Evaluation of antifilarial activity of *Berberis aristata* roots

Waseem Rizvi¹*, Anil Kumar¹, Razi Ahmad¹, Mohammad Shahid², Anwar Shazad¹ and Nakhat Haider⁴

¹Department of Pharmacology J. N. Medical College, Aligarh Muslim University, Aligarh, (UP), India; ²Department of Microbiology, J. N. Medical College, Aligarh Muslim University, Aligarh, (UP), India; ³Department of Botany, Aligarh Muslim University, Aligarh, (UP), India; ⁴University Health Service, Aligarh Muslim University, Aligarh, (UP), India

Received for publication December 12, 2006; accepted October 19, 2007

SUMMARY

The effect of aqueous and alcoholic extract of roots of *Berberis aristata* was studied on the spontaneous movements of the whole worm (w.w) preparation and nerve muscle (n.m) complex of cattle filarial parasite *Setaria cervi* and on the survival of microfilariae. Only aqueous extract could inhibit the spontaneous movements of *S. cervi*, characterized by initial stimulation followed by reversible paralysis. The concentration required to produce similar effect on n.m complex was less as compared to the w.w. The lethal concentration 50 and lethal concentration 90 for aqueous extract were 48 ng/ml and 65 ng/ml.

Key words: Antifilarial activity; *Berberis aristata*; *Setaria cervi*

INTRODUCTION

*Berberis aristata* is an erect, glabrous shrub found in Nepal at an altitude of 6,000 - 8,000 ft. The alkaloids in the bark and root bark of *Berberis* are berberine, berbamine, aromoline, karachine, palmatine, oxyacanthine and oxyberberine (Chopra, 1982).

In Ayurvedic medicine the roots of *B. aristata* is reported to possess anti-inflammatory and antibacterial activities, and is used as laxative, diaphoretic, antipyretic, antiseptic and antiulcer agent (Chopra, 1982). Clinical studies with berberine in Cholera patients showed that it was effective in both bacteriologically positive and negative patients, it reduces mortality rate, volume and duration of diarrhea (Lahiri and Dutta, 1967; Sharda, 1970). It is also effective in giardiasis (Choudhry et al., 1972) and gastroenteritis in children. Berberine is also known to possess antidiabetic activity (Hua and Wang, 1994; Hua et al., 2001). Despite the medicinal properties of *B. aristata* the antifilarial activity, especially against *S. cervi* has not yet been explored. Therefore, the present preliminary study was designed to see the antifilarial potential of aqueous and alcoholic extracts of roots of *B. aristata*.

MATERIALS AND METHODS

The shrub was collected from Forest Research Institute (F.R.I), Dehradun, and identified by Dr. Badar Alam Senior Lecturer in the department of Botany, Aligarh Muslim University and the
specimen was deposited in the same department via Herbarium/Account No. 1705.

