

## IN VITRO ANTIFILARIAL ACTIVITY OF *SENCIO NUDICAULIS* BUCH. HAM. - EFFECT ON *SETARIA CERVI* (NEMATODA FILARIOIDEA)

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**Abstract :** The effect of aqueous and alcoholic extracts of the leaves of *Sencio nudicaulis* Buch. Ham. was studied on the spontaneous movements of the whole worm and nerve-muscle preparation of *Setaria cervi* and on the survival of micro-filariae *in vitro*. Aqueous as well as alcoholic extracts caused inhibition of spontaneous motility of the whole worm and nerve-muscle preparation of *S. cervi* characterized by decreased amplitude, rate and tone of contractions. The concentration required to inhibit the movements of n.m. preparation was 1/3rd for aqueous and 1/20th for alcoholic extract suggesting a cuticular permeability barrier. The effect of *S. nudicaulis* extracts was different than that produced by calcium channel blocker nifedipine on the whole worm and n.m. preparation. While nifedipine blocks the stimulant effect of Ach the extracts of *S. nudicaulis* fails to do so. While the response bears similarity with DEC which also does not block Ach response. Both aqueous and alcoholic extracts exhibited microfilaricidal action *in vitro* LC<sub>50</sub> and LC<sub>90</sub> being 10 and 15 ng/ml for aqueous extract, 5 and 12 ng/ml for alcoholic extract.

**Key words :** *sencio nudicaulis* *setaria cervi* antifilarial activity  
nerve-muscle preparation microfilaricidal

### INTRODUCTION

*Sencio nudicaulis* Buch. Ham. of family Compositae is indigenous to temperate Himalaya. Ragwort poisoning due to several species is well known in animals, various species produced hepatic cirrhosis (1). Phytochemical studies of the *S. nudicaulis* species revealed the presence of alkaloids (2), 3 $\alpha$ , 6 $\beta$ -bis (angeloxy) furanoeremophilane and  $\gamma$ -humulene (3). However, no pharmacological activity has been reported for *S. nudicaulis*.

*Setaria cervi*, a cosmopolitan nematode parasite of cattle, which is used to assess the efficacy of potential antifilarial agents bears close similarity to human filarial worms in response to drugs (4-6). The present study was designed to observe the effect to aqueous and alcoholic extracts of *S. nudicaulis* on the spontaneous movements of the whole worm and nerve-muscle preparation (n.m. preparation) of *S. cervi* (7, 8) and on the survival of microfilariae *in vitro*.

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## METHODS

Motile adult female *S. cervi* (Nematoda Filarioidea) were collected from the peritoneal cavity of freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing modified Ringer's solution (9) at 37°C. The time period between the removal of the worms from the host to the laboratory was less than 5 hrs.

**Whole worm preparation:** Adult *S. cervi* was suspended in an isolated organ bath of 20 ml capacity in modified Ringer's solution at 37°C. Spontaneous movements of the worm were recorded on a slow moving drum (4). 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 the worm to stabilize before eliciting the response of drug. The drug was added in increasing concentrations to the bathing fluid and allowed it to remain in contact for 15 min. If there was no response, it was considered as inactive. Fresh worm was used to test the higher concentration of the extract.

**Nerve-muscle preparation:** A worm was placed in a petri dish containing modified Ringer's solution (37°C). Two dissecting needles were inserted into the worm at one end, and the cuticle was split longitudinally. The intestine and the uterus were severed 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°C. This preparation served to expose the nerve-muscle complex directly to the action of the drugs, and also could exhibit spontaneous rhythmical movements similar to those of the intact worm (8).

**Collection of microfilariae:** The uterus of a female *S. cervi* was cut at its junction with the vagina and just below the bifurcation, and removed from the worm. The uterus was teased with a needle in the solution and microfilariae were freed. The microfilariae were suspended in a human serum-Ringer mixture, the count

was adjusted to 100 ml, and 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw cap bottles containing aqueous or alcoholic extracts of *S. nudicaulis* in equal serum : Ringer mixture (v/v). The bottles were kept in an incubator at 37°C and examined under a microscope after 6 h, to count the living and dead microfilariae. The  $LC_{50}$  and  $LC_{90}$  was calculated from a concentration death graph.

In a preliminary experiment, aqueous and alcoholic extracts of *S. nudicaulis* were added to microfilariae in concentrations of 5, 10, 15, 20 and 25 ng/ml to determine the limits of activity within 6 h at 37°C. Within these limits, 6 concentrations were selected to observed the survival of microfilariae.

Dried and powdered leaves of *S. nudicaulis* were extracted with ethanol and water, separately. The crude extracts were dried and dissolved in ethanol and water before use.

