

**IN VITRO ANTIFILARIAL POTENTIAL OF THE LEAF EXTRACTS OF
FICUS INFECTORIA ROXB ON CATTLE PARASITE SETERIA CERVI**

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Summary

To find out the invitro antifilarial activity of alcoholic and aqueous extracts of the leaves of *Ficus infectoria* on *Setaria cervi* and to evaluate the LC₅₀ and LC₉₀ values for both extracts. The effect of alcoholic and aqueous extracts of the leaves of *Ficus infectoria* were studied on the spontaneous movements of both the whole worm and nerve muscle preparation of *setaria cervi* and on the survival of microfilaria in vitro. Alcoholic and aqueous extracts produced inhibition of spontaneous motility of worm and nerve muscle preparation of *setaria cervi* characterized by decrease in amplitude and tone of contractions followed by paralysis. The alcoholic and aqueous extracts of the leaves of *Ficus infectoria* showed significant antifilarial activity on whole worm, nerve muscle preparation of *setaria cervi* in vitro and showed the death of microfilaria. The concentrations required to inhibit the movement of the whole worm and nerve muscle preparation for alcoholic extract of leaves of *Ficus infectoria* were 150µg/ml and 30µg/ml respectively where as aqueous extract caused inhibition of the whole worm and nerve muscle preparation at 260µg/ml and 130µg/ml, respectively suggesting a cuticular barrier. Both alcoholic and aqueous extracts caused death of microfilaria in vitro, LC₅₀ and LC₉₀ were 10 and 21 ng/ml respectively for alcoholic, which were 14 and 26 ng/ml for aqueous extracts. Both the alcoholic and aqueous extracts of leaves of *Ficus infectoria* showed potent antifilarial activity on *setaria cervi* in vitro. On microfilariae of *setaria cervi*, alcohol extract of the leaves of *Ficus infectoria* reduced survival time in a concentration related manner. If this concentration is found effective to the microfilariae in vivo, the extract could be useful tool for the treatment of filariasis.

Keywords: *Setaria cervi*, Whole worm, Nerve muscle preparation, *Ficus infectoria* leaves, Microfilaricide.

Introduction

Filariasis is a disease of man and higher animals caused by thread like parasite round worms called filarial, belonging to genus onchocercidae. In India, about 300 million populations are exposed to the risk of filariasis infection with over 22 million infected individuals. The most common causal organisms in India are *Wuchereria bancrofti* and *Brugia malayi* which accounts for 99% of the infection. Its distribution is generally restricted to the states of Assam, Andhra Pradesh, Bihar, Gujarat, Kerala, Madhya Pradesh, Orissa, Tamil Nadu, Uttar Pradesh and west Bengal. Uttar Pradesh has been reported to have maximum number of microfilaria carriers and patients with manifestations¹. The drugs like Diethyl carbamizine citrate and Ivermectin are still the major drugs in the management of filariasis. Only few drugs are available for the treatment of filariasis from natural sources in continuation of the efforts in providing information on various tropical diseases prevalent in India, an attempt has been made for the review of antifilarial activity of the *Ficus* plants. So the present study is mainly aimed to study *Ficus infectoria* leaf extracts for its anti filarial activity.

Ficus infectoria Roxb, synonym *Ficus lacor* Buch- Ham is a large deciduous, fast growing densely foliaceous tree upto 20 m tall with well shaped crown. It is distributed in tropical and subtropical regions of the world and grown in humid tropics of India². The bark of the plant is used for leucorrhoea, for expelling round worms and for treatment of ulcers. The leaves are used for treatment of skin diseases^{3,4}.

PhytoChemical studies of *Ficus infectoria* leaves have revealed the presence of several compounds including lupeol, α - amyirin, β - amyirin, stigmasterol and campesterol. The other compounds such as infectorin, scutellarein, scutellarein glucoside, sorbifolin, bergapten, bergaptol were isolated from the whole plant^{5,6,7}.

Methods

The plant material of *Ficus infectoria* was collected from the Regional Forest Research Centre (RFRC), Rajamundry, A.P, where the voucher specimen was deposited. The plant was authenticated by Prof. M. venkaiah Department of Botany, Andhra Pradesh University, Visakhapatnam. Fresh leaves of *Ficus infectoria* were

collected during October washed, shade dried and powdered. The powder (500g) was Soxhlet extracted with alcohol and distilled water separately. The alcohol and aqueous extracts were dried and resuspended in ethanol (95%) and distilled water before use.