The dried roots were ground in an electric grinder, the powder obtained was transferred to ordinary filter paper in Soxhlet apparatus. Ethyl alcohol was used as a solvent for alcoholic extract whereas distilled water for aqueous extract. The solvent obtained was allowed to evaporate in a vacuum dessicator and after the complete evaporation of the solvent, the residual material was diluted with saline to make a stock solution of 1 mg/ml. 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 (Sodium chloride 9 g, Potassium chloride 0.42 g, Calcium chloride 0.24 g, Sodium bicarbonate 0.5 g, glucose 0.25 per liter) 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 (Singhal, 1975). Air or Oxygen was not bubbled through the solution, as it did not improve the movements of the worm. Approximately 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 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 teased with a fine needle in the solution and m.f. were freed. The m.f. were suspended in a human serum : Ringer mixture and the m.f. count was adjusted to 100 mL. 0.5 mL aliquots of the m.f. suspension were placed in sterilized screw capped bottles containing aqueous extract of *B. aristata* in equal serum : ringer mixture (v/v) (Singhal, 1975). Extract was added in doubling 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 h to observe the survival/mortality of microfilariae. The lethal concentration 50 (LC 50) and lethal concentration 90 (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 in the suspending medium did not influence the survival/mortality of the m.f. In a preliminary experiment, the aqueous and alcoholic extract of *B. aristata* were added to m.f. in concentration of 5, 10, 15, 20, 25 ng/ml to determine the limits of activity within 6 h 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 *B. aristata* on the
spontaneous movements of w.w preparation and n.m complex of S. cervi. On addition of 500 mg/ml of aqueous extract there was initial stimulation characterized by increase in amplitude and rate of contraction lasting for 30 min (Upper panel, Fig. 1), followed by a decrease in rate and amplitude at 60 min, leading to complete cessation of movements at 90 min. The motility was reversed on repeated changes of bath fluid i.e., the paralysis is reversible in nature (Lower panel, Fig. 1). The response of n.m complex to aqueous extract was different in nature. Addition of 350 mg/ml of extract to the bath fluid produced inhibition of spontaneous movements characterized by decrease in amplitude and rate of contraction (Upper panel, Fig. 2) followed by complete cessation of movements at 60 mins. The paralysis produced was reversible in nature as repeated changes of bath fluid caused reversal of movements (Lower panel, Fig. 2). The alcoholic extract failed to produce any effect on the movements of the w.w preparation or the n.m complex. Effect of aqueous extract of B. aristata on the survival of m.f in vitro. Aqueous extract caused a concentration related inhibition of survival of m.f of S. cervi. The time related lethal effect at a concentration of 25 ng/ml is shown in Fig. 3. The LC50 and LC90 observed after 6 h are 48 ng/ml.

Fig. 1. The initial stimulatory effect of 500 µg/ml of aqueous extract from roots of Berberis aristata on spontaneous movements of w.w preparation of S. cervi leading to reversible paralysis.

Fig. 2. Reversible paralysis of movements of n.m complex of S. cervi with 350 µg/ml of aqueous extract from roots of Berberis aristata.

Fig. 3. Survival of m.f of S. cervi In vitro at a concentration of 25 ng/ml of aqueous extract from roots of Berberis aristata.
Evaluation of antifilarial activity of Berberis aristata roots

Aqueous extract of B. aristata produced inhibition of spontaneous movements of both w.w preparation and n.m complex of S. cervi. The concentration required to produce the same effect on n.m complex was less than that required for w.w preparation. This suggests that cuticle can reduce permeation of extract into the intact filarid. Substance with low lipid solubility penetrate to a lesser extent across the cuticle of the nematode, has been shown for Ascaris (Fetterer, 1986) and Dipetalonema vitae (Christ et al., 1990). The onset of action was rapid in both cases with a difference that there was initial stimulatory phase in case of w.w preparation, this could be due to the irritant effect of extract on cuticle as has been shown with other substances as well (Christ et al., 1990). It has been shown that the nematodes possess both the excitatory (acetylcholine) as well as inhibitory (GABA, 5-HT) neurotransmitters which can paralyse the worm (Singhal et al., 1975). A number of anthelmitics interfere with neuromuscular transmission, like piperazine which mimics the action of GABA and causes hyperpolarization of Ascaris and S. cervi muscle cell (Singhal et al., 1975; Aubry, 1970; Del et al., 1963). Diethylcarbamazine (DEC) a piperazine derivative produces reversible paralysis by antagonizing voltage sensitive potassium channels (Martin, 1982). The aqueous extract of B. aristata also produced inhibition followed by reversible paralysis, this response resembles that of DEC (Singhal et al., 1978). To conclude we can say that aqueous extract of B. aristata possess potential antifilarial activity having action somewhat similar to that of DEC this can be further confirmed by in vivo studies.

REFERENCES


Choudhry VP, Sabir M, Bhule VN. (1972) Berberine in giardiasis. Indian Pediatr 9, 143-146.


