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Potential antifilarial activity of the fruit, leaf and stem extracts of *Melia azedarach* Linn. on cattle filarial parasite *Setaria cervi in vitro*

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SUMMARY

The effect of aqueous and alcoholic extracts of the fruit, leaf and stem of *Melia azedarach* Linn. (Meliaceae) on the spontaneous movements of both the whole worm and the nerve-muscle (n.m.) preparation of *Setaria (S.) cervi* and on the survival of microfilariae *in vitro* was studied. Alcoholic extracts of fruit, leaf and stem caused inhibition of the spontaneous movements of the whole worm and the n.m. preparation of *S. cervi*, while only aqueous extract of fruit caused inhibition of the spontaneous movements of the whole worm and the n.m. preparation of *S. cervi*. The initial stimulatory effect was not observed by the aqueous and alcoholic extracts of fruit on n.m. preparation. The concentrations required to inhibit the movements of the whole worm and n.m. preparation for alcoholic extracts of fruit, leaf and stem were 250, 40 μg/ml; 280, 40 μg/ml and 270, 25 μg/ml respectively, whereas an aqueous extract of fruit caused inhibition of whole worm and n.m. preparation at 200 μg/ml and 40 μg/ml respectively. Alcoholic extracts of the fruit, leaf and stem and aqueous extract of the fruit of *M. azedarach* caused concentration related inhibition on the survival of microfilariae (m.f.) of *S. cervi*. The LC_{50} and LC_{90} as observed after 6 h were found to be 5, 15, 10, 20 ng/ml and 10, 25, 20 and 35 ng/ml, respectively. This work was conducted in view of the exploration of potential antifilarial herbal drug.

Key words: *Melia azedarach* L.; Antifilarial activity; *Setaria cervi*

INTRODUCTION

Filarial nematodes are responsible for chronic health and economical blight. Lymphatic filariasis has been identified by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability (WHO, 1997). The filarid worm, *Setaria (S.) cervi* is a cosmopolitan nematode parasite inhabiting the peritoneal cavity of buffaloes *(Bubalis bubalis* Linn.). The parasite is also known to "cause cerebrospinal nematodiasis" in the parasitized hosts (Pachauri, 1972). *S. cervi* resembles closely to human filarial worms in its response to drugs and can therefore be used for the screening of potential antifilarial agents (Singhal *et al.*, 1969, 1972, 1973). *S. cervi* exhibits vigorous rhythmical movements, which can be recorded on a kymograph by suspending the

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worm in an isolated organ bath. Also, the split preparation or nerve-muscle (n.m.) preparation of the worm exhibits similar movements (Singhal, 1977).

Melia (M.) azedarach L., commonly known as Persian lilac or China berry has shown wide range of pharmacological activities against different organisms. Various extracts (aqueous, methanol, ethanol, acetone, ethyl acetate, chloroform, dichloromethane, benzene, petrol and hexane) of leaf, fruit, seed, stem and root and different biological active phytoconstituents of *M. azedarach* have shown strong insecticidal activity viz., larvicidal, pupicidal, adulticidal, fecundity suppression, antiovipositional activity, attractancy against *Aedes aegypti, Anopheles stephensi, Helicoverpa armigera, Earias vittela, Oneridia volxemi, Spodoptera frugiperda* and *Plutella xylostella* (Mulla *et al*., 1999; Rani *et al*.,1999; Cespedes *et al*., 2000; Bounechada *et al*., 2004; Wandscheer *et al*., 2004; Charleston *et al*., 2005; Nathan *et al*., 2005). The fruit extract and limonoid known as meliartenin isolated from it showed strong antifeedant activity against *Epilachna paenulata* and *Spodoptera eridania* (Carpinella *et al*., 2002, 2003). Azedirachtin, an active principle isolated from the plant has been approved universally as a powerful antifeedant. Powder of leaf and bark of *M. azedarach* has been found beneficial in the treatment of leprosy and for the control of scrofula (Kataria, 1994). Seed extract has inhibited folliculogenesis in albino rats (Roop *et al*., 2005) while ethanolic extract of the root has shown strong pregnancy interceptive activity in adult female Sprague-Dawley rats (Keshri *et al*., 2003, 2004). Different extracts and several different types of novel limnoids from this plant have been explored to exhibit significant antitumor activity against different cancer lines. (Bhakuni *et al*., 1969; Itokawa *et al*., 1995; Ahn *et al*., 1996; Takeya *et al*., 1996; Alche *et al*., 2003; Zhou *et al*., 2004, 2005). A significant molluscacidal activity of juice extracts of fruit has also been observed on *Lymnaea cubensis*, host snail of fascioliasis (Pina Perez *et al*., 1998). Meliacine and 28-deacetylsendanin isolated from leaf and fruit respectively, exhibited potent antiviral activity against herpes simplex virus type 1 (HSV-1), junin virus in vero cells, and meliacine was also found to inhibit plant proteins, interferon production and the multiplication of foot and mouth disease virus (FMDV) in BHK-21 cells, respectively (Wachsman *et al*., 1987, 1998; Andrei *et al*., 1990; Castilla *et al*., 1998; Kim *et al*., 1999; Alche *et al*., 2002). The methanol extracts of leaf, root and stem barks and an unsaponified matter obtained from the fixed oil of *M. azedarach* seeds have shown a broad spectrum of antibacterial activity against many gram positive and gram negative pathogenic bacteria. (Shrivastava *et al*., 1997; Khan *et al*., 2001) and methanol extract of flower has shown potent antibacterial action in rabbits suffering from a skin infection produced by *Stapyhlococcus aureus* (Saleem *et al*., 2002). Scopoletin, a hydroxycoumarin isolated from fruit has shown potent antifungal activity against *Fusarium verticillioides* and hexanic and ethanolic extracts from fruit, seed kernels, and senescent leaf also exhibited fungistatic activity against *Aspergillus flavus,Diaporthe phaseolorum var. meridionales, Fusarium oxysporum, Fusarium solani, Fusarium verticillioides, and Sclerotinia sclerotiorum* (Abou-Jawdah *et al*., 2002; Carpinella *et al*., 2003, 2005). The antiulcer effect of the lipid components of *M. azedarach* fruits has also been reported on gipsing-restraint stress-induced ulcers in rats (Hanifa Moursi *et al*., 1984). Phytochemical investigations of *Melia azedarach* have revealed the presence of a number of biological active terpenoids (Frederic *et al*., 1969; Oelrichs *et al*., 1983; Marklee *et al*., 1987), limnoids (Santosh *et al*., 1985; Han *et al*., 1991; Takeya *et al*., 1996; Nakatani *et al*., 1998; Fukuyama *et al*., 2000; Zhou *et al*., 2005) and flavonoid glycosides (Nair *et al*., 1975; Mishra *et al*., 1984).

