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In vitro antifilarial potential of the flower and stem extracts of *Leucas cephalotes* on cattle filarial parasite *Setaria cervi*

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Abstract:

Objective: To assess the potential antifilarial activity of the aqueous and alcoholic extracts of flower and stem prepared from *L. cephalotes.* Materials and methods: The effect of extracts was studied on the spontaneous movements of wholeworm and the nerve-muscle preparation of *Setaria cervi* and on the survival of microfilariae *in vitro*. Results: Alcoholic extracts of the flower and stem caused inhibition of the spontaneous movements of the wholeworm and of the nerve-muscle preparation of *S. cervi.* However, aqueous extracts of the flower and stem failed to modify the movements of the whole worm while caused irreversible paralysis of nerve muscle preparation characterized by decrease in rate, tone and amplitude of contractions. This may be due to failure of penetration of cuticular barrier by the aqueous extract. The alcoholic extract also decreased the duration of survival of microfilariae of *S.cervi.* Conclusion: *L. cephalotes* exhibits potential antifilarial activity against both the adult worms and the microfilariae of *Setaria cervi*. Further work is required to find its utility in human filarial infections.

Keyword: *Leucas cephalotes, Setaria cervi* antifilarial activity, microfilaricidal.

1. Introduciton

Leucas cephalotes Spreng. (Labiatae) is an erect, scaberulous or pubescent, stout annual plant, 30-100 cm. hight, found as a common weed in cultivated grounds and waste lands throughout the greater part of India, ascending upto 1,800 m. in the Himalayas [1]. In Ayurvedic medicine, the plant is described as stimulant, diaphoratic, laxative, anthelmintic, antiseptic and insecticidal. A syrup of the flowers is used as a domestic remedy for coughs and cold [2]. Phytochemical investigation of *L. cephalotes* reveals the presence of glycoside of β-sitosterol [3], iridoid glycosides [4] and laballenic esters [5].

During routine screening, the ability of the flower and stem extracts to inhibit the spontaneous mobility of filarial parasite

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Setaria cervi generated interest and it was thought worthwhile to explore anthelmintic potential of the two extracts (alcohol and aqueous). *S.cervi*, cosmopolitan nematode parasite of cattle water buffalo (*Bubalis bubalus* Linn.) resembles closely to human filarial worms in its response to drugs and can therefore be used for the screening of potential antifilarial agents [6, 7]. Setaria exhibits vigorous rhythmical movements which can be recorded on a kymograph by suspending the worm in an isolated organ bath.

Also, the nerve-muscle preparation of the worm exhibits similar movements [8]. The present study was designed to observe the effect of the aqueous and alcohol extracts of *L. cephalotes* on the spontaneous movements of the whole worm, nerve-muscle preparation and on the survival of microfilariae of *S.cervi in vitro*.

2. Materials and methods

2.1 Plant material

The plant material of *L. cephalotes* 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. The deposited voucher specimen No. is 1120.

2.2 Preparation of extract

Dried and powdered flower and stem of *L. cephalotes* 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, respectively. The addition of 0.2 to 0.5 ml vehicle (95% ethanol or water) to the organ bath containing 20 ml Ringer's solution had no effect on worm motility.

2.3 Collection of Setaria cervi

Motile adult *S.cervi* (Nematoda : Filarioidea) of average length 6.0 ± 1.0 cm and of average weight 35 ± 6.0 mg were obtained from the freshly slaughtered cattle (*B. bubalus* Linn.) and brought to the laboratory in a vacuum flask containing modified Ringer's solution (NaCl 9 g, KCl 0.42 g, $NaHCO_3$ 0.5 g, $CaCl_2$ 0.24 g, glucose 0.25 g in 1L distilled water) at 37°C [7]. The time period between the removal of the worms from the host to the laboratory was less than 3 h. In the laboratory, the worms were repeatedly washed with the same solution to free them of any extraneous material.

2.4 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 [9], 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 concentrations 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.

2.5 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 and 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.

2.6 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/ml, and 0.5 ml aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing aqueous or alcohol extracts of *L. cephalotes* in an equal serum : Ringer mixture (v/v). *L. cephalotes* 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 6 h, to count the living and dead microfilariae. The LC_{50} and LC_{90} was calculated from a concentration *vs.* death graph.

In a preliminary set of experiments it was ascertained that the concentration of alcohol/ water in the suspending medium did not influence the survival/motility of the microfilariae.

In a preliminary experiment, the aqueous and alcoholic extracts of *L. cephalotes* were added to microfilariae in concentrations of 5, 10, 15, 20 and 25 ng/ml to determine the limits of activity within 6 hours at 37°C. Within these limits, six 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.