Present study deals with the effect of alcoholic and aqueous extracts of the leaves of *Ficus infectoria* on motility and survival of adult worm preparation, nerve muscle preparation and the survival of microfilariae in vitro of the filarial nematode parasite, *Setaria cervi*.

Setaria cervi, a nematode parasite of cattle such as buffalo (*Bubalus bubalus* Linn) resembles closely to human filarial worms in its response to drugs and can therefore, be used for the screening of potential Antifilarial agents⁸. *Setaria* exhibits vigorous rhythmic 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 movements⁹.

Collection of *Setaria cervi*:

The motile adult *Setaria cervi* (nematode 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 solution (NaCl 9g, KCl 0.42g, NaHCO₃ 0.5g, CaCl₂ 0.42g, Glucose 0.25 g dissolved in one liter of distilled water at $37 \pm 1^\circ\text{C}$)¹⁰.

The time period between the removals of the worms from the host to the laboratory was less than three hours. In the laboratory the worms were repeatedly washed with Ringers solution to remove any extraneous matter.

Whole worm preparation:

Adult *Setaria cervi* was suspended in an isolated organ bath (20 ml) containing modified Ringer solution at 37°C. Spontaneous movements of the worm were recorded on a slow moving drum¹¹. Aeration was not required as it did not improve the motility of the worm. The movements of the worm were allowed for 15 minutes to stabilize eliciting the response to the test extract. The test extract was added in increasing concentration to the bathing fluid and allowed to remain in contact for 15 minutes. 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 test extract in the bath.

Nerve –muscle preparation:

A worm was placed in a Petridish containing modified Ringer solution maintained at 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 served at both ends and removed. The anterior one 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 solution at 37°C¹². 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. The drug concentrations were tested for their response as with whole worm preparation. The concentration of the extract which modifies the movements was tested in at least six preparations.

Collection of Microfilariae:

The uterus of a female *Setaria 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 (mf) were freed. The microfilariae were suspended in 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 extracts in an equal serum: ringer mixture (v/v). The extracts were added in doubly increasing concentrations of 5ng/ml. The bottles were kept in an incubator at 37°C and examined under microscope every 30 min, un till 6 hours to count the living and dead microfilariae. The LC₅₀ and LC₉₀ were calculated from a concentration/ 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 alcoholic and aqueous extracts of the selected plants were added to microfilariae in a concentration of 5, 10, 15, 20 and 25ng/ml to determine the limits of activity with in 6 hrs at 37°C. Within these limits of activity, 6 concentrations were selected to observe the survival of microfilariae. The effect of each dose was observed ten times. The mean of the values were plotted on a graph.

Results

Effect of alcoholic extract of leaves of *Ficus infectoria* on spontaneous movements of whole worm of *Setaria cervi* in vitro

The alcohol extract was added in a concentration of 150 $\mu\text{g/ml}$ to the bath fluid modified the movements of the whole worm. The response was characterized by stimulation followed by paralysis. The initial stimulatory response was characterized by an immediate increase in tone of contractions, while rate and amplitude of contractions decreased. The stimulant effect lasted for about 30 minutes, when the tone of contractions started declining till it attained pre drug level after about 45 minutes. At this time the amplitude and rate of contractions started decreasing and continued to do so till the movements of the worm ceased completely. After about 60 minutes, the paralysis of the worm was complete and continued for more than 6 hrs. The movements were however not restored despite repeated changes of the bathing fluid (W). This indicates that the paralysis caused was irreversible in nature. The results were presented in Fig.1

