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Investigation of in Vitro Anthelmintic activity of *Azadirachta Indica Leaves*

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Abstract

The aqueous extract of Azadirachta Indica Leaves was investigated for anthelmintic activity using earthworms (Pheretima posthuma), tapeworms (Raillietina spiralis) and roundworms (Ascaridiagalli). Various concentrations (10-70 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control.

Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at the concentration of 40 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as an anthelmintic. Therefore, the anthelmintic activity of the aqueous extract of Azadirachta Indica Leaves has been reported .

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Azadirachta Indica Leaves, , vermicidal, anthelmintic activity

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Introduction

Infections with helminth are among the most widespread infections in humans and other domestic animals affecting a large number of world population. The majority of these infections due to worms are generally restricted mainly to the tropical

regions and the occurance is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symtomps like anaemia, eosinophilia and pneumonia1. Parasitic diseases cause ruthless morbidity affecting principally in population Neem [http://www.gits4u.com/agri/agri5a.htm] is a native tree of India, found in every parts in India especially in semi-arid conditions. The Neem Tree is an incredible plant that has been declared the "Tree of the 21st century" by the United Nations. In India, it is variously known as "Divine Tree", "Life giving tree", "Nature"s Drugstore", "Village Pharmacy" and "Panacea for all diseases". It is one of the major components in Ayurvedic medicine, which has been practiced in India since many centuries. The neem is an ancient Indian cure-all due to its antibacterial, antifungal, antiviral, antihistamine and antiseptic properties.The neem leaves, flowers, seeds, roots, bark and fruits are utilized to treat inflammation, infections, skin diseases.

In India's ancient Ayurvedic Medical texts it is explained that every part of the Neem tree has health promoting benefits. What is clear from the above information is that the general population of India for, over 5000 years, has used Neem safely and effectively. In fact the people of India call the Neem Tree "The Village Pharmacy". It is also called Holy Tree (Azadirachta indica.). In ancient times neem was the most celebrated medicinal plant of India and found mention in a number of Puranic texts like the Atharava Veda, Upanivahod, Amarkosha and Ghrysutra. They all dealt with the outstanding qualities of the neem tree as a source of medicine pesticide.

Today, neem is once again steadily becoming an agro-scientific celebrity. It has figured as the priority in seminars and serious agricultural workshops all over the world. Modern western medicine is finally discovering what the ancient Indians have known for thousands of years: that the neem tree has superb pharmaceutical and pesticide controlling qualities. Its effectiveness, availability and safety have made agro-scientists promote cultivation of neem forests. The azadirachton compound in neem has been recognized as an effective insecticide that is biologically selective, not harming the useful pest-predators but keeping almost 250 harmful ones at bay. Scientists recommend coating urea with neem cake to kill nitrifying bacteria. Even water management with neem to control vectors of Japanese encephalitis was the success of neem over DDT.

The neem tree, Azadirachta indica, is a tropical evergreen with a wide adaptability. Native to India and Burma, it has Beentransplanted to Africa, the Middle East, South America and Australia.

It is especially suited to semi-arid conditions and thrives even in the poorest soil with rainfalls as little as 18 inches (450 mm) per year and temperatures up to 50° C (120° F). It may grow up to 50 feet (15 m) tall and live for 200 years. The lifespan of the Neem tree is described to be anywhere between 150 to 300 years.

Its blossoms are small, white flowers with a very sweet, jasmine-like scent. Its edible fruit — is about 3/4 of an inch (2 cm) long, with white kernels. A neem tree generally begins bearing fruits at three to five years of age, and can produce up to 50 kg (110 lbs.) of fruit annually when mature. The pinnate leaves have a very bitter taste and a garlic-like smell.

Sanskrit **Name**: Nimba, which nourishes health in the body. **Lt. Name is** Azadirachta indica – Meliaceae **Rasa** (Taste) – Tikta (Bitter); Kashaya (Astringent) **Guna** (Characteristics) – Laghu (Light **Veerya** (Potency) – Sheeta (Cold) **Vipaka** (Post digestion effect) – Katu (Pungent).

More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds.

The compounds have been divided into two major classes: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc.

The most significant part of the neem (Azadirachta Indica) is the neem leaf which has been used in multiple uses, in the form of raw neem leaves, neem leaves powder, neem leaf extracts and also neem leaf juice. Azadirachta Indica Neem leaves are widely used to cure a number of human and animal diseases. The physio-chemical properties of neem leaves help to maintain the overall well being. Neem leaves are further processed and used in the manufacturing of a number of drugs and medicines. Raw neem have been traditionally used to give bath to patients suffering . Neem leaves are generally gathered only from organic trees, this is so, because it ensures the protection of natural elements and reduction of contamination by environmental/synthetic toxins.

Neem is useful in diarrhea and frequent passage of stool, ignites the digestive fire in stomach, improves vision (eye sight) of a fellow, is useful in all skin diseases, loosens the impacted and solidified stool in rectum, useful in all metabolic disorders including Diabetes mellitus,a cardiac rejuvenating herb, one of the best Ayurveda herbs which promote healing of wounds [http://www.neem-products.com/leaf-extract.html]. Ational Research Council (NRC), Washington, USA, has released a report with title as " Neem: Tree for Solving Global Problems." The NRC Panel considers the Neem to be "one of the most promising of all plants and the fact that it may eventually benefit every person on this planet. Probably no other plant yields as many strange and varied products or has as many exploitable by-products. "

The chloroform extract of stem bark is effective against carrageenin-induced paw oedema in rat and mouse ear inflammation.The plant also possesses analgesic activity mediated through opioid receptors in laboratory animals.