Aqueous and alcoholic extracts of the leaf of *M. azedarach* have shown anthelmintic activity against tapeworm and hookworm *in vitro* (Neogi *et al*.,

1963) while aqueous alcoholic extract (70%, v/v) of the seed did not show any significant anthelmintic efficacy in lambs infected artificially with third stage larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* (Hordegen *et al*., 2003). No earlier attempt has been made to study the activity of *M. azedarach* extracts against any filarial parasite. This study was planned to observe the effect of alcohol and aqueous extracts of *M. azedarach* on the movements of whole worm and n.m. preparation of *S. cervi* and on the survival of microfilariae (m.f.) *in vitro* in view of the exploration of potential antifilarial herbal drug.

MATERIALS AND METHODS

Plant Materials

The fruit, leaf and stem of *M. azedarach* were collected from the Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India. Dr. Athar Ali Khan, Department of Botany, AMU, Aligarh, confirmed the authenticity of the plant, where the voucher specimen (# 1150) has been deposited.

Preparation of Extract

Dried and powdered fruit, leaf and stem (250 g each) of *M. azedarach* were extracted with double-distilled ethanol and water, separately (yields : 1.50, 1.65 and 1.35 g, respectively). The crude ethanolic and aqueous extracts were dried and dissolved in 95% distilled ethanol and distilled water before use. In the controlled experiment, addition of 0.2 to 0.5 ml vehicle (95% distilled ethanol or distilled water) to the organ bath, containing 20 ml Ringer's solution had no effect on worm motility observed up to 3 h.

Collection of *S. cervi*

Motile adult S. *cervi* (Nematoda: Filariodea) of average length 6.0 ± 1.0 cm and average weight cattle *(Bubalis bubalis* L.) and brought to the laboratory in a vacuum flask, containing modified Ringer's solution (NaCl 9 g, KC1 0.42 g, $NaHCO_3$ 0.5 g, $CaCl_2$ 0.24 g, glucose 0.25 g in 1 l, distilled water) at 38 ± 1°C (Singhal *et al*., 1969). The time period between the removal of the S. *cervi* from the host to the laboratory was less than 3 h. In the laboratory, the S. *cervi* were repeatedly washed with the fresh modified Ringer's solution without applying any tension on it to free them of any extraneous material. Control recording of 4 - 6 parasites was also carried out to differentiate the effect of the extract and time-dependent fading of the muscular activity.

Whole worm preparation

Adult *S. cervi* was suspended in an isolated organ bath of 20 ml capacity, in modified Ringer's solution at 38 ± 1°C. *S. cervi* exhibits vigorous rhythmical movements for more than 6 h, which can be recorded on a kymograph by suspending the worm in an isolated organ bath. 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 were 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. The effect of each dose was observed 5 times. 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. Every experiment was repeated for five times.

