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 (Ascaridia gali). 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.

**Key words:**

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 occurrence is accelerated due to
unhygienic lifestyle and poverty also resulting in the
development of symptoms like anaemia, eosinophilia
and pneumonia. 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 azadirachtion 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 been transplanted 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].

Atonal 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 antitandruff, 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.
the worms and 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 worms Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°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 Neem which is used traditionally to treat intestinal worm infections, exhibited significant anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

Acknowledgments
We wish to thank the Director Dr Professor T.K Basu of my institute The Institute of Technology & Marine Engineering for his moral support and blessings and I also express my heartfelt thanks to him for motivating me with strength and spirit.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>mg/ml</th>
<th>Pheretima posthuma Groups</th>
<th>Ascaridia galli Groups</th>
<th>Raillietina</th>
<th>(P) in min. (Mean &amp; SEM)</th>
<th>Time taken for death (D) in min. (Mean &amp; SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Water Only)</td>
<td>--</td>
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<tr>
<td>2</td>
<td>Neem</td>
<td>10</td>
<td>31±0.43</td>
<td>52±0.22</td>
<td>28±0.34</td>
<td>55±0.47</td>
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<td></td>
<td></td>
<td>20</td>
<td>25±0.15</td>
<td>46±0.26</td>
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<td>30</td>
<td>18±0.55</td>
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<td></td>
<td>40</td>
<td>17±0.32</td>
<td>30±0.11</td>
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<td>22±1.15</td>
<td>61±0.75</td>
<td>13±1.50</td>
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<td>45±0.29</td>
<td>23±0.76</td>
<td>39±0.27</td>
<td>25±0.50</td>
</tr>
<tr>
<td>3</td>
<td>Piperazine citrate</td>
<td>10</td>
<td>22±1.10</td>
<td>60±0.75</td>
<td>12±1.50</td>
<td>38±1.10</td>
<td>24±0.50</td>
</tr>
</tbody>
</table>

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

**REFERENCES:**


12) Mali RG, Wadekar RR. In Vitro anthelmintic activity of Baliospermum montanum Muell.