ANTIFILARIAL ACTIVITY OF MALLOTUS PHILIPPENSI S LAM. ON SETARIA CERVIE (NEMATODA: FILARIOIDEA) IN VITRO

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Abstract: The effect of aqueous and alcoholic extracts of the leaves of Mallotus philippensis (Lam.) Muell. Arg. was studied on the spontaneous movements of the whole worm and nerve-muscle (n.m.) preparation of Setaria cervi and on the survival of microfilariae in vitro. Both the extracts caused inhibition of spontaneous motility of whole worm and the n.m. preparation of S. Cervi characterized by initial stimulation followed by depression in amplitude. The tone and rate of contractions remained visibly unaffected. Aqueous extract at higher concentration showed immediate reduction in tone. The concentration required to inhibit the movements of n.m. preparation was 1/5th for aqueous and 1/11th for alcoholic extract compared to that for the whole worm, suggesting a cuticular permeability barrier. The stimulatory response of acetylcholine was blocked by aqueous extract on whole worm movements. On the microfilariae the LC50 and LC90 were 18 and 20 ng/ml for aqueous and 12 and 15 ng/ml for alcoholic extracts respectively.

Key words: filariasis, mallotus philippensis, setaria cervi, microfilariae

INTRODUCTION

Alcoholic and ethereal extracts of Mallotus philippensis fruits have shown taenicidal action against Hymenolepis nana and H. diminuta, both in vitro and in vivo. The extracts also exhibited lethal efficacy against trematodes (Fasciolopsis buski) but not on nematodes (Ascaris lumbricoides) in vitro (1, 2). Mallotus philippensis powder traditionally used in Ayurvedic system of medicine was found effective in conversion of stools from positive to negative in 96 percent of children harboring H. nana. Plant powder of M. philippensis is also used by external application for parasitic infection of skin and also as aphrodisiac, lithotriptic and styptic (3–6). However, there is no report on anthelmintic activity of M. philippensis leaves in literature. Further the plant has not been explored for antifilarial activity. It was, therefore, thought worthwhile to test alcoholic and aqueous extracts of M. philippensis for potential antifilarial activity in vitro on whole worm, nerve muscle preparation and microfilariae of S. cervi (Nematoda : Filarioidea).

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METHODS

Adult Setaria cervi (Nematoda filarioidea) were collected from the peritoneal cavity of freshly slaughtered cattle and brought to laboratory in a vacuum flask containing modified Ringer's solution (7) (NaCl 9g, KCl 0.42g, CaCl₂ 0.24g NaHCO₃ 0.5g, glucose 0.25g per litre) at 37°C.

Dried and powdered leaves of M. philipensis (Euphorbiaceae) were extracted with alcohol and water, separately. The crude extract was dried and dissolved in alcohol and water before use.

Whole worm preparation: Adult S. cervi were 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 kymograph drum (8). 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 worm to stabilize before eliciting the response of drug. The drug was added in increasing concentration to the bath fluid and allowed 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 petridish 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 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. The preparation served to expose the n.m. complex directly to the action of the drugs, and also could exhibit spontaneous rhythmic movements similar to those of the intact worm. The drug concentrations were tested for their response as with whole worm preparation. The concentration of extract which modified the movements was tested in at least six preparations.

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. It was teased with a fine needle in the solution and microfilariae (mf) were freed. The microfilariae were suspended in a 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 cap bottles containing aqueous or alcoholic extracts of M. philippensis in equal serum : Ringer mixture (v/v). M. philippensis extract was added in doubly increasing concentration from 5 ng/ml. The bottles were kept in an incubator at 37°C and examined under a microscope every 30 min till 210 min to count the living and dead microfilariae. Each concentration was tested in six sets of experiments. The LC₅₀ and LC₉₀ was calculated from mean concentration - death graph.

In a preliminary experiment, aqueous and alcoholic extracts of M. philippensis were added to microfilariae suspension in increasing concentrations starting from 5 upto 50 µg/ml. The survival/motility of
microfilariae was observed till 6 hr to determine limits of activity. There was no mortality at a concentration of 5 μg/ml and 100% mortality at 50 μg/ml. Six concentrations were selected beginning from a minimum which would kill not more than 10% microfilariae and maximum which would kill more than 90% but not all the microfilariae at 6 hr. These concentrations were 15, 20, 25, 30, 35 and 40 ng/ml.