Split preparation or n.m. preparation

A worm was placed in a petri-dish, 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 1cm 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 the ends and suspended in the isolated organ bath containing modified Ringer's solution at 38 ± 1°C. The split preparation showed rhythmical spontaneous movements, which were recorded on the slow-moving kymograph.

Collection of m.f.

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 Ringer's solution and microfilariae were freed. The m.f. were suspended in human serum: Ringer mixture, the count was adjusted to 100/ml, and 0.5 ml aliquots of the m.f. suspension were placed in sterilized screw capped bottles containing aqueous or alcoholic extracts of *M. azedarach* in an equal human serum: Ringer mixture *(v/v) and M. azedarach* 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 m.f. The LC_{50} and LC_{90} 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 m.f.

In preliminary experiments, the aqueous and alcoholic extracts of the *M. azedarach* were added to m.f. in concentrations of 5, 10, 15, 20 and 25 ng/ml to determine the limits of activity within 6 h at 38 ± 1°C. Within these limits, six concentrations were selected to observe the survival of m.f. The effect of each dose was observed 5 times. The mean of the values was plotted on a graph. All analyses were done using the SPSS 7.5 for windows. Experimental values were expressed as Mean ± SEM (Standard Error of Mean). Statistical significance was considered, indicating a *P*-value of less than 0.05 in all cases.

RESULTS

Effect of aqueous extract of the fruit of *M. azedarach* on the spontaneous movements of the whole worm & n.m. preparation of *S. cervi*

The addition of 200 μg/ml of aqueous extract of fruit of *M. azedarach* to the bath fluid modified the movements of the whole worm of *S. cervi,* while at lower concentration it was inactive. The response was characterized by initial stimulation followed by paralysis. The initial stimulatory response was characterized by an increase in tone of contractions with no definite change in rate and amplitude of contractions. The effect was evident immediately after the addition of the extract. The stimulant effect was short-lived and lasted for about 5 min when tone of contractions started declining till it attained pre-drug level. After about 30 min, the amplitude and rate of contractions started decreasing and continued to do so till the movements of the worm ceased completely after about 2 h. The paralysis of the worm was complete and continued for more than 6 h. There were no spontaneous twitching, contractions or

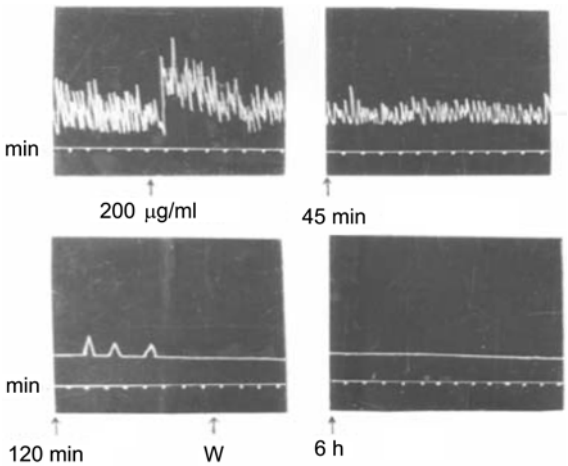

Fig. 1. Initial stimulation followed by irreversible paralysis by 200 μg/ml aqueous extract of the fruit of *M. azedarach* on the spontaneous movements of the whole worm preparation of *S. cervi.* The response was characterized by initial stimulation followed by irreversible paralysis.

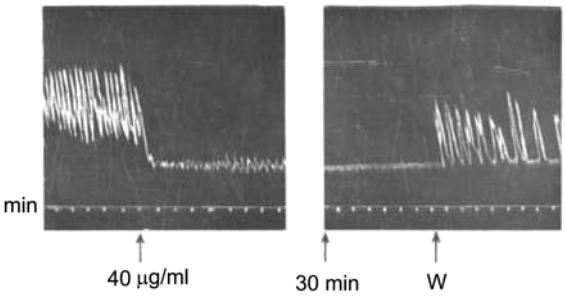

Fig. 2. The reversible paralysis by 40 µg/ml aqueous extract of the fruit of *M. azedarach* on the spontaneous movements of the n.m. preparation of *S. cervi.* The response was characterized by an immediate decrease in tone, rate and amplitude of contractions.

recovery. The movements were however not restored despite repeated changes of the bathing fluid (W). This indicates that paralysis caused was irreversible in nature (Fig. 1).

On the n.m. preparation the effect of the aqueous extract of fruit of *M. azedarach* was manifested at a concentration as low as 40 µg/ml of bath fluid. The response was characterized by an immediate decrease in tone, rate and amplitude of contractions. The effect was evident immediately after the addition of extract (Fig. 2). The initial stimulant effect was not observed as seen in the whole worm preparation. The movements of the n.m. preparation, though not restored to normal, showed a spurt of short-lived activity, which was followed by a complete cessation of movement after about 30 min. Repeated washing with extract free bathing fluid restored the movements to normal.