## RESULTS

Effects of aqueous extract of *S. nudicaulis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : A typical response of aqueous extract of *S. nudicaulis* on the whole worm (upper panel) and n.m. preparation (lower panel) of *S. cervi* is shown in Fig.1. At a concentration of 30 µg/ml the extract caused decrease in the spontaneous movements of the whole worm. The depressant effect was characterized by decrease in tone, amplitude and rate of contractions. After about 5 min the worm movements ceased completely. Repeated changes of the bathing fluid failed to restore the movements. The paralysis caused by is therefore irreversible in nature. Addition of Acetylcholine (Ach) in a concentration of 5 µg/ml failed to elicit its typical stimulant effect.

On n.m. preparation the depressant effect was similar in nature but was evident at a lower concentration of 10 µg/ml. The amplitude, tone and rate of contractions showed a steady decline leading to complete paralysis of the n.m. preparation about 10 min after the addition of drug. Repeated changes of the bathing fluid

EFFECT OF AQUEOUS EXTRACT OF SENCIO NUDICAULIS ON THE SPONTANEOUS MOVEMENTS OF SETARIA CERVI

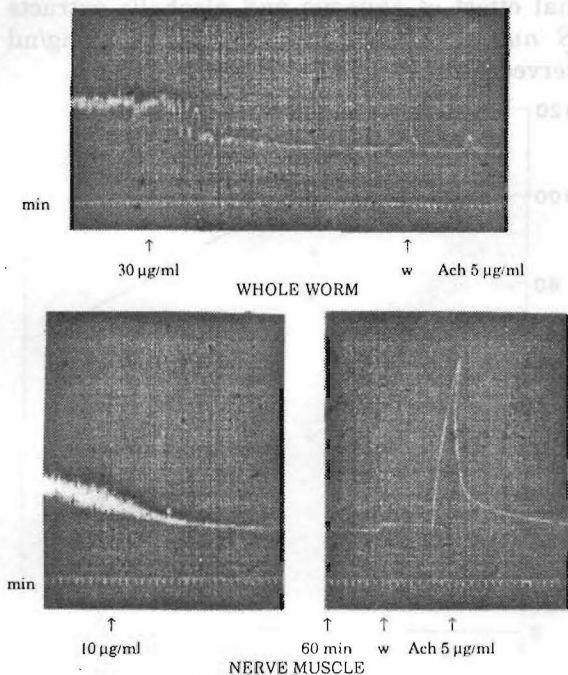


Fig. 1 : Effect of aqueous extracts of the leaves of *S. nudicaulis* on the spontaneous movements of the whole worm (upper panel) and the n.m. preparation (lower panel) of *S. cervi*. A concentration of 30 µg/ml produced irreversible paralysis of whole worm while only 10 µg/ml was required to paralyze n.m. preparation. The stimulant effect of Ach was not blocked.

failed to restore the movements till upto six hours. The preparation however responded to the addition of Ach. The response was in the form of a single spike and the preparation continued to be paralysed thereafter.

The effect of alcoholic extract of *S. nudicaulis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : The response to alcoholic extract of *S. nudicaulis* on the whole worm and n.m. preparation was not similar to that observed with the aqueous extract. Addition of alcoholic extract of *S. nudicaulis* on the spontaneous movements of whole worm in a concentration of 80 µg/ml to the bath fluid caused initial stimulation characterized by increase in amplitude which lasted for nearly five minutes

EFFECT OF ALCOHOLIC EXTRACT OF SENCIO NUDICAULIS ON THE SPONTANEOUS MOVEMENTS OF THE WHOLE WORM OF SETARIA CERVI

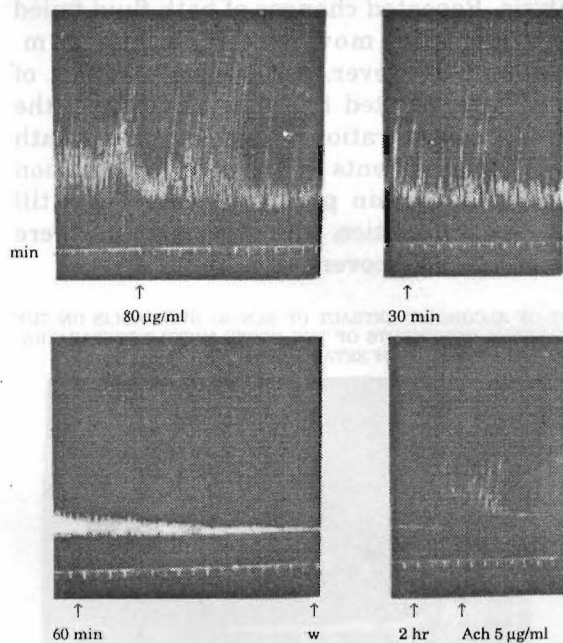


Fig. 2 : Effect of alcoholic extract of the leaves of *S. nudicaulis* on the spontaneous movements of the whole worm of *S. cervi*. Both applied concentration of 80 µg/ml caused stimulation characterized by increase in amplitude lasting for about 45 min (upper panels). Thereafter the amplitude started decreasing leading to irreversible paralysis. Response to Ach is seen in the lower right panel.

