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RESEARCH ARTICLE

Effect of *Azadirachta indica* on *Pheretima posthuma*

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ABSTRACT

Soil-transmitted helminth (STH) infections are worm infections that can be facilitated by one or more intestinal parasitic worms such as roundworm (*Ascaris lumbricoides*), hookworm (HW; *Ancylostoma duodenale* and *Necator americanus*), threadworm (*Strongyloides stercoralis*), and whipworm (*Trichuris trichiura*). More than two billion people are thought to be affected by STH infections globally. Eggs in human feces, which are commonly seen in low-resource countries, spread STH by contaminating the soil and water in unhygienic places. The transmission is by human exposures to the infective stages, either by direct skin penetration or through ingestion that could result in a serious illness, though infections remain asymptomatic in the majority of cases with light-intensity infections. *Azadirachta indica*, usually called Indian neem, has been extensively used in ayurveda, unani, and homoeopathic medicine since time immemorial. A. inidica has been used ethnopharmacologically to treat diseases such as cancer, diarrhea, gastric ulcers, wound infections, trypanosomiasis, malaria, and tuberculosis. *A. indica* leaf extracts in methanol were tested for their ability to treat *Pheretima posthuma* worms. From the experimental outcome, we find that the leaf extracts of *A. indica*, when compared to the usual medication, the plant was found to exhibit substantial anthelmintic activity. The present research concludes that neem has a variety of bioactive compounds. The findings of this study unequivocally show that *A. indica* is a significant source of helminthicide.

KEY WORDS: Clinical and laboratory findings, Factor to increase the transmission of helminthes, Introduction, Mode of transmission of helminthes, Pharmacological activity of *Azadirachta indica*.

INTRODUCTION

Helminths are complex organisms that comprise approximately three hundred thousand species that can be either free-living or parasitic.[1] Soil-transmitted helminth (STH) infections are worms that can be facilitated by one or more intestinal parasitic worms such as[2] roundworm (*Ascaris lumbricoides* [AL]), hookworm (HW; *Ancylostoma duodenale* and *Necator americanus*),[3] threadworm (*Strongyloides stercoralis*), and whipworm (*Trichuris trichiura* [TT]).[4] Soil is a good medium for egg development and can affect human health, although it rarely causes death.[5] In areas where there is poor hygiene, STH transmission occurs by ingesting eggs present in human feces, which invariably contaminate the soil, or through skin penetration.[6] It is estimated that STH infections affect more than two billion people worldwide.[7] Large numbers of cases occur in impoverished rural areas of Sub-Saharan Africa, Latin America (WHO, 2012),[8] China, and East Asia.[9] Approximately 267 million pre-school children are affected, and in 2022, treatment and preventative measures will be necessary for more than 568 million school-age children who reside in locations where these parasites are widely disseminated.[10]

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Types of helminths

The majority of neglected tropical diseases (NTDs) are caused by infections with STHs, which include AL, TT, *S. stercoralis*, and the hookworms *N. americanus*,

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Table 1: Disease caused by different types of helminthes

No.	Helminths	Morphology	Disease	References
1.	Nematodes (Round worms)	Elongated and cylindrical, tapering at both ends and extremities.	Primarily affect livestock feed consumption and the efficiency of the animals. Severe ailments result in the death of the affected animals.	[12]
2.	Cestodes (Tape worms)	Elongate, dorsoventrally flattened, divided into the anterior part with attachment function known as scolex, proliferative region, that is neck, and long strobila.	Blockage of the intestine and severe pathological changes, leading to anorexia, emaciation, anemia, reduced growth, condition, and survival	[13]
3.	Trematodes (flukes)	Resembling coffee beans in size and shape, encapsulate in pairs and produce eggs.	liver flukes, lung flukes, schistosoma	[14]

A. duodenale, and *Ancylostoma ceylanicum*. Types of helminths and diseases caused by helminths are mentioned in Table 1.[11]

Clinical and laboratory findings

Many indications and symptoms, particularly gastrointestinal ones, are associated with STH infections. The following clinical symptoms were most frequently reported: Abdominal bloating, diarrhea, iron deficiency anemia, and pale ocular conjunctiva.[15] Malabsorption syndrome, intestinal obstruction, chronic dysentery, rectal prolapse, respiratory complications, and poor weight gain.[16]

Factor to increase the transmission of helminths

STH are transmitted by eggs present in human feces, which in turn contaminate soil and water in areas with poor sanitation, conditions often found in low-resource countries.[17] Soil polluted with parasite ova, infective larvae, cysts, oocysts, open defecation, place of defecation, frequency of biting nails, absence of household latrine, rural residence, illiterate mother.[18] Poverty, inadequate sanitation, and hygiene.[19] Untrimmed fingers, shoe-wearing habits, playing on contaminated soil, and lack of knowledge about the route of transmission.[20] Poor environmental sanitation, unsafe human waste disposal systems, poor personal hygiene, frequent outdoor exposures, a lack of a safe water supply, and the low socio-economic status of the country are considered risk factors for STH infections.[21]

