

## ***In vitro* antifilarial potential of the leaves and stem extract of *Pluchea lanceolata* on the cattle filarial parasite *Setaria cervi***

Vandna Mishra<sup>1</sup>, Nazneen Parveen<sup>2</sup>, Nizam U Khan<sup>1\*</sup> and KC Singhal<sup>3</sup>

<sup>1</sup>Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India; <sup>2</sup>Senior Secondary School (Girls), Aligarh Muslim University, Aligarh-202002, India; <sup>3</sup>Department of Pharmacology (J.N. Medical College), Aligarh Muslim University, Aligarh-202002, India

### SUMMARY

The effect of alcohol and aqueous extracts of the leaves and stem of *Pluchea lanceolata* on the spontaneous movements of both the whole worm and the nerve muscle preparation of *Setaria cervi*, and on the survival of microfilariae *in vitro* was studied. Alcohol and aqueous extracts of the leaves and stem of *P. lanceolata* caused the inhibition of spontaneous movements of the whole worm and the nerve muscle preparation of *S. cervi*, characterized by short lasting small increase in tone and amplitude of contractions followed by paralysis. The concentrations required to inhibit the movements of whole worm and nerve muscle preparations for alcohol extract were 200 and 25 µg/ml, and for aqueous extract were 250 and 100 µg/ml, respectively, suggesting a cuticular permeability barrier. Both the extracts (alcohol and aqueous) caused death of microfilariae *in vitro*, LC<sub>50</sub> and LC<sub>90</sub> being 12 and 18 ng/ml for alcohol extract and 25 and 40 ng/ml for aqueous extract, respectively.

**Key words:** *Pluchea lanceolata*; *Setaria cervi*; Antifilarial activity; Microfilaricidal

### INTRODUCTION

Filariasis is prevalent in many tropical and subtropical countries. In India, a large population is exposed to the risk of filariasis and social stigma is attached to the disease. Therefore, the plants that find ethnopharmacological uses in eastern India can provide locally available and economical alternate remedy for filariasis. The present study is an effort towards the search for potential antifilarial herbs.

*Pluchea lanceolata* (Asteraceae or Compositae), is an erect, 30-60 cms. High perennial weed found throughout India. It is an important medicinal

plant, which is widely used in indigenous system of medicine (Ayurveda) for rheumatism and allied disorders, diseases of dyspepsia, bronchitis, arthritis and inflammations.

Plant extract showed acetylcholine like action, spasmolytic action on different smooth muscle preparations and potentiated barbiturate hypnosis (Rastogi and Mehrotra, 1960). Anti-inflammatory activity has been reported from the petroleum extract of the stem and leaves (Chawla *et al.*, 1991) and roots (Chawla *et al.*, 1990) of *Pluchea lanceolata*. A pentacyclic triterpene, neolupenol, isolated from flowers of *P. lanceolata* also showed anti-inflammatory activity (Kaith, 1996). No attempt has been made to study the activity of *P. lanceolata* extracts against any filarial parasite. Hence in the present study the ethanol and aqueous extracts of *P.*

\*Correspondence: Nizam U Khan, Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India. Tel: +91-09358255577; E-mail: nizamukhan@yahoo.com

*lanceolata* were screened to observe the effect on whole worm and nerve muscle (n.m.) preparation of *Setaria cervi* and on the survival of microfilariae *in vitro*. *S. cervi*, a cosmopolitan nematode parasite of cattles, such as, water buffalo (*Bubalis bubalus* Linn.), resembles closely to human filarial worm in its response to drugs, and can therefore be used for the screening of potential antifilarial agents (Singhal *et al.*, 1973). *Setaria* exhibits vigorous rhythmical movements, which can be recorded on a kymograph by suspending the worm in an isolated organ bath. The nerve muscle preparation of the worm also exhibits similar movements (Singhal *et al.*, 1977).

## MATERIALS AND METHODS

### Plant and extraction

The plant material of *P. lanceolata* was collected from the survey of medicinal plant unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India. The plant was identified by Dr. Athar Ali Khan, Department of Botany, A.M.U., Aligarh, where the voucher specimen has been deposited (Voucher no. 19605).