Effect of alcoholic extract of the fruit of *M. azedarach* on the spontaneous movements of the whole worm and n.m. preparation of *S. cervi*

The response of the alcoholic extract of fruit of *M. azedarach* on the whole worm was different than that observed with the aqueous extract. Addition of alcoholic extract in a concentration of 250 µg/ml caused an immediate increase in tone of contractions, decrease in amplitude, while rate of

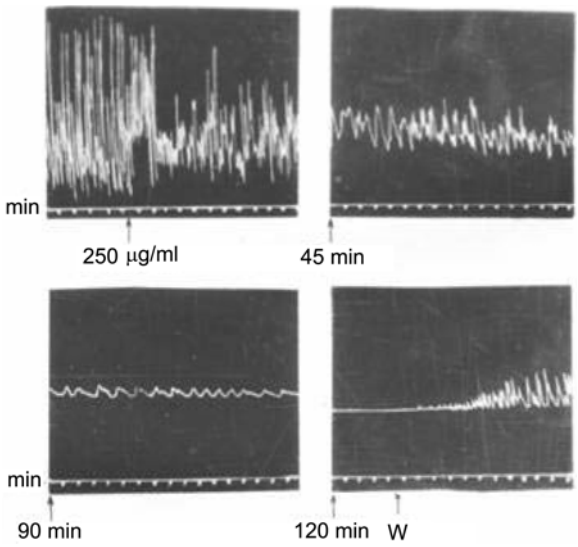

Fig. 3. Initial stimulation followed by reversible paralysis by 250 µg/ml alcoholic extract of the fruit of *M. azedarach* on the spontaneous movements of the whole worm of *S. cervi.* Addition of alcoholic extract caused an immediate increase in tone of contractions and decrease in amplitude while the rate of contractions was unaffected followed by reversible paralysis as the repeated washings slowly restored the movements to normal.

contractions was unruffled. The stimulant effect lasted for about 5 min when tone of contractions started declining till it attained pre-drug level. 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 2 h, the paralysis of the worm was complete and continued for more than 6 h. However, with repeated changes of the bathing fluid, the movements of the worm were slowly restored to normal. This indicates that the paralysis caused was reversible in nature (Fig. 3).

The effect on the n.m. preparation (Fig. 4) was almost similar as was observed on n.m. preparation by aqueous extract. Addition of the alcoholic extract in a concentration of 40 µg/ml caused a reduction in tone, rate and amplitude of contractions. Nearly 25 min. after the addition of the drug, the n.m. preparation was completely

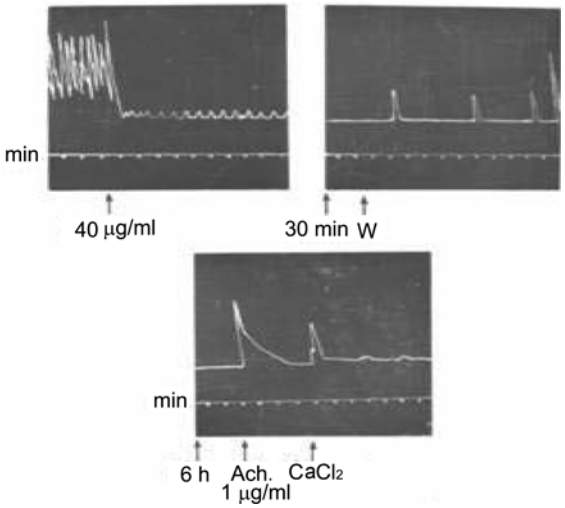

Fig. 4. The partial reversible paralysis by 40 μg/ml alcoholic extract of the fruit of *M. azedarach* on the spontaneous movements of the n.m. preparation of *S. cervi*. Alcoholic extract caused reduction in tone, rate and amplitude of contractions (upper panel). Ach. and $CaCl_2$ to the bath fluid could elicit the response (lower panel).