Mode of transmission of helminths

The transmission is by human exposures to the infective stages, either by direct skin penetration or through ingestion, which can lead to serious illness, though infections remain asymptomatic in the majority of cases with light-intensity infections.[22] The eggs of these parasites can survive for several weeks in warm, damp soil and pollute the environment through indiscriminate defecation, inappropriate sewage disposal, and the use of infected animal dung as manure. In addition to the fecal-oral route of transmission, such as in the case of roundworms, transmission can also happen when an infectious larva, such as a hookworm or *S. stercoralis*, actively penetrates the skin. Due to its significant impact on STH distribution, contaminated soil is one of the main sources of helminth egg reservoirs.[23]

Introduction to *A. indica*

A. indica, usually called Indian neem, originated in Myanmar and Southern India. They are tropical evergreen trees that grow up to a height of 15–50 m,[24] in tropical and semi-tropical regions.[25] The term Nimba, later abbreviated to Neem, was used in traditional Sanskrit. *A. indica* is the Latinized name for neem, which comes from Persia. It is known as "the free tree of India" since "Azad" means free, "Dirakht" means tree, and "Indica" means of India.[26] Due to its therapeutic and insecticidal qualities, it is one of the most adaptable medicinal plants and a member of the *Meliaceae* family (Table 2).[27] *A. indica* is popularly known as the Indian neem or margosa tree. It has been extensively used in ayurveda, unani, and homeopathic medicine since time immemorial.[28]

Phytoconstituents of *A. indica*

Phytochemicals present in the leaves of *A. indica* mainly include tannins, amino acids, alkaloids, steroids, saponins, gallic acid, flavonoids, 6-desacetylnimbinene, 4a,6a-dihydroxy-A-homo-azadiradione, 3-desacetyl-3-cinnamoyl azadirachtin, 3-acetyl-7-tigloyl-lactone-vilasinin, 3-diacetyl solanine, hyperoside, beta-sitosterol, beta-sitosterol, azadirachtanin, and azadirachtanin-A.[29] The major representative phytochemical compounds are oxidized tetranortriterpenoids, such as azadirachtin A (azadirachtin), azadirachtin B (3-tigloylazadirachtol), azadirachtin D (1-tigloyl-3-acetyl-11-hydroxy-meliacarpin), azadirachtin H (11-demethoxycarbonyl azadirachtin), azadirachtin I (1-tigloyl-3-acetyl-3-acetyl-11-demethoxycarbonyl meliacarpin), azadiriadione, azadirachtolide, deacetylnimbin, epoxyazadiradione, isoazadirolide, margosinolide, nimbin, nimbolin A, nimbandiol, nimocinol, nimbinene, nimbocinone, nimbocinolide, nimocin, nimbolide, salannin, and related derivatives.[30]

Table 2: Taxonomical classification of *Azadirachta indica*[27]

S. No.	Taxonomic Rank	Azadirachta indica
1.	Kingdom	Plantae
2.	Sub-Kingdom	Tracheobionta
3.	Division	Magnoliophyta
4.	Class	Eudicot
5.	Sub-class	*Rosidae*
6.	Order	Sapindales
7.	Family	*Meliaceae*
8.	Genus	*Azadirachta*
9.	Species	*Azadirachta indica*

Pharmacological activity of *A. indica*

The ethnopharmacological applications of *Azadirachta indica* include the management of tuberculosis, trypanosomiasis, malaria, cancer, diarrhea, gastric ulcers, wounds, and fungal infections,[31] anti-inflammatory, antibacterial, immunomodulatory activity, antidiabetic activity, and hepatoprotective activity.[32]

The aim of the present study is to evaluate the anthelmentic potential of the plant *A. inidica*.

MATERIALS AND METHODS

Methodology involved in this study was categorized into the following four steps:

I. Selection of plant
II. Collection of plant material
III. Authentication of plant material
IV. Preparation of extract.

Pharmacological evaluation

Screening of plants for antihelmentic activity by using the following animal model:
Adult Motility Assay

Plant material collection

The samples (*A. indica*) were obtained from Moga, Punjab; they were identified using local and regional floras, and the voucher specimens were deposited at the ISF Faculty of Pharmacy, Moga, Punjab. The plant material was cleaned with water, dried in the shade, ground using a mechanical grinder, and then stored in an airtight container pending more extractions.

