

## Antifilarial activity of *Nigella sativa* on *Setaria cervi*-an *in vitro* study

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### SUMMARY

The effect of aqueous and alcoholic extract of seeds of *Nigella sativa* (*N. sativa*) was studied on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m) complex of *Setaria cervi* (*S. cervi*). Both the extracts caused inhibition of spontaneous movements of the w.w and n.m complex characterized by initial stimulation followed by irreversible paralysis, with the exception that aqueous extract produced a partially reversible paralysis of w.w preparation. Lesser concentration of both the extracts was required to inhibit the movements of n.m complex than the w.w. Suggesting a cuticular permeability barrier. The lethal concentration 50 (LC<sub>50</sub>) and lethal concentration 90 (LC<sub>90</sub>) were 30 and 55 ng/ml for aqueous and 45 and 60 ng/ml for alcoholic extracts respectively.

**Key words:** *Nigella sativa*; Antifilarial; *Setaria cervi*

### INTRODUCTION

*N. sativa* Linn (family- Ranunculaceae) commonly known as Kalajira, Kalongi, Habbet el beraka (Arabic name), is a grassy plant, cultivated in many parts of India for its seeds which contain essential oil, fixed oil and saponin (Watt, 1965). It is believed to have several medicinal properties like carminative, digestive, astringent and diuretic (Kirtikar and Basu, 1933; Stlnmtez, 1957; Nadkarni, 1960; Atal and Kapoor, 1977). Several therapeutic effects in digestive disorders, gynecologic diseases, and respiratory system have been ascribed to the seeds of *N. sativa* in old Iranian medical texts (Ave-Sina, 1990). Al-Ghamdi (2001) has reported anti-inflammatory, analgesic, and antipyretic activity of *N. sativa*. Seeds of *N. sativa* also possess antibacterial, antifungal, and anthelmintic properties

(Topozada *et al.*, 1965; Rakesh *et al.*, 1979). In the present study, the aqueous and alcoholic extract of seeds of *N. sativa* was evaluated for its anthelmintic potential using a cattle filarial parasite *S. cervi*.

### MATERIALS AND METHODS

Seeds of *N. sativa* were obtained from commercial source authenticated by a botanist, cleaned and ground with the help of an electric grinder. The powder obtained (100 g) was filtered through a fine muslin cloth and was transferred to thimbles of Whatman filter paper No.1 in Soxhlet apparatus, ethyl alcohol was used as a solvent for alcoholic extract whereas distilled water for aqueous extract. The apparatus was allowed to run for 48 - 72 hours. Later the solvent was allowed to evaporate in a vacuum dessicator and after the complete evaporation of the solvent, the residual material obtained (22.5 g of alcoholic and 2 g of aqueous) was diluted with saline to make a stock solution of 1 mg/ml. Motile adult *S. cervi* (Nematoda filarioidea)

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of average length  $6.0 \pm 1.0$  cm were collected from the peritoneal cavity of freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing modified Ringer's solution (NaCl 9 g, KCl 0.42 g,  $\text{CaCl}_2$  0.24 g  $\text{NaHCO}_3$  0.5 g, glucose 0.25 g per liter) at  $37^\circ\text{C}$ .

#### Whole worm (w.w.) preparation

Adult *S. cervi* were suspended in an ideal isolated organ bath of 20 ml capacity, in modified Ringer's solution at  $37^\circ\text{C}$ . Spontaneous movements of the worm were recorded on a slow moving kymograph drum. Air or Oxygen was not bubbled through the solution as it did not improve the movements of the worm. About 15 min were allowed for the movements of worm to stabilize before eliciting the response of drug. The drug was added in increasing concentration to the bath fluid and allowed to remain in contact for 15 min. If there was no response it was considered inactive.

#### Nerve-muscle (n.m) complex

A worm was placed in a petridish containing modified Ringer's solution ( $37^\circ\text{C}$ ). Two dissecting needles were inserted into the worm at one end, and the cuticle was split longitudinally. The intestine and uterus were cut at both ends and removed. The anterior 1 cm of the worm was removed to eliminate the influence of the nerve ring and cephalic ganglia. The remaining part was tied at either end and suspended in an isolated organ bath, containing modified Ringer's solution at  $37^\circ\text{C}$ . The preparation served to expose the n.m. complex directly to the action of the drugs, and also could exhibit spontaneous rhythmical movements similar to those of the whole worm. The drug concentrations were tested for their response as with whole worm preparation. The concentration of extract which modified the movements was tested in at least six preparations.