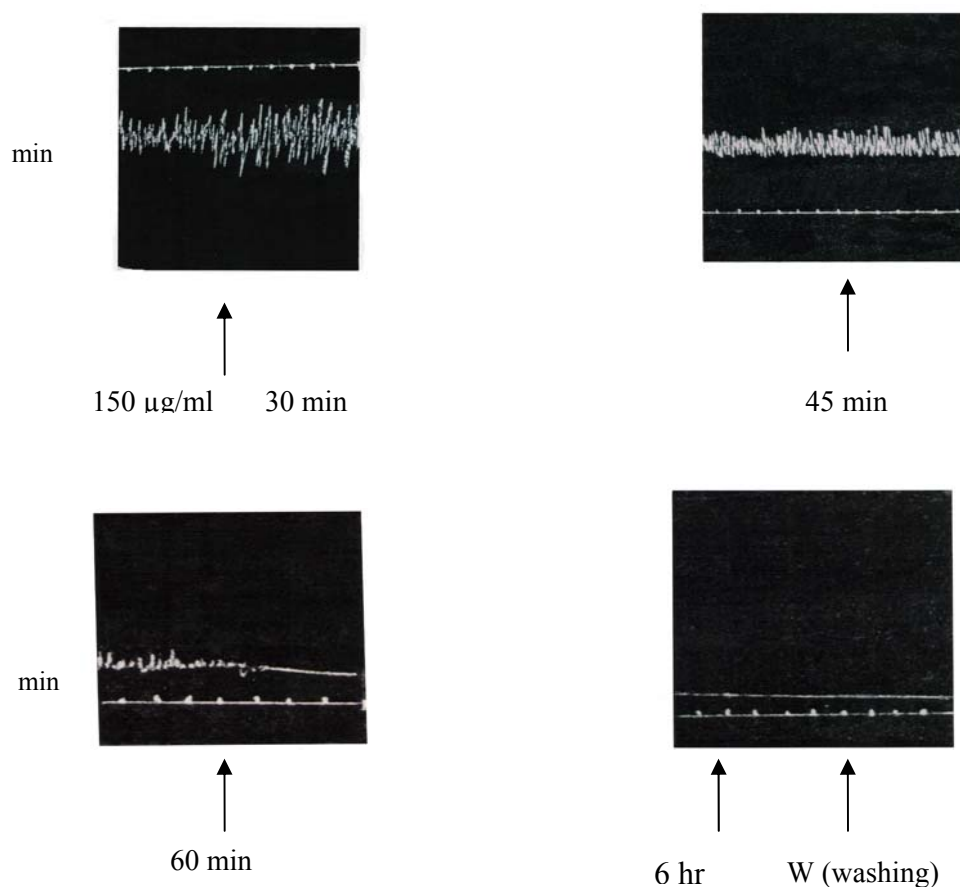


Figure 1 The irreversible effect of alcoholic extract of leaves of *Ficus infectoria* on the spontaneous movements of the whole worm.

Effect of alcoholic extract of leaves of *Ficus infectoria* on spontaneous movements of nerve muscle preparation of *Setaria cervi* in vitro

The effect of the alcoholic extract of leaves was manifested on the nerve muscle preparation at a concentration of 30 μ g/ml of bath fluid. The response was characterized by decrease in rate and amplitude of contractions, while the tone of contractions remained unaffected. The effect was evident immediately after the addition of the extract. The rate and amplitude of contractions continued to decline and after about one hour the activity ceased completely. However repeated changes of the bathing fluid, the movements of the worm were not restored to normal. This indicates that the paralysis caused was irreversible in nature. The results were recorded in Fig..2

