

## Non-Effectiveness of *Azadirachta indica* (Neem Tree) Leaf Extract against the Larvae of *Angiostrongylus cantonensis* and *Trichinella spiralis*.

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**Abstract:** The effectiveness of the leaf extracts of *Azadirachta indica* (neem tree) against two nematodes, *Angiostrongylus cantonensis* and *Trichinella spiralis* was investigated in mice. Laboratory mice were inoculated with the nematodes followed by the oral administration of the leaf extracts. Two standard drugs, flubendazole and mebendazole were compared with the plant extract in the study. No significant effects of the leaf extract against the nematodes were observed. Thus, although the extract has been shown by other investigators to be animalarial, antibacterial and antifungal, no activity was observed against the two nematodes in this study.

*Key words:* *Angiostrongylus cantonensis*, *Trichinella spiralis*, *Azadirachta indica*.

### INTRODUCTION

There are some helminthic diseases for which no effective treatments is yet available. In our search for plant-derived compounds, hot water extracts of a number of medicinal plants were investigated against the nematodes *Angiostrongylus cantonensis* and *Trichinella spiralis* in mice. One plant in particular, *Azadirachta indica*, or the neem tree, was of particular interest because the various parts of the plant has been claimed to be useful in traditional medicine for the management of a large number of diseases malaria (Rochanakij et al, 1985; Kofi-Tsekpo et al, 1991; Ofulla et al, 1995) and filariases (Comley, 1990). The extracts from the leaves, stem bark, roots, and the seeds of this plant have been developed as pesticides and the leaf extracts have been shown to possess anti-bacterial and antifungal properties (Kofi-Tsekpo et al, 1991). It was hypothesized that from the many traditional uses of the extracts of the plant, some anthelmintic properties against the above mentioned nematodes

could be found. Attempts were therefore made to investigate the effectiveness of the leaf extract of the neem tree and to compare this activity with those of flubendazole and mebendazole.

The neem tree, *Azadirachta indica* A. Juss, was indigenous to India, but it has spread extensively and it is now pan-tropical, growing mainly on coast lines on the hot tropical belt of the world. The leaves of the plant were collected from the coastal areas of Mombasa, Kenya. The fresh green leaves were spread out on the bench in the laboratory, allowed to dry completely, and then stored.

#### MATERIALS AND METHODS

*Preparation of the neem tree leaf extract.* 100 gm of the unground dry leaves were added to 2 litres of boiling distilled water in an aluminium bowl placed on a gas flame. The leaves were boiled gently for one hour, and the extract was filtered. The filtrate was allowed to cool and then freeze-dried in 200-ml aliquots in round-bottomed flasks to give straw-coloured crispy residue. This material was used in the anthelmintic tests.

*Anti-nematode tests.* The nematodes (*A. cantonensis* and *T. spiralis*) were maintained in their life cycles according to the methods developed by Maki and Yanagisawa (1980, 1983) respectively. The 6-week-old female ICR mice were each orally inoculated with 50 larvae of *A. cantonensis* or *T. spiralis*. The infected mice were randomly divided into four groups corresponding to the following: 1. Leaf extract in 1% Tween 80; 2. Flubendazole; 3. Mebendazole; 4. 1% Tween 80 (serving as solvent blank). (Flubendazole and mebendazole were obtained from Janssen Pharmaceutica). Each animal group consisted of 5 mice harbouring *T. spiralis* or 6 mice harbouring *A. cantonensis*. Two days after the inoculation, the plant extract, flubendazole, and mebendazole were each suspended in 1% Tween 80 and administered orally at a dose of 100 mg/kg to each mouse. The control mice received 1% Tween 80 only.

After 15-17 days of infection, the larvae of *A. cantonensis* were recovered under a stereoscopic microscope from the brain of the mice which were killed by anaesthesia. In the case of *T. spiralis*, 61-62 days after infection, the mice were autopsied and the number of larvae in the diaphragm was counted.

#### RESULTS

The results of the effects of the substances on *Angiostrongylus cantonensis* are presented in Table 1. The neem tree leaf extract was not effective in clearing the parasites in mice, although flubendazole and mebendazole were very effective at a dose of 100 mg/kg.

**Table 1.** Efficacy of *Azadirachta indica* extract against *Angiostrongylus cantonensis*

Drugs orally administered	Worms recovered Mean $\pm$ SE (n=number of mice)
<i>Azadirachta indica</i> leaf extract (100 mg/kg)	23 $\pm$ 5 (n=6)
Flubendazole (100 mg/kg)	0.2 $\pm$ 0.2 (n=6)
Mebendazole (100 mg/kg)	1.3 $\pm$ 0.6 (n=6)
Control (1 % Tween 80)	23 $\pm$ 2 (n=6)

Female 6-week-old ICR mice were inoculated with 50 larvae of *A. cantonensis*, given drugs 2 days after inoculation (days 2) and autopsied day 15-17.

The effects of the preparations of *Trichinella spiralis* in mice are presented in Table 2. Again, the plant extract, at a dose of 100 mg/kg did not have any activity, whereas flubendazole and mebendazole showed significant activity at the dose given.

**Table 2.** Efficacy of *Azadirachta indica* extract against *Trichinella spiralis*

Drugs orally administered	Worms recovered Mean $\pm$ SE (n=number of mice)
<i>Azadirachta indica</i> leaf extract (100 mg/kg)	136 $\pm$ 17 (n=5)
Flubendazole (100 mg/kg)	0 (n=5)
Mebendazole (100 mg/kg)	0 (n=5)
Control (1 % Tween 80)	140 $\pm$ 21 (n=5)

Female 6-week-old ICR mice were inoculated with 50 larvae of *T. spiralis*, given drugs 2 days after inoculation (day 2) and autopsied on day 61-62.

## DISCUSSION

The dose of 100 mg/kg did not produce any effects on the clearance of *Angiostrongylus cantonensis* or of *Trichinella spiralis* in mice. The dose used was similar to the average dose of 142.9 mg/kg of the neem leaf extract that is traditionally taken by humans. Thus this preparation of the neem tree leaf extract has been shown to be non-effective against the two nematodes. The use of flubendazole and mebendazole was useful in validating the in vivo experimental system applied in this study.

## CONCLUSION

Although the leaf extract of *Azadirachta indica* has been shown to be non-effective against the nematodes *A. cantonensis* and *T. spiralis*, it could be speculated that any nematocidal effects of the extracts in a traditional medicine could be indirect. Thus, due to the combined anti-protozoal, anti-bacterial and anti-fungal properties that are known for this

extract, it may resist the infectivity of the nematodes in man. But such indirect effects have to be established.

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