#### Collection of microfilariae (m.f.)

The uterus of a female *S. cervi* was cut at its

junction with the vagina just below the bifurcation, and removed from the worm. It was teased with a fine needle in the solution and microfilariae (mf) were freed. The microfilariae were suspended in a human serum : ringer mixture and the mf count was adjusted to 100/ml. 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing alcoholic and aqueous extract of *N. sativa* seeds in equal serum : ringer mixture (v/v). *N. sativa* seed extract was added in doubly increasing concentration from 5 ng/ml. The bottles were kept in an incubator at  $37^\circ\text{C}$  and examined under a microscope every 30 min till 6 hours to observe the survival/mortality of microfilariae. The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  were calculated from a concentration vs death graph.

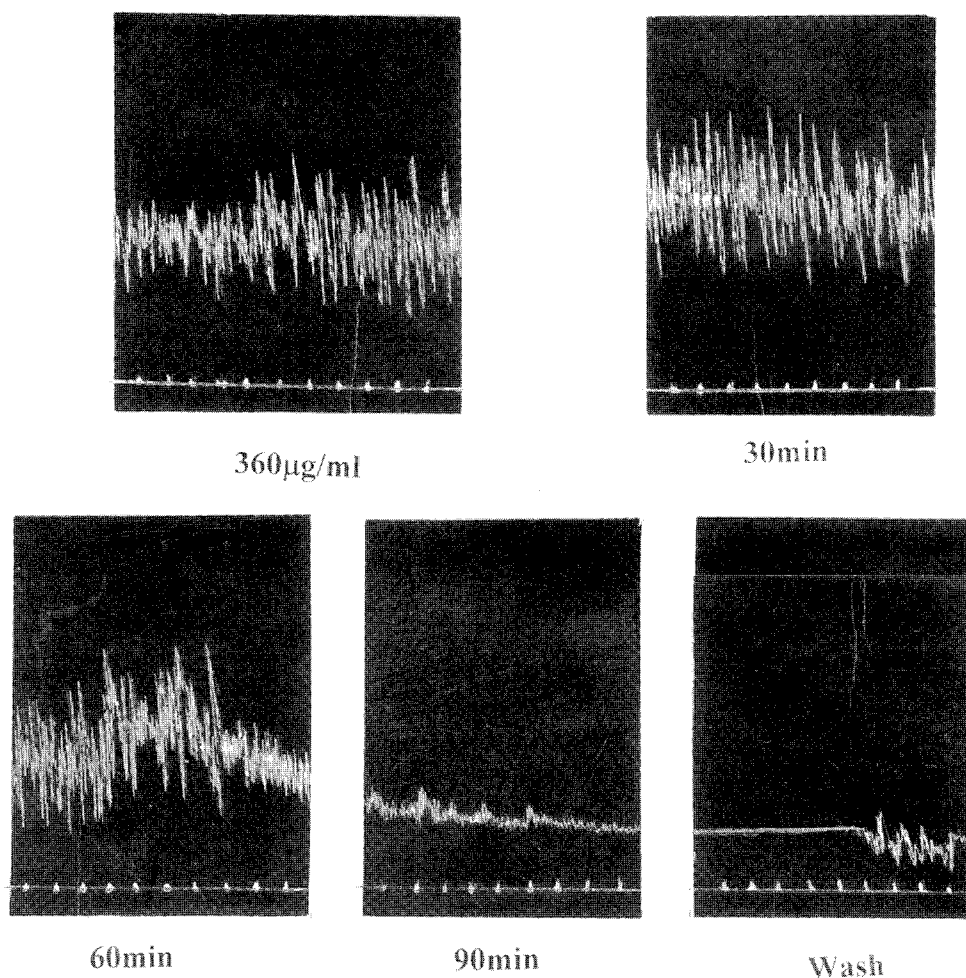
Before starting the experiment it was ascertained that the concentration of alcohol/water in the suspending medium did not influence the survival/mortality of the m.f.