3. Results

3.1 Effect of alcoholic extract of the flowers of L. cephalotes on the spontaneous movements of the wholeworm and nerve-muscle *preparation of S.cervi.*

A typical response of alcoholic extract of flowers of *L. cephalotes* on the spontaneous movements of the wholeworm of *S.cervi* is shown in Fig. 1. The addition of 160 μg/ml of alcoholic extract to the bath fluid modified the movements, while at lower concentrations, it was inactive.

The response was characterised by initial stimulation followed by paralysis. The initial stimulatory effect was characterised by an increase in tone and amplitude of contractions, while the rate of contraction was unaffected. The effect was evident immediately after the addition of the drug. The increase in amplitude was observed initially for about 30 min, thereafter it started declining and in another 1 h it was reduced to about 80% compared to predrug level.

After about 160 min, activity of the wholeworm ceased completely. The paralysis of the worm continued for more than 6 h. There was no spontaneous twitching, contractions or recovery. The movements were however restored by repeated changes of bathing fluid (w). This indicates that the paralysis caused was reversible in nature.

However, at high concentration, *viz.*, 260 μg/ ml, the initial stimulant effect was not observed. The effect was characterised by the decrease in amplitude and tone while rate of contractions remained unaffected (Fig. 2). After about 30 min the contractions became less frequent and ceased completely after about 1 h. The paralysis continued for more than 6 h, however, repeated changes of the bathing fluid (w) restored the movements to predrug level. This indicates that the paralysis caused was reversible in nature.

On the nerve-muscle preparation the effect of the alcohol extract was manifested at a concentration as low as 30 μg/ml of bath fluid. The alcoholic extract produced decrease in spontaneous movements characterised by decrease in amplitude, rate and tone of

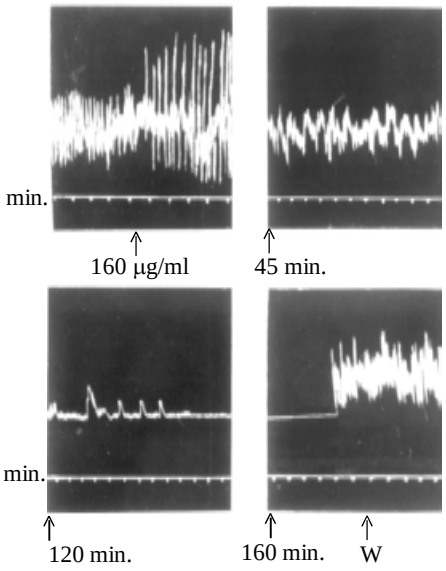

Figure 1. The stimulatory effect of 160 µg/ml alcohol extract of the flower of *L.cephalotes* on the spontaneous movements of the whole worm preparation of *S. cervi* (at low concentration).

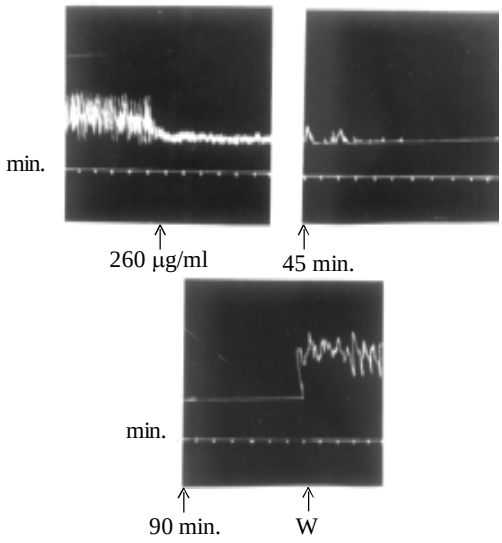

Figure 2. The reversible effect of 260 µg/ml alcholic extract of the flower of *L.cephalotes* on the spontaneous movements of the whole worm preparation of *S.cervi* (at high concentraction)

contractions. The effect was evident immediately after the addition of the drug (Fig. 3). The initial stimulant effect was not observed as seen in the whole worm preparation.

It took about 30 min for the concentration of 30 µg/ml to completely paralyse nerve-muscle preparation. The paralysis caused was partially reversed by repeated changes of the bathing fluid (w). However, the movements of the nerve-muscle preparation failed to attain predrug level. There was no indication of restoratiton of movements even after 6 h. Addition of acetylcholine (Ach) and $CaCl_2$ (both stimulants) to the bath fluid elicited their response. The response to Ach was concentration related being more with a higher concentration. (Fig. 3, lower panel).