Neem extracts possess anti-diabetic, antibacterial and anti-viral properties. The tree stem, root and bark possess astringent, tonic and anti-periodic properties. The bark is beneficial in malarial fever and useful in cutaneous diseases.It is used for external applications in skin diseases. It possesses antidandruff, antibacterial, anti viral and fungicidal properties.

Neem aqueous leaf extract significantly ($P<0.05$) prevented changes in the serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Similarly it significantly prevented the histological changes as compared to the group receiving antitubercular drugs. It also significantly reversed the biochemical and histological changes. So ***Neem*** aqueous leaf extract significantly prevents and reverses the hepatotoxic damage induced by antitubercular drugs in rats.

Azadirachta indica (Neem) is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India, Burma. Neem has the following. Phylum is Magnoliophyta ,Class is Magnoliopsida ,Order is Sapindales ,Family is Meliaceae Genus is Azadirachta.Bengal is Neem, *Neem* is a branched tree, 10 to 12 meters high.

The leaves of neem plants can be categorized as the tender young leaves which is for distorting the growth pattern of the dermatophytes. *Neem,* a tropical tree, widely distributed in villages of West Bengal and reported as medicinally important tree.

We have chosen this tree for the detail pharmacological studies to prove whether this plant can be used as an enormous source for future medicinal development.

On the side of the river Hoogly in West Bengal there are many villages situated. Therefore, this area is lack of the light of modern medicine. Therefore, the poor people are dependent on several plants or plant-based preparations for the treatment of various ailments in their traditional system. During our course of studies on ethnomedicine of this area, the plant being used as anthelmintic is leaves of Neem. This plant has a wide reputation among natives of being curative for intestinal-worm infections. This plant is being used by the villagers of West Bengal as an anthelmintic in the form of extract, prepared by soaking powdered material in water for 10-12 hours. This extract is taken orally once a day for three days to treat intestinal-worm infections. We have also watched that the poor villagers of West Bengal are using the raw juice of the leaves of Neem as anthelmintic for domestic animals such as cow, dog, goat etc. Based on this, an attempt has been made to evaluate the anthelmintic potential of Neem.

Materials and methods

Plant Materials

The tree Neem was collected from the village (Amtala) south 24 paraganas district, West Bengal, India. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Kolkata. A voucher specimen has been preserved in our laboratory for future reference. The plant material was dried in shade, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

Preparation of extract

Aqueous extract (Maceration method)

Mature green leaves are collected and allowed to dry partially. These dried leaves are then crushed and powdered. The crushed leaves are then subjected to either aqueous or organic solvent to get a concentrated extract. For making neem leaf extract, certain extraction process utilizes carbon dioxide at critical temperatures and pressures to extract the active ingredients of the neem leaf, the usual high temperatures or harsh chemicals are done away with, resulting in a better concentrated and potent extract.

The plant leaves were dried in shade, pulverized and then powdered material of *Neem* leaves (500gm) were kept for maceration with 1000 ml of double distilled water for 24 hours. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be about 4%.

Experimental Animals

Adult earthworms (*Pheretima posthuma),* Roundworm (*Ascaridia galli) and* Tapeworms (*Raillietina spiralis)* were used to evaluate anthelmintic activity in *vitro*. Earthworms were collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and double distilled water as control [10,11,12,13] .

This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when

the wormsand worms were collected and kept in normal saline solution.

The average size of round worm was 5-7 cm and average size of tapeworm was 6-8 cm.

Earthworm and helminths were identified in Dept. of Zoology, Vivekananda College, Thakurpukur, Kolkata.

Drugs & Chemicals

Piperazine citrate (Glaxo Smithkline) was used as standard anthelmintic during the experimental protocol.

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al 4. The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity [5,6,7]. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier [8,9]. Test samples of the extract was prepared at the concentrations, 10, 20,30,40 and 50 mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard was advocated earlier [8,9].Test samples of the extract was prepared at the concentrations, 10, 20,30,40 , 50, 60 and 70 mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard and double distilled water as control [10,11,12,13] . This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the wormsTime for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^{o} C).

All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

RESULTS AND DISCUSSIONS:

From our observations, higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Aqueous extract of Neem exhibited anthelmintic activity in dose-dependent manner showing maximum efficacy at 40 mg/ ml concentration for all three types of worms. Our plant extract exhibited more potent activity at lowest concentration (10 mg/ml) against (roundworm) *Ascaridia galli*. Anthelmintic activity of the extract was compared with the standard drug Piperazine citrate (Table.1). From the above results, we can conclude that *N*eem which is used traditionally to treat intestinal worm infections, exihibited significant anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

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Table 1: Anthelmintic activity of *Neem leaves* aqueous extract.
(Each value represents mean ± SEM (N=6))

Sl. No.	Group	mg/ml	Pheretima posthuma Groups		Ascaridia galli Groups		Raillietina	
			Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	(P) in min. (Mean & SEM) Time taken for death (D) in min. (Mean & SEM)
1	Control (Water Only)	--	--	--	--	--	--	--
2	Neem	10	31±0.43	52±0.22	28±0.34	55±0.47	33±1.60	61±1.40
		20	25±0.15	46±0.26	22±0.77	51±0.33	24±0.42	46±0.53
		30	18±0.55	30±0.62	15±0.19	38±0.55	20±1.65	40±1.17
		40	17±0.32	30±0.11	13±0.85	38±1.20	19±0.50	40±0.50
		50	17±0.50	32±0.25	13±0.37	39±1.12	19±0.75	41±1.20
		60	22±1.15	61±0.75	13±1.50	38±1.15	25±0.52	52±0.15
		70	19±0.50	45±0.29	23±0.76	39±0.27	25±0.50	49±0.12
3	Piperazine citrate	10	22±1.10	60±0.75	12±1.50	38±1.10	24±0.50	52±0.10

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