In a preliminary experiment, the aqueous and alcoholic extract of *N. sativa* seeds were added to m.f. in concentration of 5, 10, 15, 20, 25 ng/ml to determine the limits of activity within 6 hours at  $37^\circ\text{C}$ , within these limits six concentrations were selected to observe the survival of m.f. The effect of each dose was observed 10 times. The mean of the values were plotted on a graph.

## RESULTS

#### Effect of aqueous extract of *N. sativa* seeds on spontaneous movements of whole worm (w.w) preparation and nerve muscle (n.m) complex of *S. cervi*

On addition of aqueous extract in a concentration of  $360 \mu\text{g/ml}$  of bath fluid the response was immediate. There was initial stimulation characterized by increase in amplitude and rate of movements, tone was not affected (Fig. 1, upper panel), the effect lasted for 60 min, was followed by marked decrease in amplitude and tone, leading to complete cessation of movements after 90 min



**Fig. 1.** The initial stimulatory effect of 360 µg/ml of aqueous extract of *N. sativa* seeds on spontaneous movements of w.w preparation of *S. cervi* leading to partial reversible paralysis.

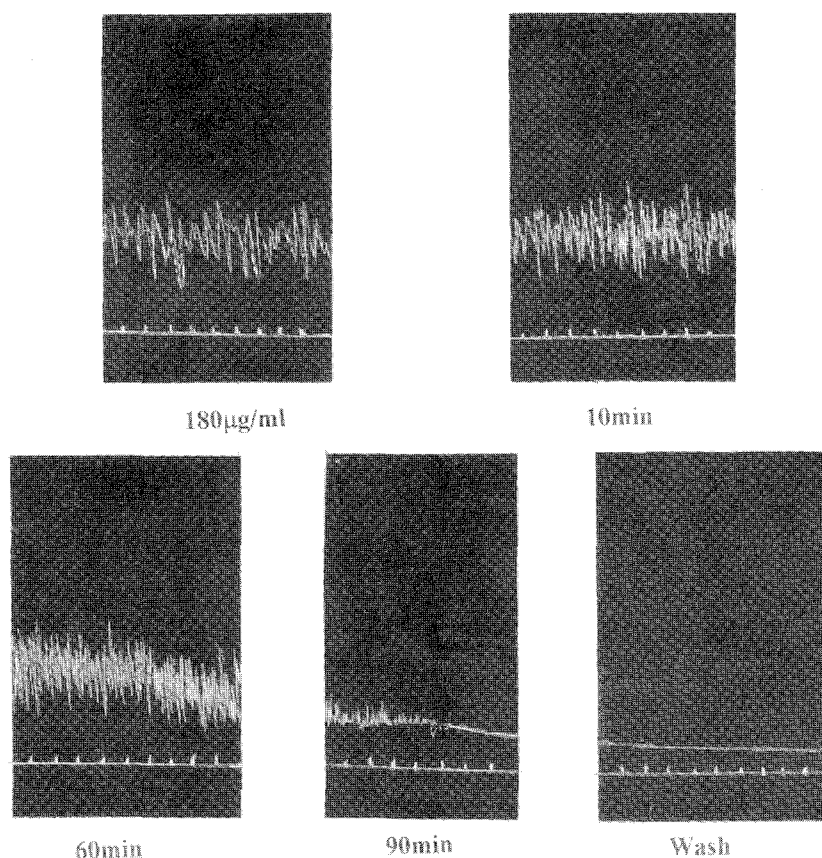
followed by reversible paralysis of worm (Fig. 1, lower panel). Motility did not reverse spontaneously till 6 hours (time for which it was observed). However repeated changes of the bath fluid restored the activity.

The response to aqueous extract could be elicited on n.m complex in a concentration of 180 mg/ml of bath fluid which is half the concentration required for the w.w. The initial stimulant effect characterized by increase in frequency of movements only (upper panel Fig. 2), was followed by a decrease in tone at 60 min, movements ceased at 90 min leading to irreversible paralysis (Fig. 2, lower

panel). Repeated changes of bath fluid failed to restore the movements. Addition of acetylcholine (Ach) during paralysis did not restore the movements.