Dried and powdered leaves and stem of *P. lanceolata* were extracted with ethanol and water, separately. The crude ethanol and aqueous extracts were dried and dissolved in 95% ethanol and distilled water before use. The addition of 0.2 to 0.5 ml vehicle (95% ethanol or water) to the organ bath containing 20 ml of Ringer's solution had no effect on worm motility

### Collection of *S. cervi*

Motile adult *S. cervi* (Nematoda: Filarioidea) of average length ( $6.0 \pm 1.0$  cm) and of average weight ( $35 \pm 6.0$  mg) were obtained from the freshly slaughtered cattle (*B. bubalus* Linn.). It was stored in a vacuum flask containing modified Ringer's solution (NaCl 9 g, KCl 0.42 g, NaHCO<sub>3</sub> 0.5 g, CaCl<sub>2</sub> 0.42 g, glucose 0.25 g in 1 litre distilled water) at 37°C (Singhal *et al.*, 1973). The time between the removals of the worms from the host to the

laboratory was less than three hrs. In the laboratory, the worms were repeatedly washed with the same solution to free them of any extraneous material.

### 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 (Singhal *et al.*, 1975), aeration was not required as it did not improve the motility of the worm. About 15 min. was allowed for the movements of the worm to stabilize before eliciting the response to the drug. The drug was added in increasing concentration to the bathing fluid and allowed to remain in contact for 15 min., if there was no response it was considered inactive. A fresh worm was used to test each concentration of the extract, this precaution was taken to avoid a cumulative response of the residual drug in the worm.

### Nerve muscle preparation

A worm was placed in a petridish containing modified Ringer's solution. Two dissecting needles were inserted at one end of the worm and the cuticle was split longitudinally in one stroke. The anterior 1 cm of the worm was cut off to eliminate the influence of the nerve ring in the cephalic ganglia. The remaining part was tied at both ends and suspended in the isolated organ bath containing modified Ringer's solution at 37°C.

### 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 human serum: Ringer mixture, the count was adjusted to 100 microfilariae/ml, and 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing alcoholic and aqueous extracts of *P.*

*lanceolata* in an equal serum: Ringer mixture (v/v). *P. lanceolata* extract was added in doubly increasing concentrations of 5 ng/ml. The bottles were kept in an incubator at 37°C and examined under a microscope after six hrs, to count the living and dead microfilariae. The LC<sub>50</sub> and LC<sub>90</sub> was calculated from a concentration/death graph. In a preliminary set of experiments it was ascertained that the concentration of alcohol/water in a suspending medium did not influence the survival/motility of the microfilariae. Both the extracts (alcohol and aqueous) caused death of microfilariae *in vitro*. LC<sub>50</sub> and LC<sub>90</sub> were found to be 12 and 18 ng/ml for alcohol and 25 and 40 ng/ml for aqueous extract, respectively. These values were the concentration mean of 10 results.

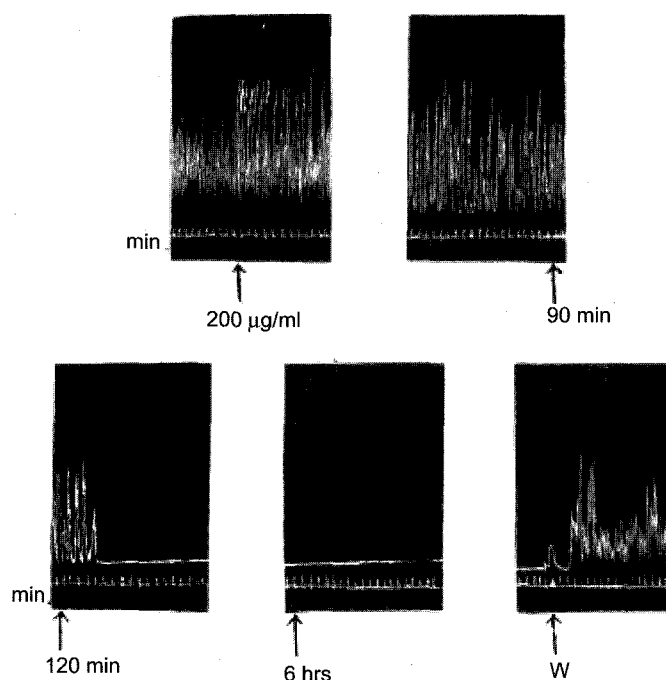
In a preliminary experiment, the alcohol and aqueous extracts of *P. lanceolata* were added to microfilariae in concentrations of 5, 10, 15, 20 and 25 ng/ml to determine the limits of activity within 6 hrs. at 37°C. within these limits, 6 concentrations

were selected to observe the survival of microfilariae. The effect of each dose was observed 10 times. The mean of the values was plotted on a graph.

