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Invitro antifilarial potential of the leaf extract of Oscimum sanctum on cattle filarial parasite Setaria cervi

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SUMMARY

The effect of aqueous and alcoholic extract of the leaves of Ocimum. sanctum was studied on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m.) complex of Setaria cervi (S. cervi) and on the survival of microfilariae (m.f.) in vitro. Both the extracts caused inhibition of the spontaneous motility of the w.w. and n.m. complex of S. cervi characterized by initial stimulation followed by reversible paralysis, aqueous extract at a higher concentration showed immediate effect and irreversible paralysis. The concentration required to inhibit the movements of n.m. complex was $1/4^{th}$ for aqueous and $1/3^{rd}$ for alcoholic extract compared to that for the w.w., suggesting a cuticular permeability barrier. On the m.f. the lethal concentration (LC 50 and LC 90) were 35 and 50 ng/ml for aqueous whereas, 60 and 85 ng/ml for alcoholic extracts respectively.

Key words: Oscimum sanctum; Setaria cervi; Filariasis; Microfilariae

INTRODUCTION

Ocimum sanctum (Tulsi) is an aromatic hairy annual herb found throughout India,upto an altitude of 1,800 meters in the Himalayas, cultivated also in temples and gardens. In Ayurvedic medicine, Tulsi is discribed as a diaphoretic and stimulating expectorant. The essential oil of Tulsi is reported to have antibacterial activity, it inhibits growth of Staphylococcus aureus, Escherichia coli and Klebsiella (Jansen *et al.*, 1988) Godhvani *et al.* (1987), have reported antiinflammatory, antipyretic, analgesic and immunoregulatory activity of methanolic extract and aqueous suspension of O. sanctum leaves. Devi and Ganasoundari. have shown that O. sanctum leaves have antioxidant property and have a protective role in radiation induced injury. Anthelmintic activity against poultry worms, Ascaridia galli and Heterakis gallinae and other nematodes has also been found in the leaves of O. sanctum (Kavindra Singh and Shalini Nagaich 2002, Saxena *et al.*, 1994). It was, therefore, thought worthwhile to test the efficacy of O. sanctum leaves against cattle filarial nematode Setaria cervi, which resembles closely in its reaction to drugs with human filarial infections. (Singhal *et al.*, 1972).

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MATERIALS AND METHODS

Fresh leaves of Ocimum. sanctum were thoroughly washed and shade dried. The leaves were ground with the help of an electric grinder. The powder obtained was filtered throgh a fine muslin cloth and was transferred to thimbles of Whatman filter paper No. 1 in Soxhlet apparatus, ethyl alcohol was used as a solvent for alcoholic extract whereas distilled water for aqueous extract. The apparatus was allowed to run for 48-72 hours. Later the solvent was allowed to evaporate in a vacuum dessicator and after the complete evaporation of the solvent, the residual material was suspended in gum acacia and diluted with saline. Motile adult S. Cervi (Nematoda filarioidea) of average length 6.0±1.0 were collected from the peritoneal cavity of freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing modified

Ringer's solution (Nacl 9 g, Kcl 0.42 g, Cacl2 0.24 g NaHCo3 0.5 g, glucose 0.25 per litre) at 37°C.

Whole worm (w.w.) preparation: Adult S. cervi were suspended in an ideal 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 . 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 movement s 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 inactive.

Nerve-muscle (n.m) complex: 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 cut 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 rhythmical movements similar to those of the whole 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 (m.f.): The uterus of a female S. cervi was cut at its junction with the vagina just below the bifurcation, and removed from the worm. It was teasted with a fine needle in the solution and microfilariae (mf) were freed. The microfiliariae 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 capped bottles containing alcoholic and aqueous extract of O. sanctum in equal serum: ringer mixture (v/v). O. sanctum leaf 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 6 hours to observe the survival / mortality of microfilariae. The LC 50 and LC 90 were calculated from a concentration vs death graph.

