They probably cause more morbidity and even economic and social deprivation among humans and animals than any single group of parasites^[5]. Anthelmintic are drugs that act either locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminthes or developmental forms that invade organs and tissues^[6]. Most of the existing anthelmintic drugs produce side effects such as abdominal pain, loss of appetite, nausea, vomiting,

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headache and diarrhea. Mebendazole is a well tolerated drug. However, gastrointestinal side-effects, dizziness have been noted in few patients. Also prolonged use in hydrated or in cysticercosis, causes headache, fever, alopecia, jaundice and neutropenia[7]. In order to exterminate the harmful side-effects of these synthetic anthelmintic drugs, it is important for us to promote the studies of traditionally used anthelmintic plants which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects[8]. The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* (*P. posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability[9,10].

Heliotropium indicum Linn. (*H. indicum*), belonging to family Boraginaceae, commonly known as 'Indian heliotrope' is very common in India and some parts of Africa and Bangladesh, but also found in other countries. It is annual or perennial herb and is usually regarded as a weed[11]. This plant has been used in different traditional and folklore systems of medicine for the treatment of various ailments such as skin infections, ophthalmic disorders, poison bites, stomachache, nervous disorders, treat wounds, anti-inflammatory, anti-tumor and rheumatism[12–14]. In the present study, experiment was carried out in order to investigate the therapeutic potential of the plant in terms of its anthelmintic activity against *P. posthuma* using mebendazole as a reference standard.

2. Materials and methods

2.1. Collection of plant material

The leaves of *H. indicum* were collected from Dibrugarh University Campus, Dibrugarh, Assam, India during the month of April–May, 2012. Both mature and young leaves were collected, washed thoroughly with water and then dried under the shade for a week. The dried leaves were then coarsely powdered and stored in airtight containers for further study. The plant was identified and authenticated by Dr. N. Odyuo, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (Specimen no. Du/KM/2012/07, Reference no. BSI/ERC/2013/Tech/Plant identification/636) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references.

2.2. Extraction of plant material

Approximately 300 g of powdered crude drug of *H. indicum* (Boraginaceae) leaves were extracted by successive extraction procedure, using soxhlet apparatus, with petroleum ether for 18 h followed by extraction with solvents such as chloroform, methanol and water. The different solvent was recovered after extraction and the extracts were concentrated by rotary evaporator at low temperature (40–45 °C) and pressure.

2.3. Selection of Indian earthworms for experiment

Adult Indian earthworms (*P. posthuma*) were used to carry out the anthelmintic activity owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[15,16]. The worms were collected from moist soil of Dibrugarh, Assam, India and washed with normal saline to remove all dirt and faecal matters. The worms measuring 3–5 cm in length and 0.2–0.3 cm in width were used to carry out the experimental procedure[17].

2.4. Drugs and chemicals

Mebendazole purchased from Cipla Pharmaceuticals Ltd. was used as standard drug. Similarly methanolic extract of *H. indicum* (MEHI) and aqueous extract of *H. indicum* (AEHI) were used as test drug for the activity. The solvents and chemicals of analytical grade were used for the experimental protocol.

2.5. Experimental procedure

The anthelmintic assay was carried out as per the method of Panda *et al.* with minor modifications in the process[18]. Indian adult earthworm 3–5 cm in length and 0.1–0.3 cm in width were used for the *in-vitro* anthelmintic bioassay of MEHI and AEHI. A total of forty five worms were collected and were divided into nine groups each containing five earthworms in each group. Different concentrations (25 mg/mL, 50 mg/mL and 100 mg/mL) of the test (MEHI and AEHI) and standard solution were prepared in distilled water keeping the volume upto 10 mL. The earthworms were previously washed in normal saline solution before they were released into 10 mL of respective standard and test solutions. Distilled water was used as control. After releasing the worms in the respective petridishes, time of releasing was noted and thereby the motility of the worms was observed. Time of paralysis was noted when the worms showed no movement except when worms were shaken vigorously and time of death was noted after determining that worms neither moved when shaken vigorously nor when dipped in warm water (40–50 °C). The death was also determined by observing the faded body colour of the worm. During the course of experiment, patterns in worms such as colour change, movement and swelling were also observed.

2.6. Statistical analysis

The results are expressed as mean±SEM of five worms in each group. Comparisons have been made between standard against test treated group. $P < 0.05$ was considered significant.

3. Results

From the observation table given in Table 1, it was found that the higher the concentration of the extract, the faster

was the paralytic effect and the shorter was the death time for all the earthworms. The data given in the observation Table 1 showed that MEHI gave shorter paralysis and death time at 100 mg/mL as compared to AEHI. Mean±SEM values were calculated for the both extract and standard. MEHI showed anthelmintic activity in a dose-dependent manner taking shortest time for paralysis (16.26±0.23) and death (32.49±0.35) at 100 mg/mL concentration. Also in case of AEHI the dose of 100 mg/mL showed shortest time of paralysis (25.01±0.54) and death (40.34±0.64). Time of paralysis and death increased with concentration of 25 mg/mL, 50 mg/mL as compared with that of standard drug mebendazole. All the investigational extract showed the anthelmintic activity starting from a minimum dose of 25 mg/mL, its significant activity ($P<0.05$) at 25 mg/mL for time taken for paralysis and death when compared to standard drug mebendazole used at 25 mg/mL[19].