3.2 Effect of alcohol extract of the stems of L. cephalotes on the spontaneous movements of wholeworm and nerve-muscle *preparation of S.cervi.*

The response to the alcohol extract of stems of *L. cephalotes* (Fig. 4) was not quite similar to that observed with the alcoholic extract of flower. Addition of alcohol extract in a concentration of 190 µg/ml caused immediate initial stimulation and was characterised by an increase in amplitude and tone of contractions. The rate of contraction was unchanged initially but showed a decrease after about 30 min when the increase in amplitude became highly significant and visible.

The amplitude increased by nearly 3 times of the original. The rate thereafter started declining slowly while the amplitude continued to remain increased. After about 90 min there was a further decrease in the rate of contraction which became distant and the amplitude decreased. After about 130 min the movements stopped completely. The paralysis caused was continued for more than 6 h. The movements were however not restored despite repeated

changes of the bathing fluid (w). This indicates that the paralysis caused was irreversible in nature (Fig. 4).

The effect on the nerve-muscle preparation (Fig. 5) was manifest with a concentration about 9 times less than required to affect the movements of the whole worm preparation. Addition of the alcohol extract in a concentration of 20 μg/ml caused a reduction in rate, amplitude, tone of contractions.

Nearly 30 min. after the addition of the drug, the n.m. preparation was completely paralysed and there was no spontaneous twitching, contraction or recovery even after more than 6 h. The movements of the n.m. preparation however were not restored despite the repeated washing of the bathing fluid (w). This indicates that the paralysis caused was irreversible in nature. Addition of acetylcholine (Ach) and $CaCl_2$ to the bath fluid could elicit the response.

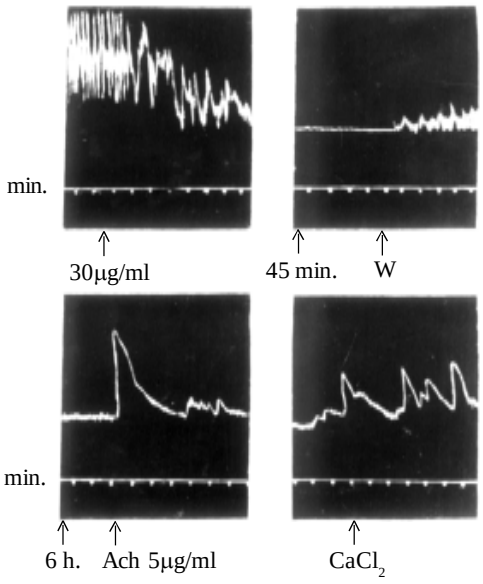

Figure 3. The partial reversible effect of 30 μg/ml alcoholic extract of the flower of *L.cephalotes* on the spontaneous movements of the nerve-muscle preparation of *S. cervi*

The response to Ach was concentration related being more with a higher concentration.

3.3 Effect of aqueous extract of the flowers of L. cephalotes on the spontaneous movements of wholeworm and n.m. preparation of S.cervi

Addition of aqueous extract of the flowers of *L. cephalotes* failed to modify the movements of whole worm of *S.cervi* at any concentration while the effect on the nerve-muscle preparation (Fig. 6) was manifest at low concentration. Addition of the aqueous extract in a concentration of 40 μg/ml caused a reduction in rate, tone and amplitude of contractions.

Nearly 40 min. after the addition of the drug, the n.m. preparation was completely pralysed. Repeated washing with the bathing fluid was not effective in restoration of the movements. This indicates that the paralysis caused was irreversible in nature. Addition of acetylcholine (Ach) and $CaCl_2$ to the bath fluid produced their stimulant effect.

3.4 Effect of aqueous extract of the stems of L. cephalotes on the spontaneous movements of wholeworm and n.m. preparation of S.cervi.

Addition of aqueous extract of the stems of *L. cephalotes* failed to modify the spontaneous movements of the whole worm of *S.cervi*, similar to that observed with the aqueous extract of flowers. However, the aqueous extract of stems on the nerve-muscle preparation produced a response similar to that observed with aqueous extract of flowers.