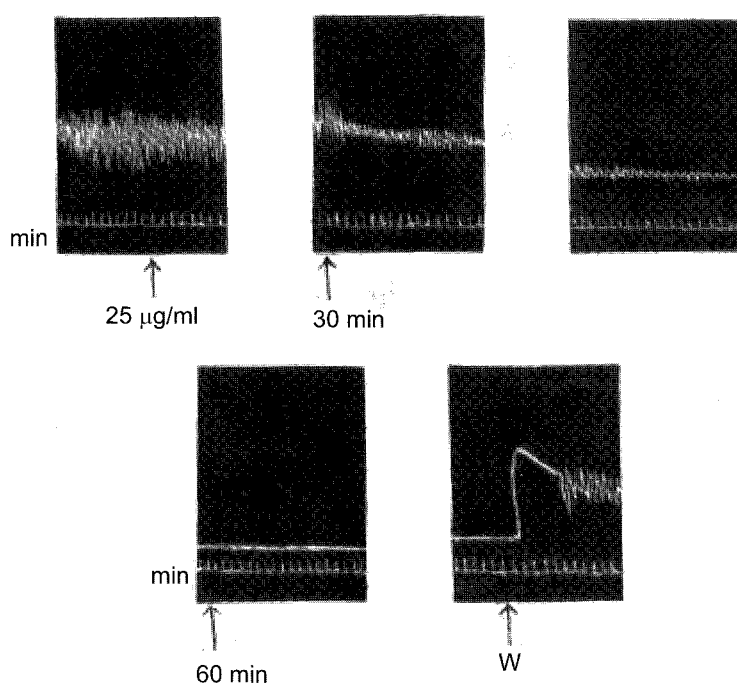
## RESULTS

### Effect of alcohol extract of the leaves and stem of *P. lanceolata* on the spontaneous movements of whole worm and nerve muscle preparation of *S. cervi*

A typical response of alcoholic extract of leaves and stem of *P. lanceolata* on the spontaneous movements of the whole worm of *S. cervi* is shown in Fig. 1. The addition of 200 µg/ml of alcoholic extract to the bath fluid modified the movements while at lower concentration it was inactive. The response was characterized by initial stimulation followed by paralysis. The initial stimulatory effect was characterized by an increase in tone and amplitude of contractions, while the rate of contractions was unaffected. The effect was evident immediately after the addition of the drug. The



**Fig. 1.** Stimulatory effect of alcohol extract (200 µg/ml) of the leaves and stem of *P. lanceolata* on the whole worm preparation of *S. cervi*. Immediate initial stimulation is observed by increase in amplitude and tone of contractions caused by spontaneous movements followed by paralysis, which is reversed after washings (W).



**Fig. 2.** Reversible effect of alcohol extract (25 µg/ml) of the leaves and stem of *P. lanceolata* shown by decrease in amplitude and rate of the contractions, and no effect on the tone of contractions on spontaneous movements of the n.m. preparation of *S. cervi*.

increase in amplitude was observed initially for about 90 min; thereafter it started declining until it attained pre-drug level.