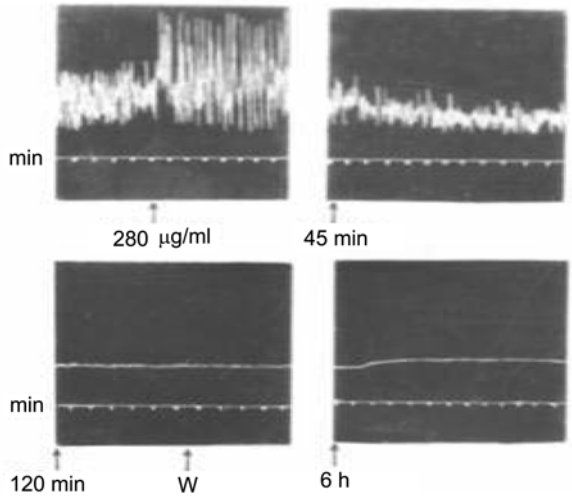

Fig. 5. Initial stimulation followed by irreversible paralysis by 280 μg/ml alcoholic extract of the leaf of *M. azedarach* on the spontaneous movements of the whole worm of *S. cervi*. Addition of the drug to the bath fluid produced a stimulated response characterized by an increase in amplitude and with a small increase in tone of contractions. However, the rate of the contractions was not affected.

paralyzed. Repeated washing with the bathing fluid was not effective in restoration of the movements. However, twitching and contractions were appearing after every few min. This response continued for about 6 h but activity was not restored to normal. This indicates that the paralysis caused was partially reversible in nature. Addition of acetylcholine (Ach.) and $CaC1_2$ (both are excitatory neurotransmitters) to the bath fluid could elicit the response (Fig. 4-lower panel).

Effect of alcoholic extract of the leaf of *M. azedarach* on the spontaneous movements of the whole worm and n.m. preparation of *S. cervi*

A typical response of alcoholic extract of the leaf of *M. azedarach* on the spontaneous movements of the whole worm of S. *cervi* is shown in Fig. 5. Addition of 280 μg/ml to the bathing fluid produced a stimulatory response characterized by an increase in amplitude and with a small increase in tone of contractions. However, the rate of the contractions was unperturbed. The effect was evident immediately after the addition of the drug. The increase in amplitude continued for nearly 30 min after that the rate and amplitude started declining and continued to do so till the movements of the worm ceased completely after about 2 h. The paralysis of the worm was complete and continued for more than 6 h. There were no spontaneous twitching, contractions or recovery of the worm. The movements were however not restored despite repeated changes of the bathing fluid (w). This indicates that paralysis caused was irreversible in nature.

On n.m. preparation, the alcoholic extract of leaf of *M. azedarach* produced the typical stimulant effect, which was similar in nature to that observed with the whole worm. However, the concentration required to produce an equivalent effect was only 40 μg/ml as compared to 280 μg/ml for the whole worm. The paralysis was complete nearly after about 90 min. The movements were, however, not

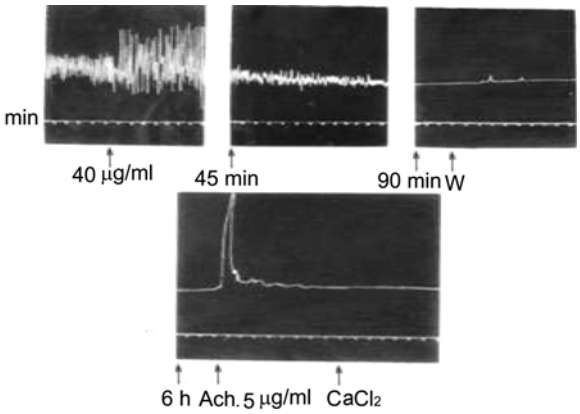

Fig. 6. Initial stimulation followed by irreversible paralysis by 40 µg/ml alcoholic extract of the leaf of *M. azedarach* on the spontaneous movements of the n.m. preparation of *S. cervi*. Addition of Ach. to the bath fluid could elicit the response whereas, $CaCl_2$ failed to show its stimulatory effect.

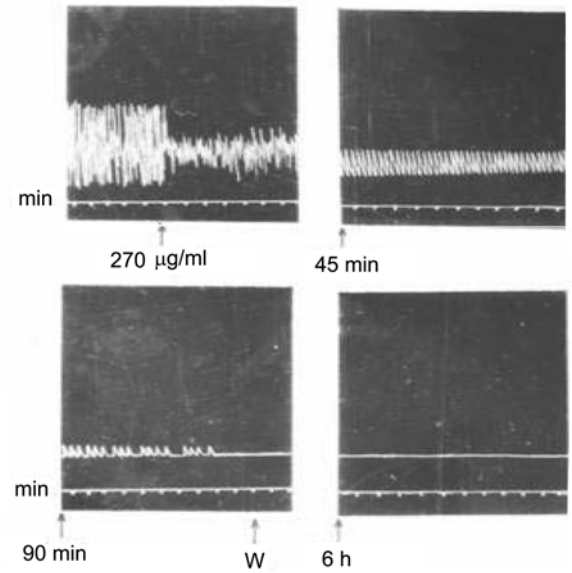

Fig. 7. The irreversible paralysis by 270 µg/ml alcoholic extract of the stem of *M. azedarach* on the spontaneous movements of the whole worm of *S. cervi*. The initial stimulatory response was characterized by an increase in tone and decrease in amplitude of contractions while rate of the contractions remained unaffected.

restored despite repeated changes of the bathing fluid (w). This indicates that the paralysis caused was irreversible in nature. There was no indication of restoration of movements even after 6 h. Addition of Ach. to the bath fluid could elicit the response where as $CaC1_2$ didn't produce any stimulatory effect (Fig. 6).