#### **Effect of alcoholic extract of *N. sativa* seeds on spontaneous movements of w.w preparation and n.m complex of *S. cervi***

Addition of alcoholic extract in a concentration of 800 mg/ml to the bath fluid caused initial stimulation characterized by increase in amplitude, tone and frequency of movements which lasted for about 60 min (Fig. 3, upper panel), followed by a



**Fig. 2.** Irreversible paralysis of movements of n.m complex of *S. cervi* with 180 mg/ml of aqueous extract of *N. sativa* seeds.

decrease in amplitude and frequency of movements at 90 min leading to irreversible paralysis. Repeated changes of bath fluid or addition of Ach failed to restore the motility (Fig. 3, lower panel)

Alcoholic extract could produce effect of similar nature i.e, stimulation (Fig. 4, upper panel) and irreversible paralysis (Fig. 4, lower panel) on n.m complex in a concentration of 560 µg/ml.

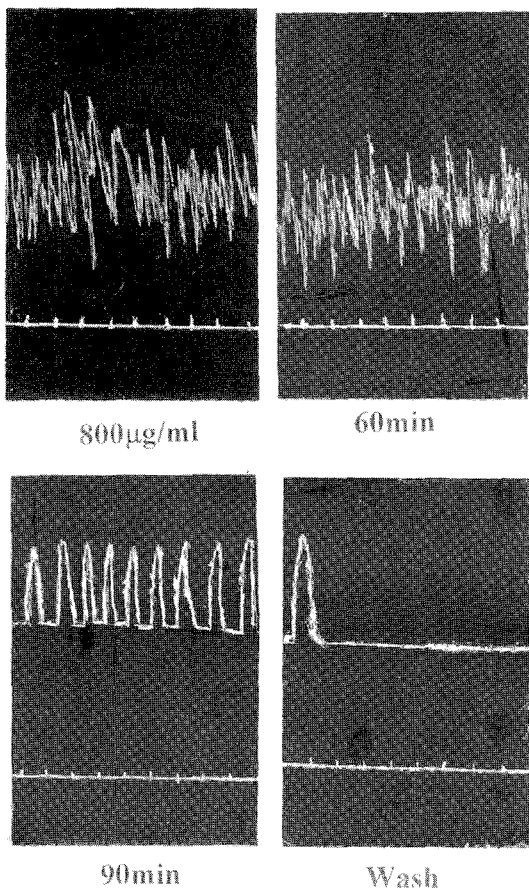
#### **Effect of extract of *N. sativa* seeds on the survival of m.f of *S. cervi* *in vitro***

The aqueous and alcoholic extract of *N. sativa* seeds caused a concentration related inhibition of survival of m.f of *S. cervi*. The aqueous extract was more lethal as compared to alcoholic extract. The time related lethal effect at a concentration of 25 ng/ml of *N. sativa* is shown in Fig. 5. The LC<sub>50</sub> and

LC 90 seen after 6 hours is shown in Table 1.

## **DISCUSSION**

The effect of aqueous and alcoholic extract of *N. sativa* seeds on spontaneous movements of *S. cervi* was different in nature, suggesting involvement of more than one active principle in the outcome of the study. Both the extracts produced inhibition of the movements of w.w and n.m complex of *S. cervi* followed by irreversible paralysis, except for the effect of aqueous extract on w.w where the inhibition is followed by partially reversible paralysis which could be due to presence of two active principles. Although the nature of response in both the intact as well as n.m complex was inhibitory, a smaller dose of the extract was