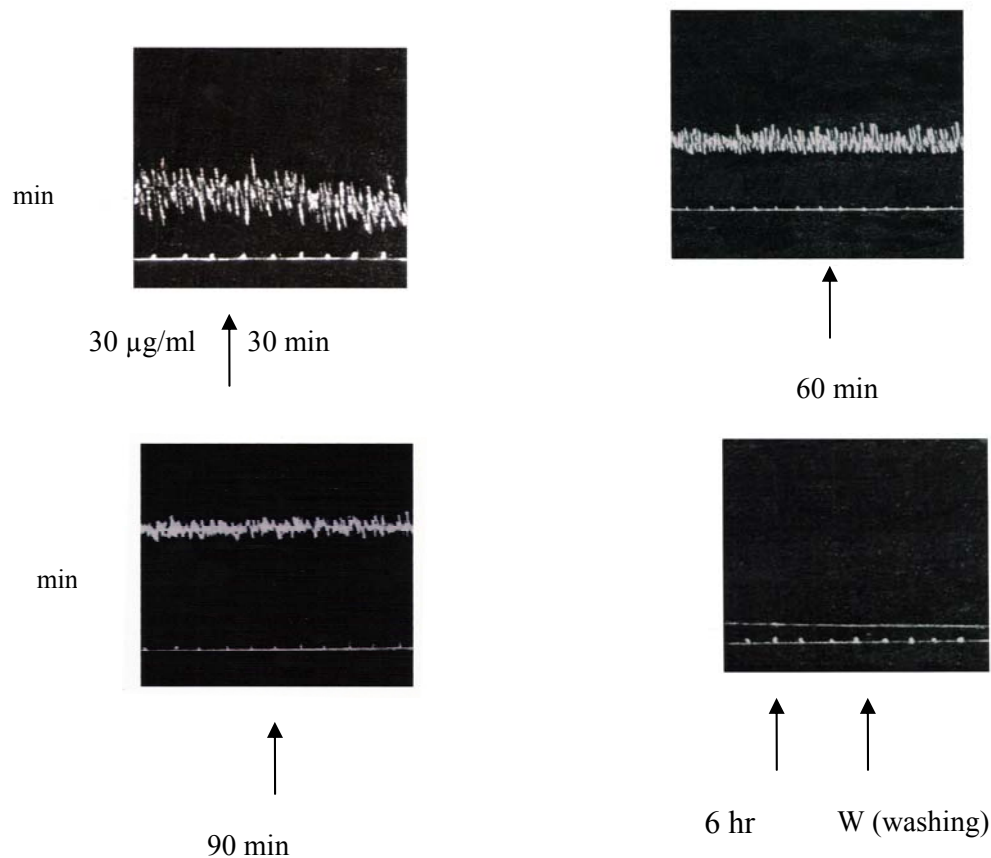


Figure 2 The reversible effect of alcoholic extract of leaves of *Ficus infectoria* on the spontaneous movements of nerve muscle preparation.

Effect of aqueous extract of leaves of *Ficus infectoria* on spontaneous movements of whole worm of *Setaria cervi* in vitro

The aqueous extract was added in a concentration of 260 $\mu\text{g/ml}$ to the bath fluid, modified the movements of the whole worm.. The response was characterized by initial stimulation followed by paralysis. The initial stimulant response was characterized by an increase in amplitude, while the rate of contractions decreased. The amplitude started declining and in another 120min, contractions ceased completely. Repeated washing with the bathing fluid failed to restore the movements of the whole worm. The results were recorded in Fig.3