RESULTS

Effect of aqueous extract of *M. philippensis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*: A typical response to aqueous extract of *M. philippensis* on the spontaneous movements of whole worm of *S. cervi* is shown in Fig. 1. Immediately after the addition of the extract in a concentration of 100 μg/ml to the bath fluid, the amplitude of the contractions showed a gradual increase till it reached its peak at about 60 min. The rate of contractions showed a corresponding decrease while the tone remained unaffected. Thereafter there was increase in the frequency but the amplitude decreased. After about 95 min movements of whole worm ceased completely. Repeated changes of bathing fluid was not beneficial in restoring the movements. Addition of acetylcholine (Ach) at this stage in concentrations of 5 and 100 μg/ml of bath fluid only induced a feeble response as did the addition of KCl.

The response to aqueous extract of *M. philippensis* could be elicited on the n.m. complex of *S. cervi* in a concentration of 20 μg/ml i.e. 1/5th of that required for the whole worm and was also different in nature. The initial stimulant effect characterized by increase in amplitude of contractions observed with whole worm was not observed with n.m. preparation. The movements were characterized by decrease in amplitude only while the tone and rate of contractions remained unchanged. The depressant effect was reversible with repeated changes of the bathing fluid which restored the movements to normal.

With a higher concentration of aqueous extract (40 μg/ml) on the n.m. preparation the response was immediate with reduction in tone characterized by lowering of base line and cessation of movements. The effect at this concentration was irreversible with washing of the preparation with bathing fluid. However, the n.m. preparation...
responded with a well defined typical response of Ach (lower panel of Fig. 2)

![Min](20 \mu g/ml)

![Min](40 \mu g/ml) w Ach 5 \mu g/ml

Fig. 2: Effect of aqueous extract of the leaves of *M. philippensis* on the spontaneous movements of the n.m. preparation of *S. cervi*. A concentration of 20 \mu g/ml caused decrease in amplitude and tone while the rate of contractions remained visibly unaffected. But at a higher concentration (40 \mu g/ml) the paralysis caused was irreversible as repeated washings (w) failed to restore the movements. Ach (5 \mu g/ml) produced its usual stimulant effect during the phase of paralysis.

Effect of alcoholic extract of *M. philippensis* on the spontaneous movements of the whole worm and n.m. preparation of *S. cervi*: addition of alcoholic extract of *M. philippensis* in a concentration of 230 \mu g/ml of bath fluid caused initial stimulation followed by paralysis of the whole worm of *S. cervi*. The initial stimulant effect lasted for about 45 min and was characterized by short-lasting increase in amplitude. In the later part of the stimulation phase i.e. after nearly 30 min a decrease in the frequency of movement was observed. This was followed by paralysis of the worm characterized by complete cessation of movements, which did not reverse spontaneously till about 6 h. However, repeated changes of bath fluid did restore the movements, showing that the paralysis induced by the alcoholic extract of *M. philippensis* is reversible in nature (Fig. 3)

![Min](230 \mu g/ml)

Fig. 3: Effect of alcoholic extract of the leaves of *M. philippensis* on the spontaneous movements of the whole worm of *S. cervi*. Bath applied concentration of 230 \mu g/ml caused initial stimulation characterized by increase in amplitude followed by reversible paralysis. The rate of contractions remained visibly unaffected while the tone showed initial short-lasting increase.

The effect of the alcoholic extract of *M. philippensis* on the n.m. preparation of *S. cervi* was manifest in a concentration nearly 10 times less (20 \mu g/ml) than that required for the whole worm preparation. The initial stimulant effect was characterized by an increase in amplitude alone and there was no increase in tone as seen with the whole worm. The stimulant effect lasted for only 5 min as compared to 45 min with the whole worm. The paralysis which followed was similar in nature and if the bath fluid was not changed it continued for more than 6 h (time till the preparation was observed). However, repeated changes
of the bathing fluid restored the movements to normal (Fig. 4).

**Fig. 4 :** Effect of alcoholic extract of the leaves of *M. philippensis* on the spontaneous movements of n.m. preparation of *S. cervi*. Bath applied concentration of 20 μg/ml caused initial stimulation characterized by increase in amplitude followed by reversible paralysis. The rate of contractions remained visibly unaffected while the tone showed a gradual decrease.

