Observations on in vitro and in vivo antimicrofilarial effects of Bishop's weed (*Trachispermum ammi*)

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Abstract The antimicrofilarial efficacy of *Trachispermum ammi* extracts in vitro and in vivo using *Setaria cervi* as a model, was investigated. *T. ammi* seed extracts were prepared using different solvents (with increasing order of polarity of the solvent) including petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol, and methanol. The extracts were tested for in vitro antimicrofilarial activity. The ethanolic and the methanolic extracts showed maximum activity in causing flaccidity in the microfilariae. The extracts were potent even at concentrations as low as 5 µl/ml. When orally administered to experimentally infected rats, the extracts eliminated circulating microfilariae within 2 weeks. It is inferred that the antimicrofilarial molecule(s), are polar in nature. They induce flaccidity in the microfilariae, by possibly inhibiting monoamine oxidase. This communication supplements the ethnopharmacological information for the use of *T. ammi* as an antihelminthic, and indicates that *T. ammi* could be used as a potential source of antimicrofilarial drugs.

Keywords *Setaria cervi* · Filaria · In vivo · *T. ammi* · Microfilariae

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Filariasis, including lymphatic filariasis, onchocerciasis and loiasis, caused by parasitic filarial nematodes, encompasses several chronic and painful infections and leads to considerable morbidity in tropical and subtropical countries (Katiyar and Singh 2011). Despite the fact that these infections have been known for a long period of time, the discovery of safe and novel antifilarial agents is widely spaced. Keeping in view the occurrence of filarial infections in humans and livestock and drug resistance being rampant in the field, there is a need to develop both novel non-chemical approaches for parasite control and molecular assays capable of detecting resistant worms (Kaplan 2004).

The adults of nematode parasite, *Setaria cervi* inhabit the peritoneum of the bovine host, where as its larval stages, the microfilariae, appear in the peripheral circulation of the host. Common symptoms of infection include anorexia, weakness, pyrexia, congestion of conjunctiva and watery discharge from nostrils. However, in severe cases, lumber paralysis due to pathological lesions consisting of focal malacia, microcavitation, perivascular infiltration of lymphocytes and disappearance of axis cylinder and myelin sheath is seen (Ghani 2002). A characteristic feature of this infection is the appearance of microfilariae in the body fluids of the host.

In view of the practical difficulties in procuring human strains of *Wuchereria bancrofti* and *Brugia malayi* for drug screening, the bovine filarial parasite *S. cervi*, resembling the human parasites in having microfilarial periodicity and chemotherapeutic response to known antifilarial agents, is widely used as a model to screen potential antifilarial agents (Ahmad and Srivastava 2007).

Trachispermum ammi or Bishop's weed (commonly known as Ajwain in North India) a native to Egypt, is now commonly cultivated in South East Asia (Khan et al. 2010).

The seeds of this plant, besides being used as a spice, have been extensively used as a folk medicine to clear systemic helminth infections, both in humans and domestic animals. *T. ammi* has also been shown to be effective against bacterial infections (Khan et al. 2010).

The present study was carried out to assess the efficacy of *T. ammi* seed extracts as potential antifilarial agents against filariid nematodes, using *S. cervi* as a model, both in vitro and in vivo.

Trachispermum ammi seeds were procured from the local market. The species was identified and authenticated by the Department of Botany, Aligarh Muslim University, Aligarh, India. The seeds were washed with distilled water and dried in shade. The dried seeds were powdered and the powder refluxed with absolute ethanol for 6 h. Successive extraction of the powder was done with the help of Soxhlet apparatus in different solvents with increasing order of polarity of the solvent. The solvents included petroleum ether (E1), diethyl ether (E2), chloroform (E3), ethyl acetate (E4), acetone (E5), ethanol (E6) and methanol (E7). The solvents were evaporated at a constant temperature of 60°C until a very concentrated extract was obtained, and no detectable solvent remained in the extracts. The working concentrations of the extracts were prepared in Hanks' balanced salt solution (HBSS).

Active female *S. cervi* (Fig. 1), from freshly slaughtered field infected Indian Water Buffalo (*Bubalis bubalis*) were collected in HBSS (pH 7) premaintained at $37 \pm 1^\circ\text{C}$. The worms were washed in three changes of HBSS to remove debris and were used within 2 h of harvesting from the host. The active females were placed in HBSS with 2.7% EDTA in a glass petri plate. Using a fine needle under a dissection microscope, the uterus was gently teased apart to release microfilariae in the medium. The debris of the female worm was carefully removed and the medium containing motile microfilariae was pooled. Iso-osmotic percoll was prepared by mixing 9 parts of percoll (density 1.130 g/ml, Sigma) with one part of 2.5 M sucrose. Dilution was made to obtain 30% solution of density 1.062. Over a solution of 5 ml percoll, 0.5 ml medium containing motile microfilariae and the blood of an infected host (diluted in the ratio of 1:9 with normal saline containing 2.7% EDTA) was applied and centrifuged at $500 \times g$ for 30 min at room temperature. Centrifugation resulted in separation of the microfilariae as a separate band (Fig. 2). To count the microfilariae, 20 μl of the microfilariae rich layer was taken and counted under a compound microscope. To obtain percoll free microfilariae, HBSS was added in the ratio of 1:2 and centrifuged at $500 \times g$ for 20 min. Supernatant was discarded and pellet containing the microfilariae was retained (Fig. 3).