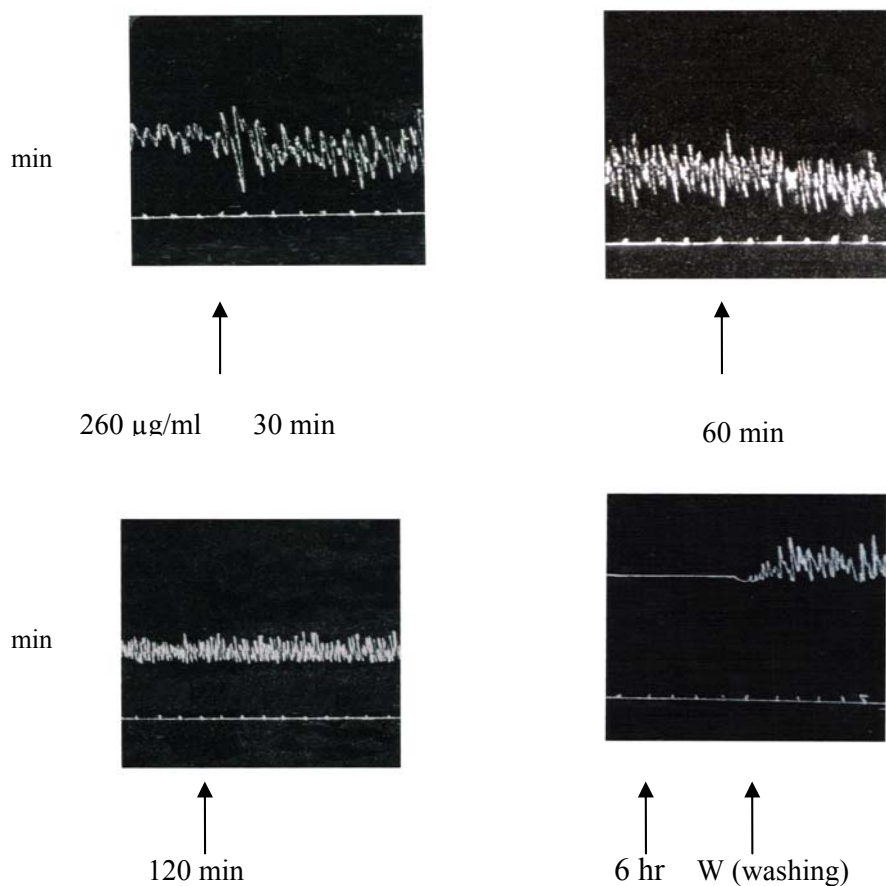


Figure 3 The reversible effect of aqueous extract of leaves of *Ficus infectoria* on the spontaneous movements of the whole worm of *Setaria cervi*.

Effect of aqueous extract of leaves of *Ficus infectoria* on spontaneous movements of nerve muscle preparation of *Setaria cervi* in vitro

The effect of aqueous extract was manifested on the nerve muscle preparation on a concentration of 130 $\mu\text{g/ml}$ of bath fluid.. The response was characterized by an increase in amplitude, while the rate and tone of contractions remained nearly unaffected. The phase of stimulation was short lived and after another 10 minutes, the amplitude started decreasing and became lower. The movements of the worm though not restored to normal, showed a spurt of short lived activity which was followed by a complete cessation of movements. Repeated washing with the bathing fluid failed to restore the movements of the whole worm. The results were recorded in Fig..4

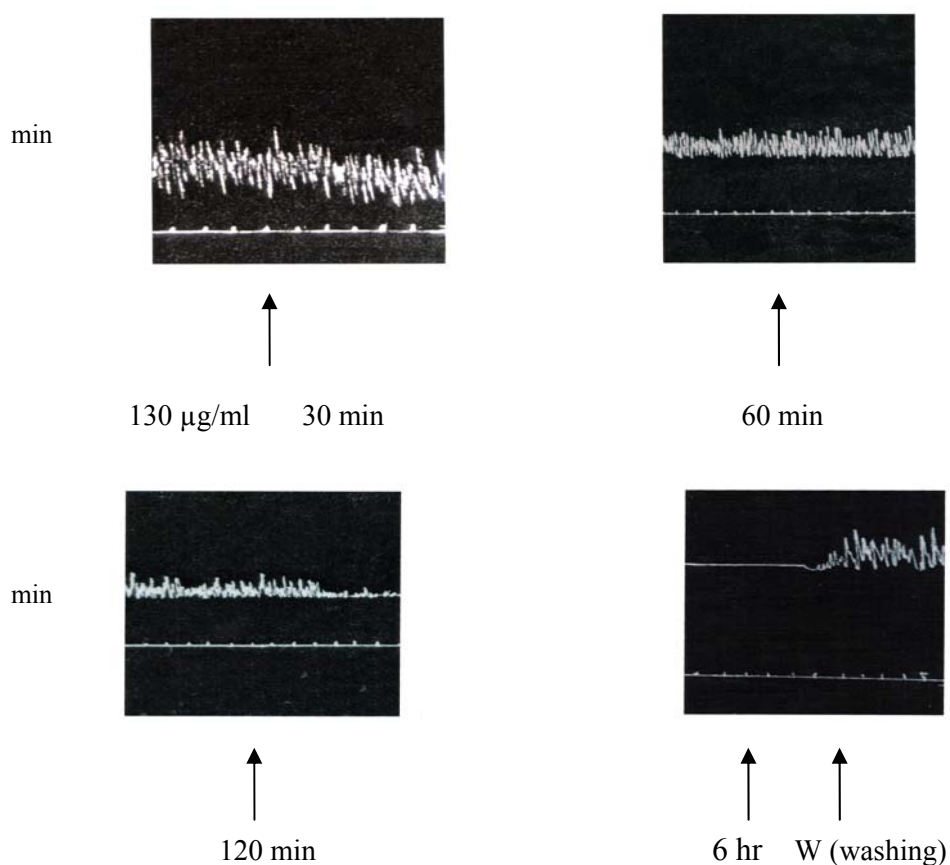


Figure 4 The reversible effect of aqueous extract of leaves of *Ficus infectoria* on the spontaneous movements of nerve muscle preparation of *Setaria cervi*.