Effect of alcoholic extract of the stem of *M. azedarach* on the spontaneous movements of the whole worm and n.m. preparation of *S. cervi*

A typical significant response of alcoholic extract of stem on the whole worm is shown in Fig. 7. The response of alcoholic extract was not similar to the extracts of fruit or leaf extracts observed on the whole worm of *S. cervi*. Addition of 270 µg/ml of alcohol extract to the bath fluid modified the movements of the whole worm. The response was characterized by initial stimulation followed by complete paralysis. The initial stimulatory response was characterized by an increase in tone and decrease in amplitude of contractions while rate of the contractions remained unaffected. The stimulant effect lasted for about 25 min when tone of contractions started declining till it attained pre-drug level after about 30 min. 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 100 min, the paralysis of the worm was complete and continued for more than 6 h. There were no spontaneous twitching, contractions or recovery. The movements were however not restored despite repeated changes of the bathing fluid (w). This indicates that paralysis caused was irreversible in nature.

On n.m. preparation, the alcoholic extract of stem of *M. azedarach* produced the stimulant effect, which was similar in nature to that observed with the whole worm. However, the concentration required to produce an equivalent inhibitory effect was only 25 µg/ml as compared to 270 µg/ml for the whole worm. The paralysis caused was reversible as the repeated washings slowly restored the movements to normal. This indicates that the paralysis caused was reversible in nature (Fig. 8).

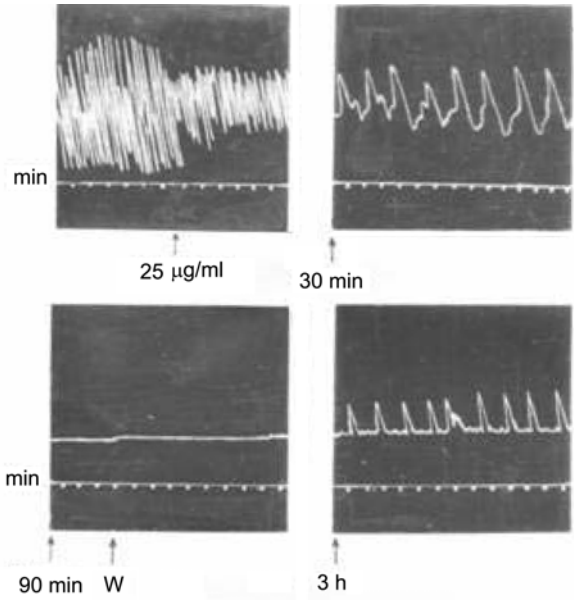

Fig. 8. The reversible paralysis by 25 μg/ml alcoholic of the stem of *M. azedarach* on the spontaneous movements of the n.m. preparation of *S. cervi.* The paralysis caused was reversible as the repeated washings slowly restored the movements to normal.

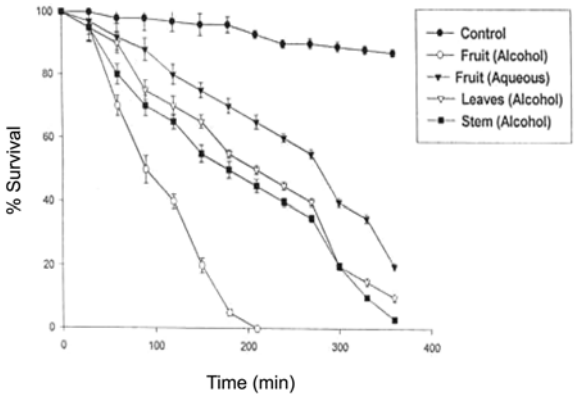

Fig. 9. Effect of alcoholic and aqueous extracts of the fruit, leaf and stem of *M. azedarach* on the survival of microfilariae of *S. cervi* at a concentration of 25 ng/ml. All experiments were performed in the pentaplate and bars representing standard errors of mean are shown.