**Effect of *M. philippensis* leave extracts on the survival of microfilariae in vitro:** Aqueous and alcoholic extracts of *M. philippensis* leaves caused concentration related inhibition of survival of microfilariae of *S. cervi*. The alcoholic extract was more potent in its lethal effect as compared to the aqueous extract. Time related lethal effect of aqueous and alcoholic extracts of *M. philippensis* in a concentration of 25 ng/ml observed for 210 min. The LC<sub>50</sub> and LC<sub>90</sub> as observed after 6 h is presented in Table I.

**TABLE I :** Effect of aqueous and alcoholic extracts of leaves of *Mallotus philippensis* on the survival of microfilariae of *S. cervi* in vitro at 6 h.

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Concentration (ng/ml)</th>
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<tbody>
<tr>
<td>Aqueous extract LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>18</td>
</tr>
<tr>
<td>Aqueous extract LC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>20</td>
</tr>
<tr>
<td>Alcoholic extract LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>12</td>
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<tr>
<td>Alcoholic extract LC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>15</td>
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**DISCUSSION**

The observations indicate that recording of spontaneous movements of the whole and n.m. preparation of *S. cervi* is an easy, convenient and reliable method to study the efficacy of antifilarial drugs. It not only provides information regarding lethal or paralysing effect of drugs but also gives an insight into the mechanism of action.

*M. philippensis* extracts were effective in lower concentrations on the n.m. preparation as compared to the whole worm. This indicates that the cuticle can reduce the penetration of the extracts of *M. philippensis* into intact filarids. Substances with low lipid solubility penetrate to a lesser extent across the nematode cuticle, has also been shown for *Ascaris* (9) and *Dipetalonema vitae* (10). The aqueous extract could cause paralysis of n.m. preparation in five times less concentration than that required for the whole worm while for the alcoholic extract the concentration required was eleven times less. This indicates that the aqueous extract has more lipid soluble constituents as compared to the alcoholic extract. The onset of action of both aqueous and alcoholic extracts was rapid and was characterized by stimulation of worm movements reflected mainly as increase in amplitude which was followed by paralysis.

A number of anthelmintics interfere selectively with neuromuscular transmission of the nematode parasite. The bath applied Ach produces stimulation of spontaneous rhythmic movements of *Setaria*, which can be partially blocked by α-tubocurarine but not by atropine (11). The Ach receptors of
Setaria are similar in nature (12) to Ascaris Ach receptors (12, 13, 14, 15, 16, 17). On S. cervi in vitro levamisole and tetramisole also produce stimulation of the movements of whole worm and n.m. preparation only in low concentration while at higher concentration the stimulatory phase though increased in intensity is reduced in duration and is followed by paralysis from which the worm does not recover.

The response with aqueous and ethanolic extracts of M. philippensis was similar qualitatively to that seen with levamisole. However, the stimulatory phase was more prolonged and the subsequent paralysis was reversible in contrast to an irreversible paralysis seen with levamisole and pyrantel pamoate.

Nematodes exhibit rhythmic vigorous movements which can be modified by neurotransmitters and anthelmintics. While Ach has been identified as an excitatory transmitter, GABA and 5-HT paralyze the worm (8). Studies have shown the presence of these substances in parasites. Anthelmintics like piperazine mimic the action of y-aminobutyric acid (GABA) and bring about hyperpolarization of Ascaris and Setaria muscle cells (8, 15, 18, 19). In Ascaris this has been shown to be modified by increase in Cl-conductance. Diethylarbamazine (DEC), a piperazine derivative, on the other hand, produces initial stimulation followed by reversible dose dependent paralysis which results from antagonism of voltage sensitive K+ conductance (20). The aqueous and alcoholic extracts of M. philippensis also produce increase in amplitude and frequency of spontaneous contractions of both whole worm and n.m. preparation of S. cervi. This is followed by reversible paralysis which is evident only at low concentrations (upper panel Fig. 2). Further the response to Ach could be elicited in both whole worm and in the n.m. preparation when they were paralysed by aqueous as well alcoholic extracts. Such a response is similar to that produced by DEC (21).

Although the response of M. philippensis extracts and DEC are similar on in vitro preparations of Setaria, it can be concluded that M. philippensis possesses antifilarial activity which can be potentially useful clinically. Further experiments are needed to evaluate the antifilarial activity in an in vivo model. The paralysis of the worm seen in vivo could only take place when adequate concentrations analogous to those producing paralysis in vitro can be achieved at the site of location of the worm in the animal, i.e. the peritoneal cavity for Setaria and lymphatics for Wuchereria bancrofti.

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REFERENCES