In a preliminary set of experiment it was ascertained that the concentration of alcohol/water /gum acacia in the suspending medium did not influence the survival/mortality of the m.f.

In a preliminary experiment, the aqueous and alcoholic extract of O. sanctum leaves were added to m.f. in concentration of 5, 10, 15, 20, 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 m.f. The effect of each dose was observed 10 times. The mean of the values were plotted on a graph.

RESULTS

Effect of aqueous extract of Ocimum. sanctum leaves on the spontaneous movements of whole worm (w.w) preparation and nerve muscle (n.m.) complex of Setaria. cervi:

Immediately after the addition of aqueous extract in a concentration of 800 μg/ml of the bath fluid the amplitude of the contractions showed a gradual increase till it reached its peak at about 30 min (upper panel Fig. 1). The rate showed a slight decrease but the tone was unaffected, thereafter both the amplitude and the frequency of contractions decreased after about 60 min. and there was complete cessation of movements at about 95 min (lower panel Fig. 1). Repeated changes of the bath fluid restored the movements to its pre drug state, suggesting that the paralysis is reversible in nature.

The response to aqueous extract of O. sanctum leaves could be elicited on n.m. complex of S. cervi in a concentration of 200 μg/ml of the bath fluid. This concentration was 1/4th of that required for the whole worm and the response was also different in nature. The initial stimulant effect characterized by increase in amplitude lasting for 15 min. was followed by a marked decrease in tone, amplitude and frequency at about 20 min. which ultimately led to complete cessation of movements after about 30 min (upper panel Fig. 2). Repeated changes of the bath fluid restored the movements to pre drug state, suggesting that the paralysis is reversible in nature (lower panel Fig.

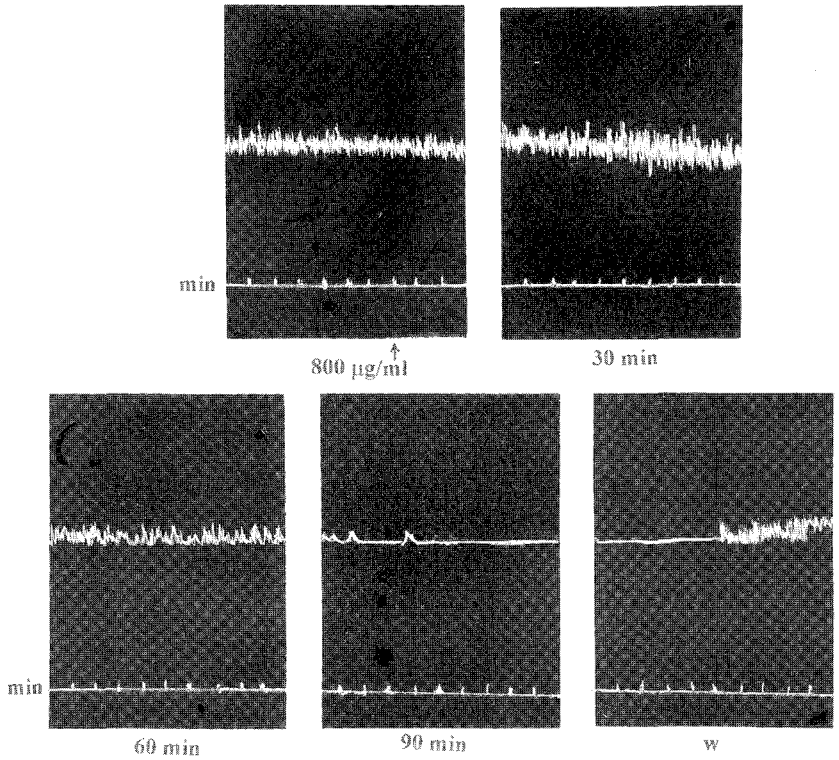

Fig. 1. The initial stimulatory effect of 800 μg/ml of aqueous extract of O. sanctum leaves on spontaneous movements of whole worm preparation of S. cervi leading to reversible paralysis.