Addition of the aqueous extract to the bath fluid in a concentration of 40 μg/ml caused a reduction in amplitude and tone of contractions while the rate of contractions remained unaffected. The amplitude and rate of contractions continued to decrease, after about 45 min the contractions became less frequent with smaller amplitude and movements ceased

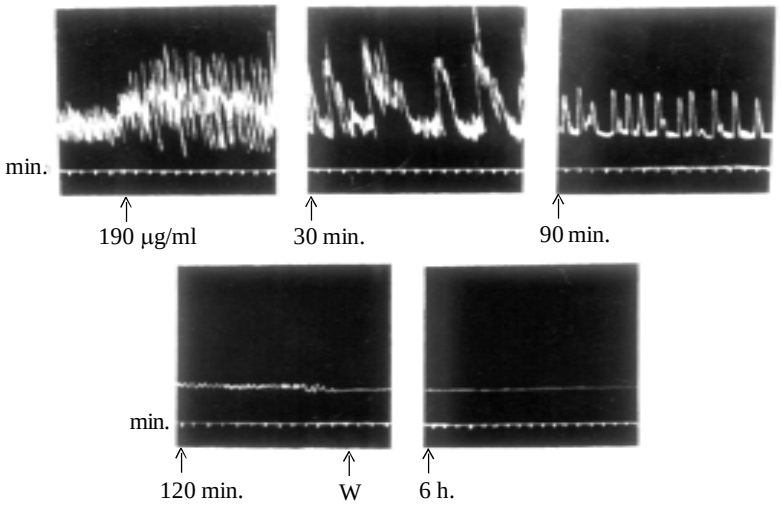

Figure 4. The irreversible effect of 190 µg/ml alcoholic extract of the stem of *L.cephalotes* on the spontaneous movements of the wholeoworm preparation of *S.cervi.*

completely after about 90 min. The movements were not restored despite repeated changes of the bathing fluid (w) (Fig. 7).

This indicates that the paralysis caused was irreversible in nature. Addition of Ach and $CaCl_2$ (both stimulants) to the bath fluid produced stimulant response. The response to Ach was concentration related being more with higher concentration (Fig. 7, lower panel).

4. Discussion

It is interesting to note that the effect of aqueous and alcohol extracts of flower and stem of *L. cephalotes* had activities different in nature from each other suggesting involvement of more than one active principle in the causation of action. While the aqueous extracts of flower and stem did not show any effect on the movements of whole worm, the aqueous extracts of flower and stem produced inhibition of the movements of the nerve-muscle preparation of *S.cervi* followed by irreversible paralysis. It is possible that the aqueous extract is not able to permeate through cuticular barrier in the intact worm. When this is removed as in case of nerve-muscle preparation, the effect is manifest.

This is seen with many substances including neurotransmitters in Setaria [8]. The alcohol extracts of flower and stem produced initial stimulation of the movements followed by paralysis of the whole worm. These too differed from each other. The alcohol extract of flower produced reversible while that of stem produced irreversible paralysis. The effect on the nerve-muscle preparation also showed variance with regard to the type of the response depending upon the part of the plant used.

Unlike to the effect of alcohol extracts of flower and stem of *L. cephalotes* on whole worm where initial stimulation was observed, on n.m. preparation it produced inhibition of movements resulting partially reversible paralysis in case of alcohol extract of flower and irreversible paralysis in case of alcohol extract of stem.

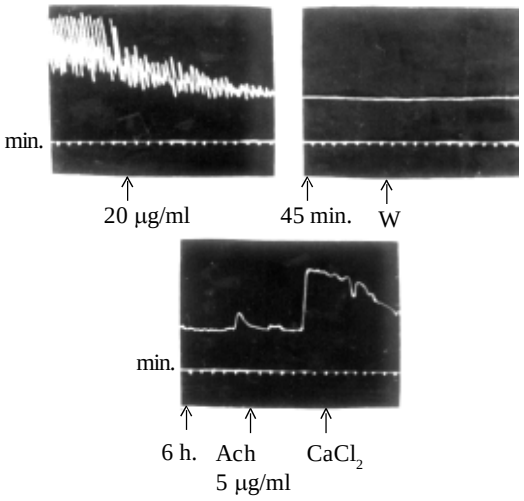

Figure 5. The irreversible effect of 20 µg/ml alcoholic extract of the stem of *L.cephalotes* on the spontaneous movements of the nerve-muscle preparation of *S.cervi.*

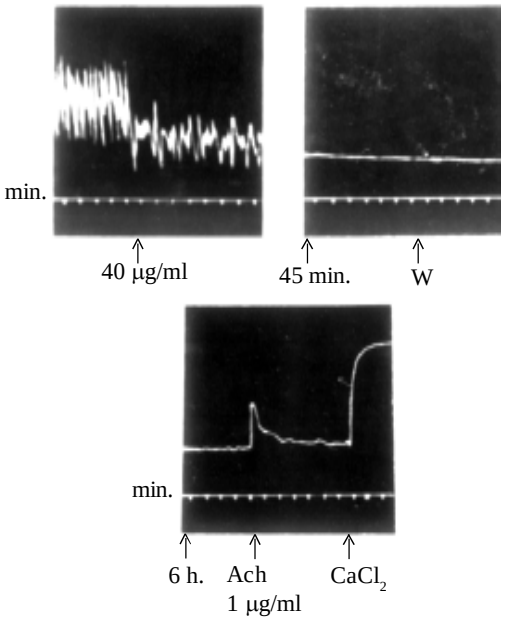

Figure 6. The irreversible effect of 40 µg/ml aqueous extract of the flower of *L.cephalotes* on the spontaneous movements of the nerve-muscle preparation of *S.cervi.*