Effect of aqueous extract of the fruit and alcoholic extracts of the fruit, leaf and stem of M. azedarach on the survival of m.f. of S. cervi *in vitro*

Aqueous extracts of leaf and stem of *M. azedarach* failed to affect the survival of m.f. to any extent up to a concentration of 500 ng/ml. However, alcoholic extracts of fruit, leaf and stem and aqueous extract of fruit of *M. azedarach* caused concentration related inhibition on the survival of m.f. of *S. cervi.* The LC_{50} and LC_{90} as observed after 6 h. were 5 and 10 ng/ml for alcoholic extract of fruits, 15 and 25 ng/ml for alcoholic extract of leaf, 10 and 20 ng/ml for alcoholic extract of stem and 20 and 35 ng/ml for aqueous extract of fruit, respectively. The survival of m.f. of *S. cervi* was significantly reduced in the presence of alcoholic extracts of fruit and leaf of *M. azedarach* with a concentration of 5 ng/ml (LC_{50}) and 15 ng/ml (LC_{50}) of the extract, the survival of m.f. was significantly reduced after remaining in contact for 120 min ($P < 0.05$) and continued to remain until the observation period of 6 h. Similarly, at a higher concentration of 25 ng/ml of each extract, the survival of m.f. was reduced but a little earlier and significant reduction in survival was observed. The concentration-related effect of alcoholic extracts of fruit, leaf and stem and aqueous extract of fruit of *M.azedarach* in a concentration of 25 ng/ml observed for 350 min. is shown in Fig. 9. Alcoholic extracts of *M. azedarach* being more potent in its lethal effects as compared to the aqueous extract (Table 1).

DISCUSSION

It is interesting to note that the effect of aqueous and alcoholic extracts of fruit, leaf and stem of *M. azedarach* had activities different in nature with each other suggesting involvement of more than one active principle in the causation of action. An aqueous extract of fruit produced initial stimulation of the movements of the whole worm followed by irreversible paralysis while an aqueous extracts of leaf and stem did not show any effect on the movements of whole worm and n.m. preparation of *S. cervi.* The alcoholic extracts of

Table 1. Effect of alcoholic and aqueous extracts of the fruit, leaf and stem of *M. azedarach* on the survival of microfilariae of *S.cervi in vitro.* [Mean ± SEM (*P* values)

Time (min.)	Survival (%)								
	Control	Aqueous extract of fruit		Alcoholic extract of fruit		Alcoholic extract of leaf		Alcoholic extract of stem	
		LC_{50}(20 ng/ml) LC_{90} (35 ng/ml)		LC_{50} (05 ng/ml) LC_{90} (10 ng/ml)		LC_{50} (15 ng/ml) LC_{90} (25 ng/ml)		LC_{50} (10 ng/ml) LC_{90} (20 ng/ml)	
0	100	100	100	100	100	100	100	100	100
30	98 ± 0.56	95 ± 2.12	93 ± 2.74	98 ± 1.22	95 ± 2.55	95 ± 2.55	93 ± 2.12	97 ± 2.12	95 ± 1.00
60	97 ± 0.47	93 ± 4.18[a]	90 ± 1.23[a]	96 ± 1.06	90 ± 2.55[a]	93 ± 2.73[a]	85 ± 1.73[a]	95 ± 1.00	90 ± 2.92[a]
90	96 ± 0.71	92 ± 2.73[a]	84 ± 2.35[a]	90 ± 3.60[a]	83 ± 3.39[a]	87 ± 1.22[a]	80 ± 2.55[a]	92 ± 2.55[a]	85 ± 3.08[a]
120	95 ± 0.84	88 ± 1.87[a]	80 ± 3.16[a]	85 ± 1.87[a]	80 ± 1.58[a]	84 ± 3.61[a]	75 ± 2.12[a]	90 ± 2.12[a]	80 ± 3.39[a]
150	93 ± 1.22	80 ± 1.22[a]	77 ± 4.18[a]	80 ± 1.87[a]	77 ± 2.74[b]	77 ± 2.55[a]	70 ± 3.39[b]	85 ± 4.30[a]	70 ± 2.92[b]
180	92 ± 0.71	75 ± 1.87[b]	65 ± 2.55[b]	75 ± 2.55[b]	70 ± 2.15[b]	72 ± 1.87[b]	60 ± 2.12[b]	79 ± 2.74[a]	62 ± 2.55[b]
210	92 ± 1.00	70 ± 2.92[b]	60 ± 4.06[b]	70 ± 3.10[b]	65 ± 1.87[b]	70 ± 2.74[a]	55 ± 1.00[b]	70 ± 4.85[b]	55 ± 2.74[b]
240	91 ± 1.58	65 ± 3.16[b]	55 ± 5.15[b]	65 ± 2.23[b]	50 ± 1.58[b]	65 ± 3.81[b]	45 ± 2.55[b]	65 ± 2.35[b]	49 ± 2.55[b]
270	90 ± 1.05	63 ± 2.35[b]	40 ± 4.06[b]	60 ± 2.62[b]	35 ± 2.62[b]	60 ± 3.08[b]	38 ± 2.92[b]	60 ± 3.16[b]	40 ± 1.58[b]
300	90 ± 1.41	60 ± 1.23[b]	35 ± 0.71[b]	55 ± 2.55[b]	25 ± 3.39[b]	58 ± 2.92[b]	30 ± 5.15[b]	58 ± 1.22[b]	30 ± 2.74[b]
330	88 ± 1.14	55 ± 2.55[b]	25 ± 2.12[b]	50 ± 2.15[b]	15 ± 1.58[b]	55 ± 1.87[b]	25 ± 1.87[b]	55 ± 1.00[b]	23 ± 2.00[b]
360	86 ± 1.14	48 ± 1.58[b]	15 ± 2.74[b]	47 ± 1.27[b]	09 ± 2.23[b]	50 ± 1.58[b]	12 ± 2.74[b]	47 ± 1.58[b]	08 ± 2.74[b]