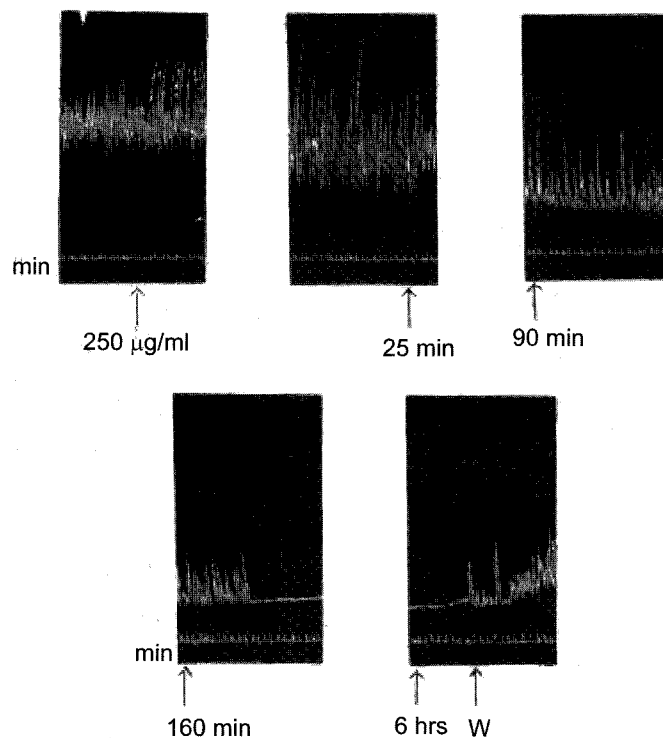
After about 130 min, activity of the whole worm ceased completely. The paralysis of the worm continued for more than 6 hours. There were no spontaneous twitching, contractions or recovery. However, on repeated changes of bathing fluid (w), the movements of the worm were restored to normal. This indicates that the paralysis caused was reversible in nature.

On the n.m. preparation, the effect of the alcohol extract of leaves and stem was manifested at a concentration as low as 25 µg/ml of bath fluid. The response was characterized by decrease in rate and amplitude of the contractions while the tone of contractions remained unaffected. The initial stimulant effect was not observed as seen in the whole worm preparation (Fig. 2). The rate and amplitude of the contractions continued to decline and after 1 hour, the activity ceased completely. However, on repeated

changes of bathing fluid (w), the movements were restored to normal. This indicates that the paralysis caused was reversible in nature.

#### **Effect of aqueous extract of the leaves and stem of *P. lanceolata* on the spontaneous movements of whole worm and nerve muscle preparation of *S. cervi***

The response to the aqueous extract of leaves and stem of *P. lanceolata* was quite similar to that observed with the alcoholic extract (Fig. 3). Addition of aqueous extract in a concentration of 250 µg/ml caused immediate initial stimulation and was characterized by an increase in amplitude and tone of contractions. The initial stimulatory effect was evident immediately after the addition of the drug. The stimulant effect lasted for about 30 minutes when amplitude of contractions started declining. The rate of contractions was unchanged initially but showed decrease after about 90 min. After about 160 min there was complete cessation of movements resulting in the paralysis. The worm



**Fig. 3.** Stimulatory effect of aqueous extract (250 µg/ml) of the leaves and stem of *P. lanceolata* on the whole worm preparation of *S. cervi*. Immediate initial stimulation is observed by an increase in amplitude and tone of contractions caused by spontaneous movements followed by paralysis, which is reversed after washings (W).

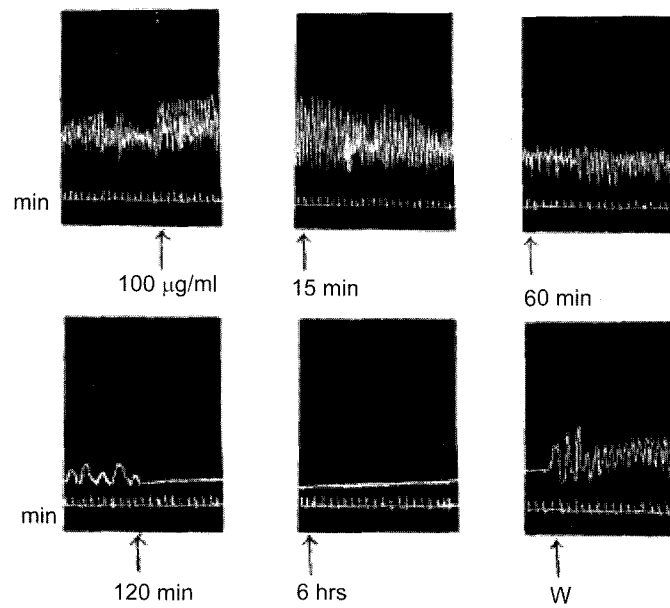
continued to remain paralysed for more than 6 hours. However, with repeated changes of the bathing fluid (w), the movements of the worm were slowly restored to normal. This indicates that the paralysis was reversible in nature.