Preparation of methanol extract

Neem leaves were ground into a powder (100 g) and exposed to a maceration extraction procedure in 70% methanol at 4°C for 24 h, followed by 5–7 percolations to complete the extraction. After filtration, methanol was removed from the extract under reduced pressure using a vacuum evaporator at 50°C. The crude extract obtained was lyophilized and stored at −20°C until subsequent use.[33]

Experimental procedure

A. indica leaf extracts in methanol were tested for their ability to treat *Pheretima posthuma* worms. Using a bioassay to test different amounts of each extract (50 and 100 mg/mL), it was possible to determine the time of the worms' paralysis and death. Salinized water served as the control, and albendazole served as the standard reference. Due to their morphological and physiological similarities to the human intestinal roundworm parasite, *P. posthuma*, mature Indian earthworms (Scientific names *Lumbricus terrestris* and *P. posthuma*) were used for the test. Due to their accessibility, earthworms have frequently been utilized for the initial *in vitro* assessment of anthelmintics. substances. To prepare the earthworms for the anthelmintic investigation, they were dug up from moist soil and thoroughly washed with ordinary saline. For every experiment, earthworms of 6–8 cm in length and 0.2–0.3 cm in breadth were utilized. Six earthworms each made up one of the two groups from which the earthworms were separated. Before beginning the studies, all of the extracts and the standard drug solution were freshly made in normal saline. On various Petri plates, various extracts and standard drug solutions were added. All of the earthworms were dispensed into a 10 mL formulation along with two different strengths of methanol extract and 40 mg/mL amounts of albendazole. The length of time it took for the worms to become paralyzed and die was observed. When no movement of any kind could be seen unless the worms were violently shaken, this condition was known as paralysis. The worms were declared dead when they stopped moving when submerged in warm water (50°C) and their body colors started to fade.[34]

Experimental design

Six worms were released into 50 mL of the solutions (which were reconstituted with sterile water) of Albendazole (40 mg/mL), the ethanol extracts of the leaves of *A. indica* (50 and 100 mg/mL), and normal saline (100 mg/mL). The endpoint of the study was the time taken for the paralysis and/or death of the worms. The worms are randomly divided into four groups (with six worms in each group).

- Group – 1 Albendazole 40 mg/mL in 50 mL solution
- Group – 2 A *A. indica* 50 mg/mL in 50 mL solution
- Group – 3 A *A. indica* 100 mg/mL in 50 mL solution
- Group – 4 Normal saline 100 mg/mL in 50 mL solution.

Table 3: Anthelmintic activity of the extracts of *Azadirachta indica*

Treatment	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Methanol	50	70	97
	100	55	67
Albendazole	40	37	52
Control	-	-	-

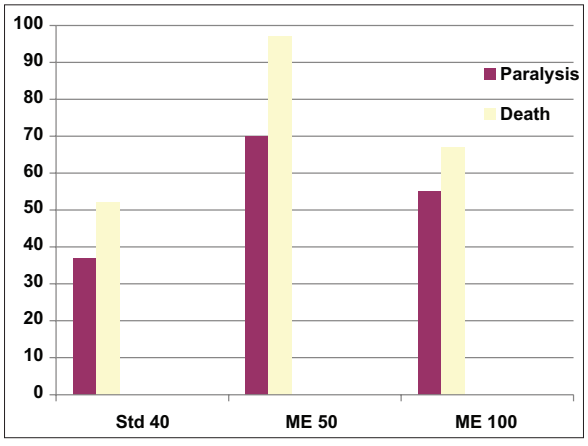

Figure 1: Paralysis and death time. Std: Standard, ME: Methanolic extract

RESULTS

From the experimental outcome, we find that the leaf extracts of *A. indica*, when compared to the usual medication, the plant was found to exhibit substantial anthelmintic activity. The methanolic extract of *A. indica* at concentrations of 50 and 100 mg/mL shows paralysis at 70 min and 55 min and death at 97 min and 67 min, respectively. The standard drug albendazole 40 mg/mL shows paralysis at 37 min, and death time is 45 min for the earthworm *P. posthuma* [Table 3 and Figure 1]. Albendazole exhibits anthelmintic activity by blocking glucose uptake and the depletion of glycogen stores in the test parasite. The methanol extracts of *A. indica* showed not only paralysis but also the death of worms considerably more quickly than Albendazole did at different concentrations of 50 mg/mL and 100 mg/mL.

DISCUSSION

The findings of this study unequivocally show that *A. indica* is a significance source of helminthicide. This finding agrees with the previous report that indigenous plants are useful in the treatment of helminthiasis.[35]

CONCLUSION

Drugs derived from neem have been used for therapeutic and health benefits. They have few adverse effects and are safe and effective.[36] More than 140 physiologically active substances with complicated structures and varied chemical compositions make up the various portions of neem.[37] The traditional use of the leaves of *A. indica* as an anthelmintic has been confirmed using a methanolic extract of different concentrations that showed significant anthelmintic activity.[38] Further, it would be interesting to isolate the phytoconstituents that are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory. This research shows that the neem has *in vitro* anthelmintic activities and recommends that further trials be conducted to evaluate *in vivo* biomedical activities.[39]

ETHICLAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST STATEMENT

In relation to the research, authorship, or publishing of this paper, the authors reported that they had no potential conflicts of interest.

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AUTHOR'S CONTRIBUTIONS

Mr. Abdul Quaiyoom, Ms. Geetanjali Kumari, and Mr. Alok Kumar Aditya are the major contributor to the writing and drafting of the manuscript; Mr. Ranjeet Kumar is the major contributor in editing and drafting the manuscript. All authors read it and gave their approval.

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