Experiments are indicated in the parenthesis. [a] = $P < 0.05$, [b] = $P < 0.001$ compared to controlled.

fruit, leaf and stem all produced stimulation of the movements of the worm movements. These too differed from each other. The alcoholic extract of fruit, produced reversible while that of leaf and stem produced irreversible paralysis on the whole worm.

The effect on the n.m. 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 aqueous extract of fruit of *M. azedarach* on whole worm where initial stimulation was observed, on n.m. preparation it produced inhibition of movements resulting in partially reversed paralysis. The aqueous extracts of leaf and stem were without any effect. The alcoholic extracts of fruit, leaf and stem all caused stimulation of the movements of the n.m. preparation followed by reversible paralysis with fruit and stem extracts and irreversible paralysis with leaf extract.

The above activity profile placidly reveals that there are at least two active ingredients in the plant, one causing a stimulation followed by reversible paralysis and the other causing stimulation followed by irreversible paralysis. During the phase of reversible paralysis, an addition of Ach. and $CaCl_2$ to the bath fluid produced their normal stimulant effect, indicating that the effect paralysis of the parasite is not due to blockade of cholinergic receptors or blockade of calcium channels in the whole worm or the n.m. preparation of *S. cervi* (Singhal *et al*., 1995; Uddin *et al*, 2003). It is likely that the response to a substance is similar in activity to diethylcarbamazine (DEC) a known antifilarial agent. Bath applied DEC produced an initial short-lasting stimulation of the movements of split preparation of *S. cervi,* followed by irreversible paralysis. DEC has also been shown to decrease the glucose uptake by the adult worm of *S. cervi,* suspended in modified Ringer's solution and produces reversible dose-dependent depolarization of the membrane

potential of another nematode, *Ascaris suum* by antagonizing voltage-sensitive K^+ conductance in the muscle. (Singhal *et al*., 1978; Hawking, 1979; Martin, 1982). Pharmacological studies have also shown that DEC interferes with arachadonic acid metabolism, blocking a number of steps in both cyclooxygenase and lipoxygenase pathways (Davies *et al*., 1984). Also, microfilariae secrete Prostaglandins E_2 (PGE_2, also known as Prostin) and I_2 (PGI_2, Prostacyclin), important vasoactive mediators in pulmonary vessels, which may facilitate their passage by increasing capillary network diameters. DEC blocks the PGE_2 and PGI_2 production from both m.f. and endothelial cells, causing a constriction of the capillaries and impeding the passage of larvae (Kanesa-thasan *et al*., 1991). Moreover, DEC presents a direct mode of action for promoting severe damage of microfilarial cells, including the presence of large vacuoles, lysis of the cytoplasm and chromatin and bodies extruding from the plasma membrane, which are indicative features of an apoptotic process (Peixoto *et al*., 2004). The human filarial parasite lives in tissues, e.g., *Wuchereria bancrofti* and *Brugia malayi* cause elephantiasis by blocking the lymphatics. It cannot be predicted whether DEC ever reaches the adult worm, but there is presumptive evidence that DEC sensitizes the adult-worm and even cause paralysis and may cause death. However, it is the microfilariae, which live in circulation are exposed to the drug. 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, 1949).

Irreversible paralysis seen with the aqueous extract of fruit in whole worm and alcoholic extract of leaf and stem on whole worm preparation though apparently similar differed in their reaction to stimulants viz. Ach. and $CaCl_2$. While the effect of these two could be elicited during whole worm, paralysant phase following aqueous extract of fruit and alcohol extracts of leaf and stem on the stimulant effect of $CaCl_2$ was not seen during paralysis following addition of alcohol extract of leaf on n.m. preparation. This indicates that possibility exists of calcium channel blocking activity in the leaf of *M. azedarach.* This needs to be explored with further experiments.

On the m.f. of the *S. cervi,* alcoholic extracts of fruit, leaf, stem and aqueous extract of fruit *of M. azedarach* reduced the survival time in a concentration-related manner. These findings reveal that the fruit, leaf and stem *of M. azedarach* extracts may provide a chemical lead for the synthesis of new derivatives, which might prove to be potential antifilarial agents.

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