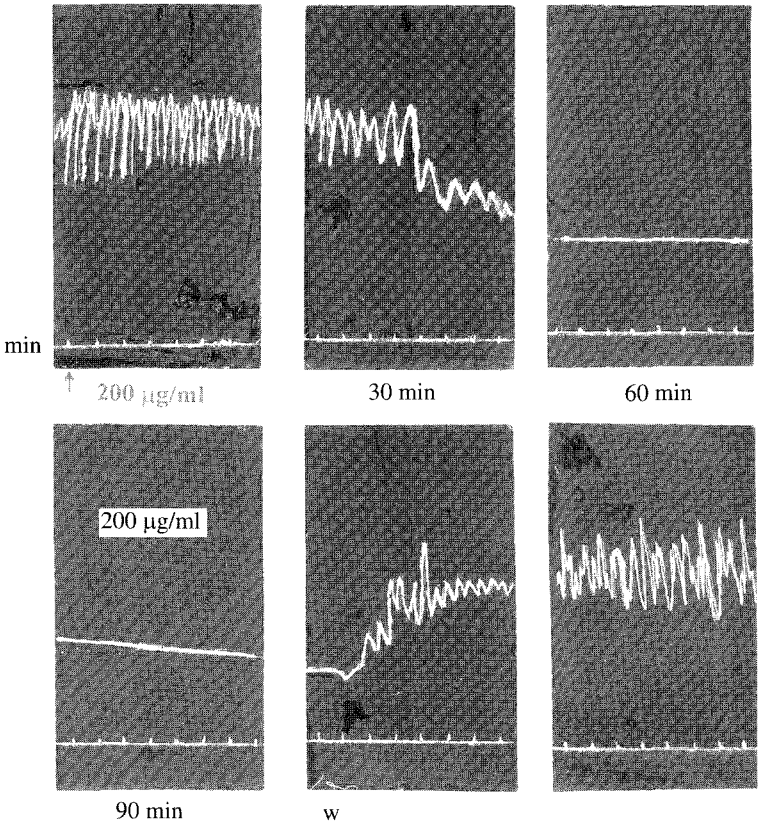

Fig. 2. Inhibitory effect of 200 μg/ml of aqueous extract of O. sanctum leaves on spontaneous movements of nerve muscle complex of S. cervi.

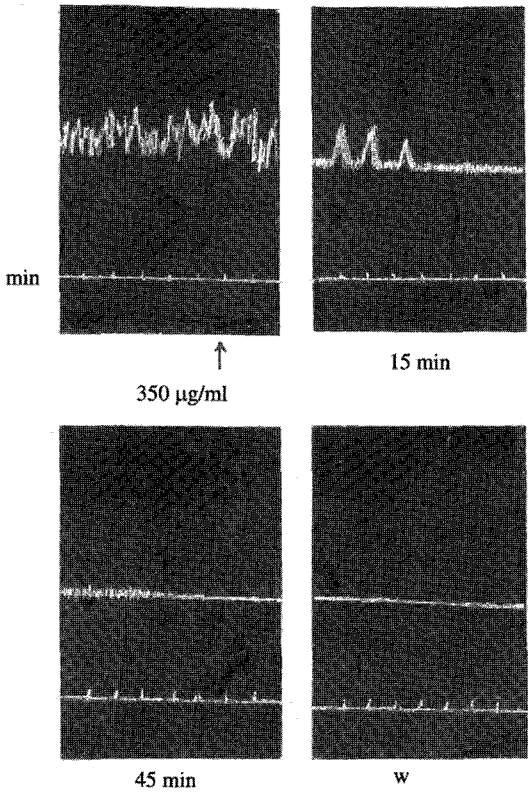

Fig. 3. Irreversible paralysis of nerve muscle complex of S. cervi caused by higher concentration (350 µg/ml) of aqueous extract of O. sanctum leaves.