Table 1

In-vitro anthelmintic activity of leaf extract of *H. indicum*.

Groups	Drugs treatment	Concentration (mg/mL)	<i>P. postuma</i> (earthworm)	
			Time taken for paralysis (min)	Time taken for death (min)
I	Control	-----	-----	-----
II	Mebendazole	25	39.34±0.19	45.56±0.18
III	Mebendazole	50	29.34±0.17	35.05±0.15
IV	Mebendazole	100	19.25±0.23	24.67±0.20
V	MEHI	25	41.23±0.21	62.45±0.29
VI	MEHI	50	25.05±0.26	47.03±0.42
VII	MEHI	100	16.26±0.23	32.49±0.35
VIII	AEHI	25	50.08±0.61	75.16±0.45
IX	AEHI	50	35.20±0.45	55.45±0.39
X	AEHI	100	25.01±0.54	40.34±0.64

MEHI=Methanolic extract of *H. indicum*, AEHI=Aqueous extract of *H. indicum*.

The result was expressed as mean±SEM. Statistical analysis was carried out with comparisons between standard and treated groups. $P<0.05$ was considered statistically significant and $n=5$ was taken in each group.

This study was performed on Indian adult earthworms as these are easily available and possess anatomical and physiological resemblance with that of intestinal round worm parasites of human beings[9,10]. The results obtained in this study have shown hopeful results on anthelmintic activity. The result of anthelmintic activity on earthworm *P. posthuma* given in Table 1 reveals that the different concentrations used for both aqueous and alcoholic extracts have shown paralysis and death of earthworms and it was compared in the same concentration with mebendazole as reference drug. Mebendazole blocks the glucose uptake in the parasite causing depletion of glycogen stores. The site of action of mebendazole appears to be the micro tubular protein 'β-tubulin' of the parasite. It binds to β-tubulin of susceptible worms with high affinity and inhibits its polymerization[7].

This plant could be used by human beings in controlling gastrointestinal nematode infections. The literature review reveals that tannins which are chemically polyphenolic compounds are present in the aqueous extract of the leaves responsible to produce anthelmintic activity[20–22]. As phytochemical analysis of AEHI revealed the presence of

the tannin among other chemical constituents. It is possible that tannins contained in the extracts produced similar effects. The reported anthelmintic effect of tannin is that they can bind to free proteins in the gastrointestinal tract of host animal[23], or glycoprotein on the cuticle of the parasite and may cause death[24]. In addition, tannins or their metabolites have a direct effect on the viability of the pre-parasitic stages of helminthes and other phytochemicals may responsible for have an anthelmintic effect include essential oils, flavonoids and terpenoids[25]. This speculation is supported by varying rates of effectiveness of *H. indicum*. Therefore from the present study conducted, it can be suggested that the MEHI and AEHI showed moderate effectiveness against helminthes possibly due to alkaloids, carbohydrates, fats and oils, glycosides, steroids, saponins, tannins and phenolic compounds present in it. Further studies needs to be carried out using *in-vivo* models to establish the pharmacological efficacy for the use of *H. indicum* as an anthelmintic drug.

The MEHI and AEHI exhibited anthelmintic activity against Indian adult earthworm (*P. posthuma*). From the biological assay performed and observations noted, it can be concluded that *H. indicum* Linn. (Boraginaceae) possesses significant anthelmintic activity to treat intestinal worm infections, when compared with the normally used drug. To broaden the studies, further *in vivo* models are necessary to prove the effectiveness for the use of leaf as anthelmintic drug. Further studies are needed to be done to isolate the active phytoconstituents responsible for bringing the pharmacological response against the Indian adult earthworm. It can be concluded that the MEHI is more effective than the AEHI against the Indian adult earthworm (*P. posthuma*).

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Helminthic infections have severe consequences for the health of millions of people worldwide. Plant-based medicines, derived from locally abundant species, are probably cheaper and more easily available to poor communities than synthetic anthelmintics. The aim of this

present study was to investigate the therapeutic efficacy of *H. indicum* against helminthiasis.

Research frontiers

Tannins or their metabolites have a direct effect on the viability of the pre-parasitic stages of helminthes. Essential oils, flavonoids, terpenoids and other phytochemicals, which are present in the plant, may be responsible for having an anthelmintic effect.

Related reports

Shoge Mansurat Oluwatoyin *et al.*, reported the potent efficacy of methanolic extract of *H. indicum* against microbial infections. From the earlier studies reported by Osungunna and Adedeji, it was clear that this plant has potent effect on microbes. Based on these related reports, the anthelmintic activity assessment for this plant was carried out.

Innovations & breakthroughs

A new prospect in the treatment of helminthiasis infections with the help of herbal drug has been found out with the help of this research.

Applications

This study is a contribution in finding out the potent efficacy of *H. indicum* against helminthiasis. This research can promote studies to isolate the compounds which are responsible for showing the anthelmintic effect.

Peer review

This paper is a good way of presenting the contribution in the field of helminthiasis. To broaden the studies, further *in vivo* models are necessary to prove the effectiveness for the use of leaf as anthelmintic drug. This paper on the whole is an excellent research in the field of microbial infections.

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