Antifilarial activity of *Azadirachta indica* on cattle filarial parasite *Setaria cervi*

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Abstract

Alcohol and aqueous extracts of flowers of *Azadirachta indica* were tested in vitro for their potential antifilarial activity against whole worm, nerve muscle (n.m.) preparation and microfilariae of *Setaria cervi*. The effects of alcohol and aqueous extracts were similar in nature on the spontaneous movements of whole worm and nerve muscle preparation. On the whole worm, the response was characterized by initial increase in tone, rate and amplitude of contractions followed by reversible paralysis. The initial stimulant effect is likely to be due to irritant effect on the cuticle. Nerve muscle preparation responded to both extracts by inhibition of spontaneous movements followed by reversible paralysis; initial stimulation phase was absent. The inhibition was concentration related. Alcohol and aqueous extracts had almost similar lethal effect on the microfilariae of *S. cervi*, the LC\textsubscript{50} being 15 and 18 ng/ml, respectively.

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1. Introduction

*Azadirachta indica* (Meliaceae) A.Juss. (neem) is a large evergreen tree, 40 to 50 ft in height, common throughout the greater parts of India and Burma. Almost every part of this tree is used for medicinal purposes in India [1]. Leaves, roots, stem have been used as antimalarial [2], antioxidant [3], antifungal [4], antinflammatory [5], antibacterial [6], antiviral [7] and for several other medicinal purposes in Ayurvedic system of medicine. The neem based common products like urea coated with nimin and aqueous extract of neem seeds showed nematocidal activity against *Meloidogyne incognite* (Kefoid and White) Chitwood [8,9]. Chemo-preventive potential of neem flowers on carcinogen-induced rat mammary and liver carcinogenesis has been reported [10].

Four prenylated flavanones possessing antimutagenic activity against heterocyclic amines in the *Salmonella typhimurium*, myricetin, quercetin and kaempferol, were isolated from the flowers of *A. indica* [11,12]. In the present paper, the results of an investigation of the efficacy of alcohol and aqueous extracts of the flowers of this plant concerning the antifilarial activity against *Setaria cervi* (Nematoda: Filarioideae) are presented.

2. Experimental

2.1. Plant material

The flowers of *A. indica*, collected from the survey of medicinal plants unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India, were identified by Dr. Athar Ali Khan, Department of Botany, A.M.U., Aligarh (Voucher specimen number 19610).

2.2. Preparation of the extracts

Dried and powdered flowers of *A. indica* (500 g each) were extracted with distilled EtOH and distilled water, separately (yields: 2.35 and 2.45 g, respectively). The extracts were dissolved in 95% EtOH and distilled water before use as the addition of 0.2 to 0.5 ml vehicle (95% EtOH or distilled water) to the organ bath containing 20 ml Ringer’s solution had no effect on worm motility.

2.3. Worms

*S. cervi*, a nematode parasite of the cattle water buffalo (*Bubalis bubalis* L.), resembles closely the human filarial worms in its response to drugs and can therefore be used for the screening of potential antifilarial agents [13]. *S. cervi* exhibits vigorous rhythmical movements, which can be recorded on a kymograph by suspending the worm in an isolated organ bath. The nerve muscle (n.m.) preparation of the worm exhibits similar movements [14].
2.4. Antifilarial activity

The present study was designed to observe the effect of the EtOH and aqueous extracts of flowers of *A. indica* on the spontaneous movements of the whole worm, nerve muscle and on the survival of *S. cervi* in vitro.

Adult *S. cervi* were picked up from the peritoneal cavity of freshly slaughtered cattle and were transported to the laboratory in a vacuum flask containing modified Ringer’s solution at 37 °C [13]. In the laboratory, worms were given repeated bath with the same solution to free them from any extraneous material. The whole worm was suspended in an isolated organ bath containing modified Ringer’s solution and movements were recorded on a slow moving kymograph [14].

N.m. preparation was prepared by the method described earlier [14] and suspended in an isolated organ bath containing modified Ringer’s solution at 37 °C to record movements. The method of the collection of microfilariae of *S. cervi* was described earlier [15]. In a preliminary experiment, the aqueous and EtOH extracts were added to microfilariae in increasing concentrations, i.e. 5, 10, 15, 20 and 25 ng/ml to determine the limits of activity within 6 h at 37 °C. Within these limits, six concentrations were selected to observe the survival of microfilariae.

![Fig. 1. The reversible effect of 150 μg/ml ethanol extract of *A. indica* on the spontaneous movements of the whole worm of *S. cervi*.](image-url)
3. Results

A typical response of ethanol extract of flowers of _A. indica_ on the spontaneous movements of whole worm of _S. cervi_ is shown in Fig. 1. Addition of extract in a concentration of 150 µg/ml produced a stimulant effect characterized by an increase in the tone and rate of contractions and decrease in the amplitude of contractions. The effect was evident immediately after the addition of the drug. The stimulant effect lasted for about 30 min when tone of contractions started declining till it attained pre-drug level after about 40 min. At this time, the amplitude of contractions started declining and continued to do so till the movements of the worm ceased completely after about 45 min. The paralysis of the worm was complete and continued for ca. 6 h. However, with repeated changes of the bathing fluid (w), the movements of the worm were slowly restored to normal. This indicates that the paralysis induced was reversible in nature.