2). When a higher concentration of aqueous extract (350 µg/ml) was added to the bath fluid the response of n.m. complex was immediate and was characterized by a decrease in amplitude and frequency in 15 min. After about 45 min. the cessation of movements was complete leading to irreversible paralysis of the worm (Fig. 3). Repeated changes of the bath fluid failed to restore the movements. Addition of Ach or Calcium Chloride during paralysant phase was ineffective in restoring the movements.

Effect of alcoholic extract of O. sanctum leaves on the spontaneous movements of the whole worm preparation and nerve muscle complex of S. cervi: addition of alcoholic extract in a concentration of 3 mg/ml of bath fluid caused initial stimulation followed by paralysis of the whole worm. The initial stimulant effect lasted for about 60 min (upper panel Fig. 4). and was characterized by increase in the amplitude only. Later on i.e around 45 min. in the stimulation phase a decrease in frequency of movement was observed, this was followed by paralysis of the worm characterised by complete cessation of movements, which did not reverse spontaneously till about 6 hours. However,

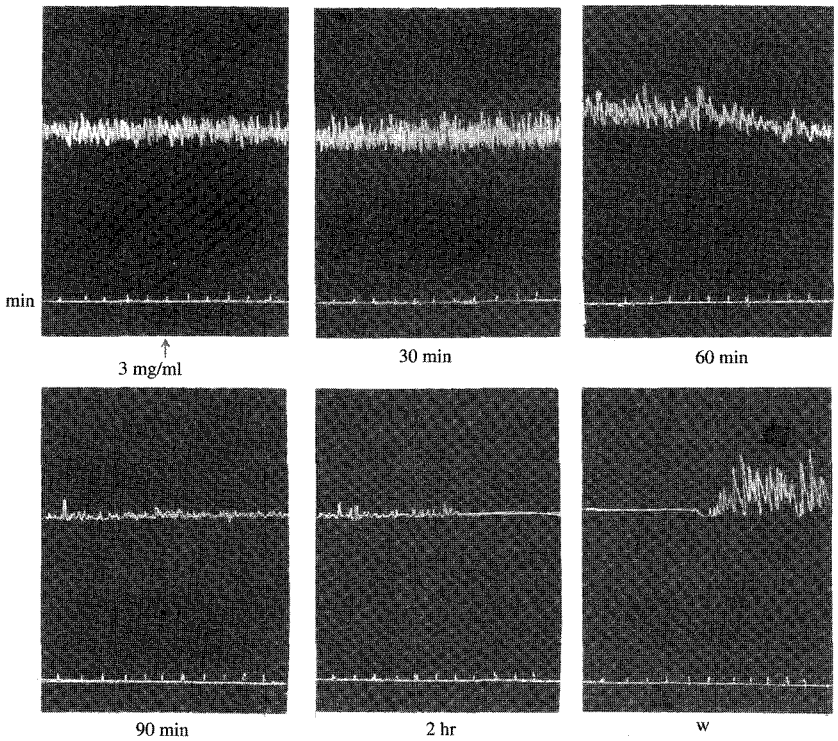

Fig. 4. Reversible paralysis of whole worm preparation of S. cervi caused by 3 mg/ml of alcoholic extract of O. sanctum leaves.