Both alcoholic and aqueous extract of leaves of *Ficus infectoria* caused concentration related effect on the survival of microfilaria of *Setaria cervi*. The alcoholic extract was more potent in its lethal effect as compared to the aqueous extract. The LC₅₀ and LC₉₀ as observed after 6 hours are presented in Table 1.

Table 1. Effect of alcoholic and aqueous extracts of *Ficus infectoria* leaves on the survival of m.f of *Setaria cervi* in vitro at 6 hours.

Extracts	Concentration (ng/ml)	Lethal concentration
Alc.ext of leaves of <i>F.infectoria</i>	10	LC ₅₀
	21	LC ₉₀
Aq.ext. of fruits of <i>F.infectoria</i>	14	LC ₅₀
	26	LC ₉₀

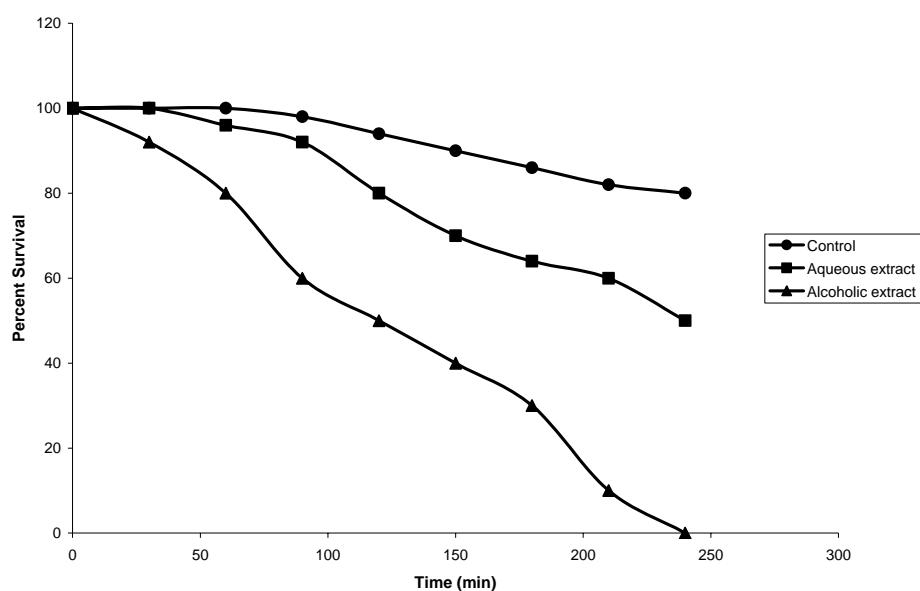


Figure.5 Effect of alcoholic and aqueous extracts of *Ficus infectoria* leaves on the survival of microfilariae of *Setaria cervi* in vitro at a concentration 25 ng/ml observed 360 minutes.

Discussion

It was interesting to note that the effect of alcoholic extract on both the whole worm and the nerve muscle preparation was irreversible in nature where as the aqueous extract of the leaves of *Ficus infectoria* produced reversible effect on whole worm and nerve muscle preparation of *seteria cervi*. This indicates that the contents of the alcoholic extract are different from aqueous extract and contain substances which have irreversible effect.

Alcoholic extract produced stimulant effect on the whole worm followed by irreversible paralysis. On the other hand no stimulation was observed on the nerve muscle preparation. We may discount the initial stimulation effect of alcohol extract of the leaves as this is not manifested on the nerve muscle preparation. In nerve muscle preparation, the drug is in direct contact with nerve muscle complex of *seteria cervi* may be the initial stimulation observed on the whole worm is due to the irritant effect on cuticle. The effect is similar with other substances as well which cause irritation to the worm.

It may be noted that during the phase of paralysis, addition of acetylcholine to the bath did not produced the typical stimulation as is seen otherwise, indicating that the effect could be due to the blockade of cholinergic receptors. Initial stimulation followed by paralysis was a response similar to that produced by diethylcarbamazine (DEC), an antifilarial agent¹³. Although the response of *Ficus infectoria* leaf extracts is similar to the DEC it can be concluded that *Ficus infectoria* leaf extracts possesses potential antifilarial activity which can provide lead for the development of a new derivative. Further studies are required to evaluate this activity in invivo models. The survival time of microfilariae was reduced in a concentration related manner, if this

concentration can be presented to the microfilariae in vivo, it could prove to be a useful tool for the treatment of filariasis

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