(Fig.2). Thereafter the amplitude started showing a decrease but occasional spikes of high amplitude were seen at irregular intervals. After about 60 min these spikes of high amplitude were not seen and the amplitude continued to decrease and in another about 20 min the movements of the worm ceased completely. Repeated washings with the bathing fluid failed to restore the movements. The paralysis induced was therefore irreversible. When Ach was added to the bath fluid at this time a typical response was observed in the form of stimulations lasting about 5 min.

On the n.m. preparation the effect was evident in a concentration 20 times less than that required for the whole worm preparation. The response was characterized by initial short

lasting (2-3 min) stimulation followed by paralysis. Repeated changes of bath fluid failed to restore the movements of the n.m. preparation. However, the stimulant effect of Ach could be elicited following addition of the drug in a concentration of 5  $\mu\text{g/ml}$  of the bath fluid. The movements of the n.m. preparation continued to remain paralysed upto 6 hrs till when the preparation was observed and there was no sign of recovery (Fig.3).

EFFECT OF ALCOHOLIC EXTRACT OF *SENCIO NUDICAULIS* ON THE SPONTANEOUS MOVEMENTS OF THE NERVE MUSCLE PREPARATION OF *SETARIA CERVI*

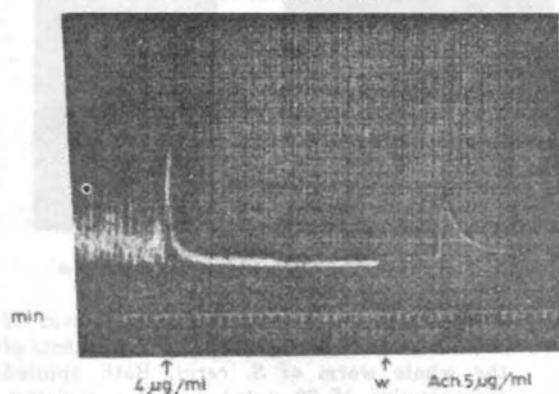


Fig. 3 : Effect of alcoholic extract of the leaves of *S. nudicaulis* on the spontaneous movements of the n.m. preparation of *S. cervi*. A concentration of 4  $\mu\text{g/ml}$  caused initial stimulation followed by irreversible paralysis. Repeated washings failed to restore the movements (w). Ach (5  $\mu\text{g/ml}$ ) produced its usual stimulant effect during the phase of paralysis.

TABLE I : Effect of aqueous and alcoholic extracts of leaves of *Sencio nudicaulis* on the survival of micro-filariae of *Setaria cervi* *in vitro*.

L.C.	Concentration ng/ml
Aqueous extract	
LC <sub>50</sub>	10
LC <sub>90</sub>	15
Alcoholic extract	
LC <sub>50</sub>	5
LC <sub>90</sub>	12

Aqueous and alcoholic extracts of *S. nudicaulis* leaves caused concentration related effect on the survival of microfilariae of *S. cervi*. The LD<sub>50</sub> and LD<sub>90</sub> as observed after 6 hrs is presented in Table I. The alcoholic extract being more potent in its lethal effect as compared

to the aqueous extract. Concentration related lethal effect of aqueous and alcoholic extracts of *S. nudicaulis* in a concentration of 35 ng/ml observed for 210 min is shown in Fig.4.

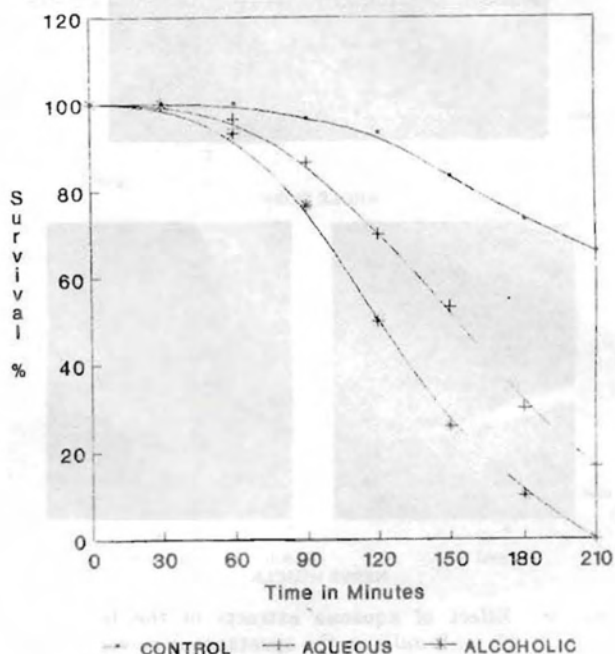


Fig. 4 : Effect of aqueous and alcoholic extracts of the leaves of *Sencio nudicaulis* on the survival of microfilariae of *Setaria cervi* *in vitro* at a concentration of 25 ng/ml. Abscissa denotes time in min and ordinate denotes percentage of survival.