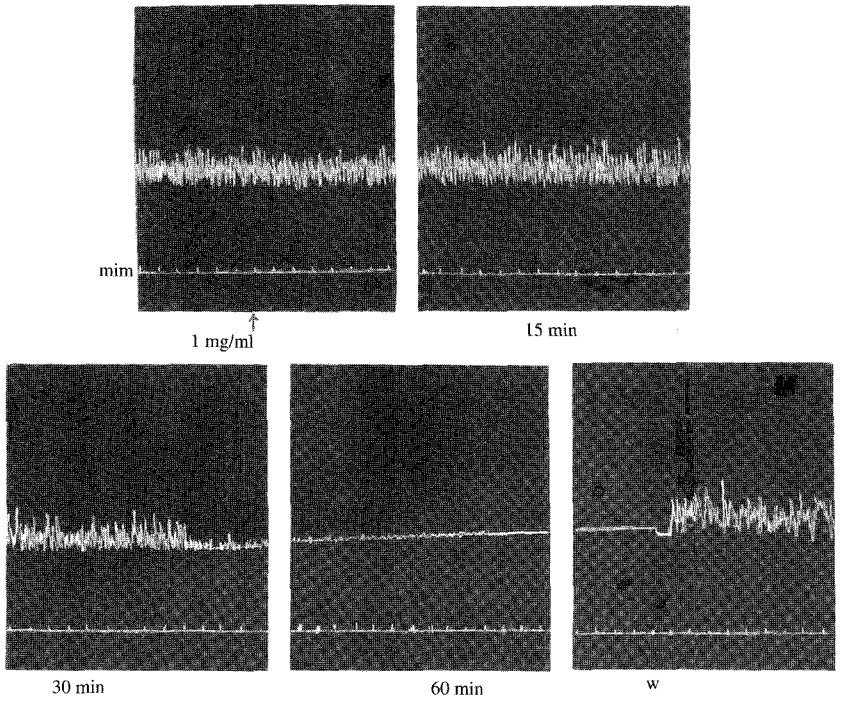

Fig. 5. Reversible paralysis of nerve muscle complex of S. cervi caused by 1 mg/ml of alcoholic extract of O. sanctum leaves.

repeated changes of the bath fluid restored the activity to the predrug state showing that the paralysis is reversible in nature (lower panel Fig. 4).

Effect of alcoholic extract on the n.m. complex was initially seen at a concentration of 1 mg/ml of bath fluid which is nearly 1/3rd of that required for whole worm. The initial stimulant effect was characterised by an increase in the amplitude alone and there was no change in the tone and frequency (upper panel Fig. 5). The stimulant effect lasted for 30 min. as compared to 60 min. with the whole worm. The paralysis which followed was similar in nature and if the bath fluid was not changed it continued for 6 hours (time till the preparation was observed). However, repeated changes of the bath fluid restored the activity to predrug state, suggesting a reversible paralysis (lower panel Fig. 5).

Effect of extract of Ocimum sanctum leaves on the survival of microfilariae (mf) Setaria. cervi in vitro : aqueous and alcoholic extract of O. sanctum leaves caused a concentration related inhibition of survival of m f of S cervi. The aqueous extract was more potent in its lethal effect compared to the alcoholic extract. The time related lethal effect of

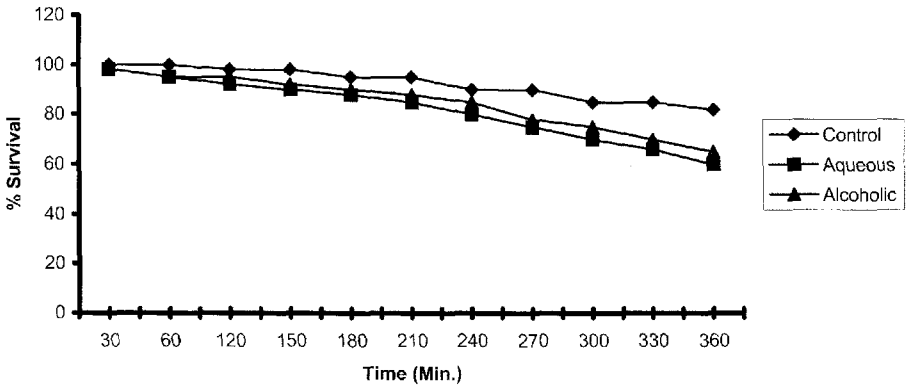

Fig. 6. Effect of aqueous and alcoholic extract of O. sanctum leaves on the survival of microfilariae of S. cervi in vitro at a concentration of 25 ng/ml.