On n.m. preparation, the effect of ethanol extract was different in nature as compared to that observed on whole worm (Fig. 2). The initial stimulant effect characterized by increase in tone was absent in the n.m. preparation. Immediately after the addition of the ethanol extract, the tone, amplitude and rate of spontaneous contractions started declining simultaneously. After about 30 min, the contractions became less frequent with smaller amplitude. It took about 60 min for a concentration of 25 µg/ml to paralyze the n.m.

![Fig. 1. The reversible effect of 25 µg/ml ethanol extract of _A. indica_ on the spontaneous movements of whole worm of _S. cervi_.](image1)

![Fig. 2. The reversible effect of 25 µg/ml ethanol extract of _A. indica_ on the spontaneous movements of the n.m. preparation of _S. cervi_.](image2)
preparation, completely. However, with repeated changes of the bathing fluid (w), the movements were restored to normal. This indicates that the paralysis was reversible.

The response of aqueous extract was different in nature to the response of ethanol extract of flowers of *A. indica*. Addition of extract in a concentration of 250 μg/ml, produced a stimulant effect characterized by increase in the amplitude and rate of contractions. However, tone of contractions was not visibly affected to any significant extent. After ca. 60 min, amplitude was reduced and the rate of contractions continued to decrease. The decrease in amplitude of contractions continued and after about 120 min the movements of the whole worm ceased completely. However, repeated changes with the bathing fluid (w), the movements were restored to normal. This indicates that the paralysis was reversible in nature (Fig. 3).

On the n.m. preparation, the aqueous extract produced a decrease in the spontaneous movements characterized by a decrease in tone and rate of contractions. However, the amplitude of contractions increased. The effect was evident after ca. 5 min of the injection of the drug. The initial stimulant effect was not observed as in the whole worm preparation. It took about 1 h for a concentration of 50 μg/ml to completely paralyze the n.m. preparation. The paralysis caused was reversible, as repeated washings (w) restored the movements to normal (Fig. 4).

Both ethanol and aqueous extracts of flowers of *A. indica* caused concentration related effect on the survival of microfilariae of *S. cervi*. The LC$_{50}$ and LC$_{90}$ as observed after 6 h

![Fig. 3. The reversible effect of 250 μg/ml aqueous extract of *A. indica* on the spontaneous movements of the whole worm of *S. cervi*.](image-url)
are presented in Table 1. The concentration-related effect of the ethanol and aqueous extracts of flowers of *A. indica* at a concentration of 25 ng/ml observed for 360 min is shown in Fig. 5.

4. Discussion

*S. cervi* whole worm as well n.m. preparation reacts to *A. indica* ethanolic and aqueous extracts almost in the same way as it does with diethylcarbamazine, a known antifilarial agent. The effect on whole worm was characterized by initial stimulation followed by paralysis, but on n.m. preparation the effect was only paralysis. The paralysis induced by *A. indica* extracts was concentration related and reversible. Diethylcarbamazine too caused reversible paralysis [16] or little or no effect on the adult worms in vitro [17].

The n.m. preparation of *S. cervi* responded at six times less concentration of *A. indica* ethanol extract and at five times less concentration of its aqueous extract as compared to

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<td>Ethanolic extract</td>
<td></td>
</tr>
<tr>
<td>LC₅₀</td>
<td>15</td>
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<td>LC₉₀</td>
<td>23</td>
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<td>Aqueous extract</td>
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<tr>
<td>LC₅₀</td>
<td>18</td>
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<td>LC₉₀</td>
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whole worm, demonstrating the effectiveness of cuticular barrier to foreign molecules. In addition to increased responsiveness of the n.m. preparation, there was a qualitative change in the response as well. The stimulation alone in small concentration or initial stimulation followed by paralysis observed with the adult worm was not obtained with any dose level in the n.m. preparation and the effect was characterized by only paralysis. The stimulant effect observed in the whole worm is possibly due to irritation, which can occur when the worm is intact and cuticular barrier is stimulated by *A. indica* extracts.

On the microfilariae of *S. cervi*, both the ethanolic and aqueous extracts had nearly similar lethal effect, indicating that the amount of active ingredients responsible for the death of microfilariae is extracted almost equally in ethanol and distilled water.

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**References**


Fig. 5. The effect of ethanol and aqueous extracts of the flowers of *A. indica* on the survival of microfilariae of *S. cervi* in vitro at a concentration of 25 ng/ml.