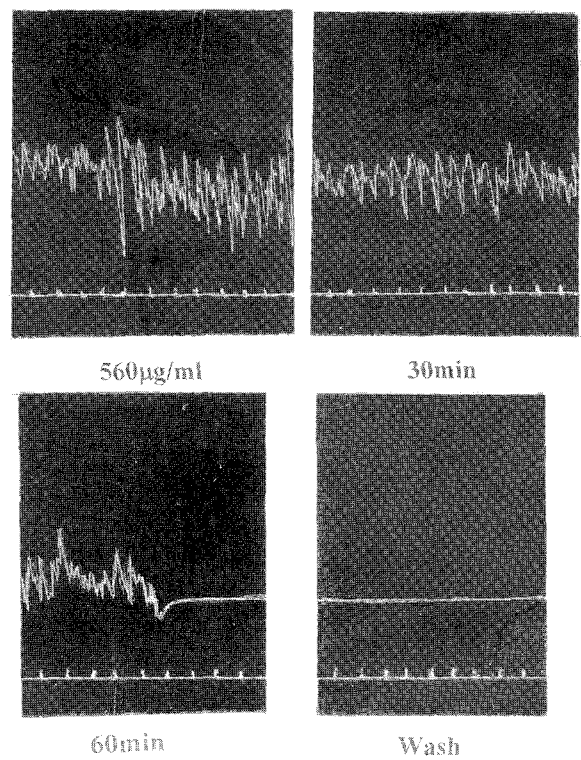


**Fig. 3.** Initial stimulation leading to irreversible paralysis of movements of w.w preparation of *S. cervi* with 800 mg/ml of alcoholic extract of *N. sativa* seeds.

required to produce an equivalent effect in the n.m complex suggesting the existence of cuticular permeability barrier for the drug.

It may be noted that during the phase of paralysis addition of acetylcholine to the bath did not produce the typical stimulation as is seen otherwise, indicating that the effect could be due to the blockade of cholinergic receptors.

A variety of anthelmintic agents selectively alters the neuromuscular transmission of the nematodes, Ach applied to the bath fluid produces stimulation of the rhythmic movements of *S. cervi*, which can be partially blocked by d-tubocurarine and not by atropine (Singhal et al., 1977). The Ach receptors of *S. cervi* are similar in nature to *Ascaris*



**Fig. 4.** Stimulatory effect of 560 mg/ml of alcoholic extract of *N. sativa* seeds leading to irreversible paralysis of movements of n.m complex of *S. cervi*.

**Table 1.** Effect of Aqueous and Alcoholic extract of *N. sativa* seeds on the survival of m.f. of *S. cervi* in vitro at 6 hours

Extract LC	Concentration (ng/ml)
Aqueous LC <sub>50</sub>	30
Aqueous LC <sub>90</sub>	55
Alcoholic LC <sub>50</sub>	45
Alcoholic LC <sub>90</sub>	60

Ach receptors (Baldwin and Moyle, 1949; Natoff, 1969; Aubry et al., 1970; Eyre, 1970; Singhal et al., 1978; Singhal and Saxena, 1978). Drugs like levamisole and pyrantel pamoate produce stimulation of movements of w.w and n.m complex of *S. cervi* followed by irreversible paralysis. The response with aqueous and alcoholic extract of seeds of *N. sativa* was similar to that produced by levamisole and pyrantel palmoate except that the stimulatory phase was

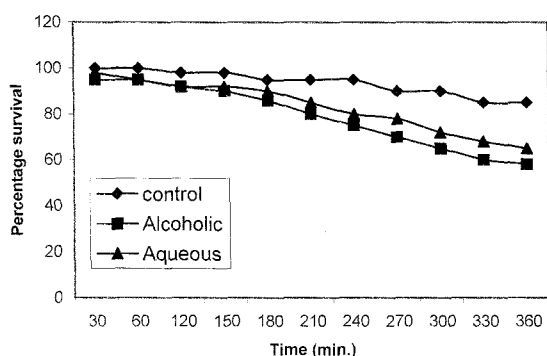


Fig. 5. Survival of m.f of *S. cervi* in vitro at a concentration of 25 ng/ml of aqueous and alcoholic extract of *N. sativa* seeds.

more prolonged. Although the response of *N. sativa* seed extract is similar to the two drugs on *in vitro* preparation of *S. cervi*, it can be concluded that *N. sativa* seeds possesses potential antifilarial activity which can provide lead for the development of a new derivative. Further studies are required to evaluate this activity in *in vivo* models. The survival time of microfilariae (mf) was reduced in a concentrated related manner, if this concentration can be presented to the m.f *in vivo*, it could prove to be a useful tool for the treatment of filariasis.

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