Table 1. Effect of Aqueous and Alcoholic extract of Ocimum sanctum leaves on the survival of m.f. of S. cervi in vitro at 6 hours

Extract LC	Concentration ng/ml
Aqueous LC50	35
Aqueous LC90	50
Alcoholic LC50	60
Alcoholic LC90	85

aqueous and alcoholic extract of O. sanctum leaves at a concentration of 25 ng/ml is shown in Fig. 6.

The LC 50 and LC 90 observed after 6 hours is shown in table No. 1

DISCUSSION

Filarial nematodes are essentially aquatic animals which exhibit spontaneous movements which help the parasite in locating and maintaining itself in the environment. S. cervi too shows spontaneous movements which can be recorded on kymograph. Nerve muscle preparation in which cuticular permeability barrier is removed by splitting the worm longitudinally and organs are removed from the body cavity exhibit movements similar to whole worm. However, this preparation is more sensitive to the action of drugs as the effect is directly on the nerve muscle complex (Singhal K.C., Madan B.R. and Saxena P.N., 1977).

O. sanctum leaf extract (both aqueous and alcoholic) inhibit spontaneous movements of whole worm preparation and n.m complex of S. cervi in vitro (Fig. 1-5), The concentration required to influence the spontaneous movements in whole worm preparation and n.m complex was different and also differed with the type of extract used. While the aqueous extract was effective in a concentration of 800 μg/ml of bath fluid on the whole worm preparation, the similar response with the alcoholic extract was obtained at a higher concentration of 3 mg/ml of bath fluid. On the n.m. complex too the efficacy of aqueous extract was higher (200 μg/ ml) than the alcoholic extract (1 mg/ml). The n.m. complex responded to lesser concentration of aqueous (1/4th) as well as alcoholic extract (1/3rd).

The findings indicate that an effective cuticular permeability barrier exist in S. cervi for O. sanctum leaf extract as it does to many other substances (Frettrer RH, 1986, Christ D, Goebel M, Saz HJ, 1990). A water soluble substance has a easy permeability across cell membrane through pores which could be the situation with aqueous extract of O. sanctum. It could also be possible that substances soluble in water may also be lipid soluble providing an added advantage to aqueous substances present in O. sanctum to penetrate the permeability barrier of the n.m. complex and cuticle of S. cervi.

Inhibition of spontaneous rhythmical movements in w.w. and n.m. of S. cervi could be due to blockade of the action of excitatory neurotransmitter acetylcholine (Singhal K.C., Madan B.R. and Saxena P.N., 1977), or potentiation of inhibitory neurotransmitters 5-hydroxytryptamine (5-HT) or gama -aminobutyric acid (GABA) (Singhal *et al.*, 1975).

Certain anthelmintics like piperazine mimics the action of GABA and bring about hyperpolarization of Ascaris and S. cervi muscle cell (Singhal KC *et al.*, 1975, Aubry ML *et al.*, 1970, Del Castillo *et al.*, 1963, 64). Diethylcarbamazine (DEC), a piperazine derivative produces initial stimulation followed by reversible dose dependent paralysis through antagonism of voltage sensitive potassium channels (Martin BJ, 1982). The aqueous and alcoholic extracts of O. sanctum leaves also produce initial stimulation of both w.w. preparation and n.m. complex of S. cervi followed by reversible paralysis, such a response is similar to that of DEC (Singhal KC *et al.*, 1978). The inhibition of spontaneous movements of n.m. complex was complete and irreversible when higher concentration of aqueous extract was presented to the bath fluid. The reason may be sought in either non competitive blockade of excitatory receptors at higher concentration or the extract contains two active ingredients. While the one at low concentration causes reversible paralysis and the other at higher concentration leads to non competitive irreversible paralysis leading to the death of parasite.

It can be argued that O. sanctum leaves possess antifilarial activity having some what similar action as that of DEC, which can be potentially useful clinically.

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