On the n.m. preparation the effect of the aqueous extract was manifested at a concentration of 100 µg/ml of bath fluid. The onset of initial stimulation was observed after an interval of few minutes (Fig. 4), but became visibly evident after about 15 min of addition of the extract to the bathing fluid. The response was characterized by an increase in amplitude while the rate and tone of contractions remained nearly unaffected. The stimulant effect lasted for about 30 minutes. At this time, the amplitude and rate of contractions started decreasing and continued to do so till the movements of the n.m. preparation ceased completely. The paralysis

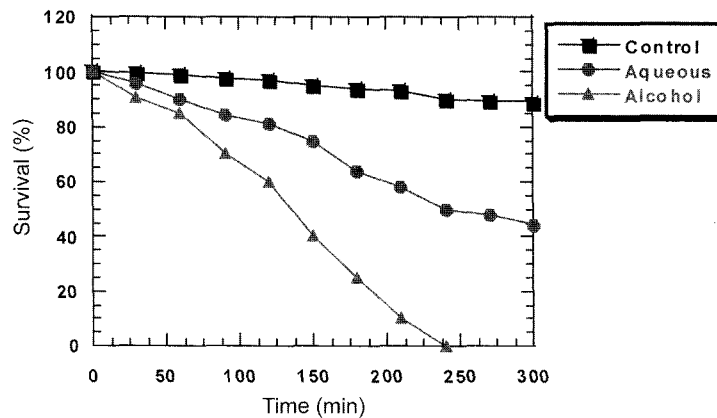
was complete and continued for more than 6 hours. The movements were however restored to normal by repeated washing of the bathing fluid (w). This indicates that the paralysis was reversible in nature.

#### **Effect of alcohol and aqueous extracts of leaves and stem of *P. lanceolata* on the survival of the microfilariae of *S. cervi***

Alcohol and aqueous extracts of leaves and stem of *P. lanceolata* caused concentration related effect on the survival of the microfilariae of *S. cervi*. The  $LC_{50}$  and  $LC_{90}$  as observed after 6 hours were 12 and 18 ng/ml and 25 and 40 ng/ml for alcohol and aqueous extract, respectively. The concentration effect of alcohol and aqueous extracts of leaves and stem of *P. lanceolata* at a concentration of 25 ng/ml observed for 300 min is shown in Fig. 5.



**Fig. 4.** Reversible effect of aqueous extract (100 µg/ml) of the leaves and stem of *P. lanceolata* on the spontaneous movements of the n.m. preparation of *S. cervi*. Initial stimulation is observed by an increase in amplitude without change in rate and tone of contractions followed by decrease in amplitude and rate of contractions. The induced paralysis was reversed after washing (W).



**Fig. 5.** The effect of alcohol and aqueous extracts of the leaves and stem of *P. lanceolata* on the survival of microfilariae of *S. cervi*.

### DISCUSSION

It is interesting to note that the effect of *P. lanceolata* extracts (alcohol and aqueous) on both whole worm and n.m. preparation was reversible paralysis. Whole worm movements were stimulated initially by both alcoholic and aqueous extracts, whereas a stimulatory action on the n.m. preparation was

only obtained with the aqueous extract. On the n.m. preparation the alcohol extract produced only paralysis without the initial stimulation. The response (i.e. initial stimulation followed by paralysis) of *Setaria* to the leaves and stem extract of *P. lanceolata* observed in the present study resembles diethylcarbamazine (DEC), a known antifilarial agent. Bath applied diethylcarbamazine, in that low doses

caused short lasting stimulation characterized by increase in amplitude followed by paralysis. Diethylcarbamazine has also been shown to decrease the glucose uptake by the adult worm of *S. cervi* suspended in modified Ringer's solution (Singhal et al., 1978). Further DEC produces reversible dose dependent depolarization of the membrane potential by antagonizing voltage sensitive K<sup>+</sup> conductance in the muscle (Martin, 1982). The effect of DEC on filarial parasite is said to be obscure (Maizels and Denham, 1992). However, DEC does not kill the microfilariae in circulation but sensitizes the microfilariae to the action of fixed macrophages, which kill them (Hawking et al., 1948).

The alcohol and aqueous extracts of leaves and stem of *P. lanceolata* reduced the survival time of microfilariae of *S. cervi* in a concentration related manner. If this concentration can be presented to the microfilariae *in vivo*, the extract of *P. lanceolata* can provide herbal remedy for the treatment of filariasis.

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