Around 10 microfilariae in 100 μl of HBSS, were placed in a 96 well microtiter plate. Different extracts of *T. ammi*

Fig. 1 Adult male (left) and female (right) of *Setaria cervi*. Bar is equivalent to 1.2 cm

Fig. 2 Separation of the viable microfilariae as a single band (white arrow) after percoll gradient centrifugation

seeds were applied at a concentration of 20, 10 and 5 $\mu\text{l}/\text{ml}$ in each well, with each concentration taken in triplicates. The control wells did not receive any treatment. The plate

Fig. 3 The viable microfilariae. Bar is equivalent to 145 μ m

was incubated at $37 \pm 1^\circ\text{C}$ for 45 min after which the microfilariae were observed microscopically.

Male Wistar rats, weighing between 75 and 100 g, were used for the in vivo experiment. The animals were housed in clean plastic cages and acclimatized to rat chow and water for a week. The animals were infected by a single dose intraperitoneal injections of 4 ml HBSS carrying about 2,000 microfilariae. 2 weeks post infection, the rats were bled from the tail vein daily, and a thin blood smear was prepared which was checked for circulating microfilariae. The animals with the presence of motile microfilariae circulating in peripheral blood for more than 3 days were considered infected. Five infected rats were treated with extracts E6 and E7 with an oral dose of 100 mg/kg body weight for 2 weeks daily and the microfilarial load in circulation was assessed at the end of the 2 weeks.

Results indicated all *T. ammi* extracts were mildly active, except the ethanolic and methanolic extracts, which caused total flaccidity of worms within 45 min (Fig. 4). The worms were non motile, except for spastic wriggles, which were of low frequency. The activity was apparent in low doses of extracts (5 μ l/ml). In the control experiment, all the worms were active and highly motile. Whether the effect was reversible or not, was not studied in this preliminary study.

Systemic infection in rats was cleared within 2 weeks of treatment. No apparent behavioural or toxicological effects were seen in any animal undergoing the treatment.

A previous study (Subramanyam et al. 1978), separated *L. carinii* microfilariae from defibrinated blood using ficollhyplaque of 1.05% density. The percoll method has also been used previously to isolate *B. malayi* and *D. immitis* microfilariae from the peripheral blood of host (Feldmeier et al. 1981). In the present study, microfilariae were

Fig. 4 The flaccidity inducing effect of different *T. ammi* extracts on microfilariae of *S. cervi*. The solvents included petroleum ether (E1), diethyl ether (E2), chloroform (E3), ethyl acetate (E4), acetone (E5), ethanol (E6) and methanol (E7). Experiments were carried out at $37 \pm 1^\circ\text{C}$ for 45 min. Hank's balanced salt solution served as a control, in which the microfilariae were motile at the end of the experiment (not shown)

directly isolated from the female worm and the blood of an infected host using a percoll gradient. The isolated microfilariae were viable and capable of surviving up to 2 weeks post infection in experimentally infected rats when injected intraperitoneally.

Our results indicated that the active molecule responsible for the microfilaricidal activity of *T. ammi* is a polar molecule, since the antimicrofilarial activity was localised in the methanolic and ethanolic extracts of *T. ammi* seeds. In a recent study, Mathew et al. (2008) have shown that the methanolic extract of *T. ammi* seeds has an antifilarial effect on the adult worms and causes female sterility in rodents experimentally infected with *B. malayi*. They have identified a phenolic monoterpene as the active molecule.

Besides the above discussed effects, our results also indicated that *T. ammi* extracts are capable of inducing flaccidity in the microfilariae. Flaccidity in flatworms is in part mediated by modulation of activity of parasite monoamine oxidase (Abidi and Nizami 2000). Results of a pilot study from our laboratory have indicated that the cause of flaccidity of microfilariae could be the partial inhibition of parasite monoamine oxidase by the ethanolic and methanolic extracts of *T. ammi* (Rizvi and Abidi, unpublished). Hence, a possible mode of action of the anti microfilarial activity in vivo, might involve the partial inhibition of monoamine oxidase of parasite origin, leading to the flaccidity of the microfilariae. This would make the parasite more susceptible to attack by phagocytic cells of the host immune system.

The present study shows that in vivo the systemic infection of *S. cervi* in experimentally infected rats is

cleared in 2 weeks. Since microfilariae appear in the peripheral circulation, where from they are picked up by the blood feeding female *Aedes* and *Culex* mosquitoes, clearance of circulating microfilariae from the circulation of experimentally infected rats is an important outcome of this study.

Thus it can be concluded that *T. ammi* seeds may serve as a potential source of developing novel antifilarial molecules which can attack the life cycle of filarial worms at several crucial junctures, namely the survivability of adults, the reproductive potential of the worms (Mathew et al. 2008) and the microfilariae and therefore may be used for successful elimination of these parasites. However, the pharmacokinetic and toxicological studies need to be undertaken if *T. ammi* products are to be used as therapeutic agents against filarial infections.

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