Table 1.
The effect of alchol extracts of the flower and stem of *L.cephalotes* on the survival of microfilariae of *S. cervi in vitro*

Extract	**Concentration (ng/ml)***
Alcohol extract of flower	
LC_{50}	30
LC_{90}	50
Alcohol extract of stem	
LC_{50}	45
LC_{90}	75

* Mean of 10 replicates

We may discount the initial stimulant effect of alcohol extracts of stem and flower, as this is not manifest on the nerve-muscle preparation. In nerve-muscle. preparation the drug is in direct contact with n.m. complex of Setaria. May be the initial stimulation observed on the whole worm is due to the irritant effect on cuticle as has been seen with other substances as well, which cause irritation to the worm [10].

On the nerve-muscle preparation the aqueous and alcohol extracts of the flower and stem of *L. cephalotes* produced different type of actions. The alcohol extract of flower produced partially reversible paralysis. The other three extracts *viz.*, alcohol extract of stem and aqueous extracts of stem and flower produced irreversible paralysis.

It may be inferred that the plant contains atleast two distinct active ingredients. One causing stimulation followed by reversible paralysis and the other causing stimulation followed by irreversible paralysis. During the phase of paralysis, the stimulant effect of acetylcholine and addition of calcium chloride to the bath fluid produced its response indicating that the effect is not due to blockade of cholinergic receptors or

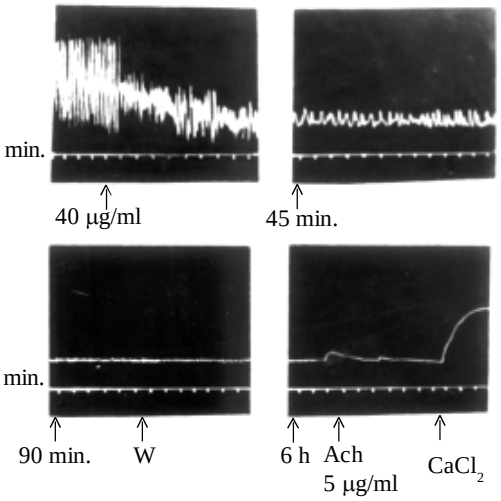

Figure 7. The irreversible effect of 40 µg/ml aqueous extract of the stem of *L.cephalotes* on the spontaneous movements of the nerve-muscle preparation of *S.cervi.*

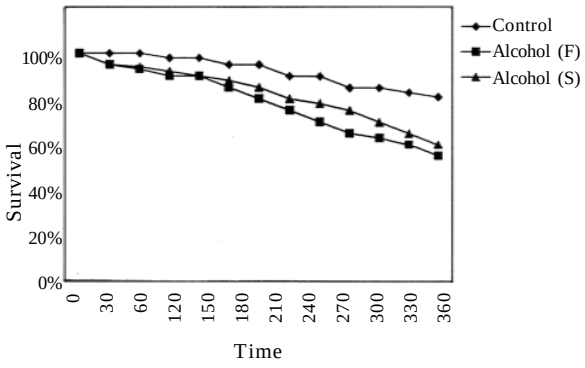

Figure 8. Effect of alcoholic extracts of the flower and stem of *L.cephalotes* on the survival of microfilariae of *S.cervi in vitro* at a concentration of 25 ng/ml

blockade of calcium channels in the whole worm or the nerve-muscle preparation of *Setaria cervi* [11].

It is likely that the response to a substance is similar in activity to diethylcarbamazine (DEC) a known antifilarial agent, in that low doses on the whole worm cause stimulation characterised by increase in amplitude followed by paralysis [12]. DEC antagonises a voltage sensitive K^+ conductance [13].

Flower and stem extracts *L. cephalotes* may provide a chemical lead for the synthesis of new derivatives which might prove to be potential antifilarial agents.

On the microfilariae of the *S.cervi*, alcohol extracts of flower and stem of *L. cephalotes* reduced the survival time in a concentration related manner. If this concentration can be presented to the microfilariae *in vivo*, the extract could be a useful tool for the treatment of filariasis. Further studies are in progress to isolate the active principle and to studies ints mechanism of action.

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