## DISCUSSION

Our experience has demonstrated that the response of isotonic contractions from the filarid, *S. cervi* is an easy and reliable method for observing the effect of drugs on the spontaneous motility. The parasite a normal inhabitant of the peritoneal cavity of buffalo *Bubalis bubalus* linn. is a thin thread like structure responds quickly to neurohumors and other drugs. The magnitude of spontaneous activity after an initial period of stabilization (about 15 min) is sustained for several hours. The nerve muscle complex prepared by slitting open the worm longitudinally and removing the nerve ring is more sensitive to drugs as compared to the intact filarid. In intact worm, cuticle presents a

permeability barrier for the drugs, and prevents totally or partially, pharmacological tools, to reach the sites of action.

Aqueous and alcoholic extracts of *S. nudicaulis* produced relaxation of spontaneous movements of whole worm and n.m. preparation of *Setaria*. The concentration required to produce an equivalent effect in n.m. preparation was 3 and 20 times less for aqueous and alcoholic extracts respectively as compared with the whole worm, indicating that aqueous extract is likely to penetrate the cuticular barrier with ease as compared to the alcoholic extract.

The presence of acetylcholine, the stimulation of whole worm and n.m. preparation (8) and the presence of cholinesterase (10) has led to the suggestion that Ach is the excitatory transmitter at neuromuscular junction in *S. cervi*. Extensive studies with other nematodes like *Ascaris suum* has assigned Ach a role of excitatory neurotransmitter (11, 12). The action of Ach is dependent upon the availability of  $Ca^{++}$  ions. In absence of calcium in the bathing fluid the spontaneous motility of both whole worm and n.m. preparation decreases and ceases altogether with the passage of time. Calcium channel blockers like nifedipine also cause decrease in spontaneous motility leading to paralysis of the whole worm as well as n.m. preparation (13). Aqueous and alcoholic extracts of *S. nudicaulis* cause concentration related decrease in spontaneous movements leading to irreversible paralysis. Repeated changes in bathing fluid failed to restore the movements. The preparation were viable at this stage too as indicated by their response to Ach. Ach produced an excitatory effect on both whole worm and n.m. preparation at a stage when these were irreversibly paralysed.

Both nifedipine and extracts (aqueous and alcoholic) of *S. nudicaulis* cause irreversible paralysis of whole worm and n.m. preparation of *S. cervi*. The former blocks the stimulant effect of Ach while extracts of *S. nudicaulis* does not. The evidence suggest that the mechanism of action of the two is different. *S. nudicaulis* does not produce its effect either

by blocking the ionic channels for calcium or by blocking Ach receptors.

*S. cervi* like *Ascaris* responds to GABA with hyperpolarization leading to relaxation and paralysis of whole worm and n.m. preparation. Anthelmintic piperazine (7) acts as a weak GABA agonist (14) and so also its derivative diethyl-carbamazine (DEC) used to treat brugian and bancroftian filariasis. The response of both extracts on whole worm and n.m. preparation of *Setaria* was similar to DEC which was characterized by initial short lived stimulation followed by irreversible paralysis (15).

The aqueous as well as the alcoholic extract of *S. nudicaulis* affected the survival of microfilariae adversely *in vitro*. The concentration required to kill 50 and 90% of microfilariae within a period of 6 hr being much less than that required to modify the spontaneous motility of the whole worm or the n.m. preparation. The observations indicate that the two extracts of plant are both macro and microfilaricidal *in vitro*. This differentiates the action of *S. nudicaulis* extracts and DEC, since the latter causes irreversible paralysis of the whole worm and the n.m. preparation but does not kill the microfilariae *in vitro* (16-19).

Phytochemical studies have revealed the presence of alkaloids which have not yet been characterized (2) and furanoreomophilane derivative (3) of which the pharmacological activity has not yet been studied, it is premature to assign these or any other ingredient present in the plant extracts for the macro or microfilaricidal activity. The macro and microfilaricidal action of extracts of *S. nudicaulis* that too at a low concentration is an encouraging observation. This needs to be tested in *in vivo* model. Further the isolation of active principles is also required to find the chemical moiety responsible for the action.

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