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Antifilarial activity in *Stereospermum suaveolens* DC leaves against Brugia malayi

Vijai Lakshmi[1,2]*, Shailja-Misra Bhattacharya[3]

[1]Department of Biochemistry, King George Medical University, Lucknow-226003, (INDIA)

[2]Division of Medicinal and Process Chemistry, Lucknow – 226001, U.P, (INDIA)

[3]Division of parasitology, CSIR-Central Drug Research Institute, Lucknow – 226001, U.P, (INDIA)

E-mail: vijlakshmius@yahoo.com

ABSTRACT

The present study is aimed to evaluate antifilarial activity of *Stereospermum suaveolens* DC (Bignoniaceae) against human lymphatic filarial parasite *Brugia malayi in vitro* and *in vivo*. The ethanolic extract of the leaves of the *S. suaveolens* was tested in vitro on adult worms and microfilariae (mf) of *B. malayi* and the active sample was further evaluated *in vivo* in *B. malayi* (intraperitoneally) i.p. transplanted in the jird model (Meriones unguiculatus) and Mastomys coucha subcutaneously infected with infective larvae (L3). The ethanolic extract was active in vitro (IC50:adult = 31.5 µg/ml; mf = 15.45µg/ml) and demonstrated 61.9% adulticidal and embryostatic effect on *B. malayi*, respectively, in Mastomys at a dose of 5 × 100 mg/kg by oral route. The antifilarial activity was at 5 × 100 mg/kg by subcutaneous route revealed excellent adulticidal efficacy resulting in to the death of 61.9% transplanted adult *B. malayi* in the peritoneal cavity of jirds respectively in addition to noticeable microfilaricidalo action on the day of autopsy. The findings reveal that the extract from the leaves of *S. suaveolens* contains promising in vitro and in vivo antifilarial activity against human lymphatic filarial parasite *B. malayi* which may be further explored to evaluate the antifilarial activity by evaluating its bioactive fractions and pure compounds from the active fractions, which may provide anew antifilarial agent.

KEYWORDS

S.suaveolens;
Antifilarial activity;
In vitro;
In vivo;
B.malayi.

INTRODUCTION

Stereospermum suaveolens DC (Bignoniaceae), commonly known as "Patala," is widely available in India. It mainly contains lapachol, dinatin, β-sitosterol, saponin and palmitic, stearic and oleic acids[1]. Traditionally, it is mainly used as analgesic, wound healing, antidyspeptic, astringent and liver stimulant[2]. Some recent literature reviews indicated

***Stereospermum suaveolens* DC**

that many flavonoids and antioxidants possess antiulcerogenic and wound healing activity[3]. Because phytochemical investigations of the methanolic stem bark extract of *S. suaveolens* also revealed the presence of flavonoids, phenols, saponins, tannins and alkaloids, an attempt has been made to investigate the antifilarial activity against *Brugia malayi* in *in vitro* and *in vivo* models of the ethanolic extract of *S. suaveolens leaves.*

MATERIALS AND METHODS

Plant material

Leaves of the *S. suaveolens* (500g.) were purchased from the market, India and was authenticated by Botany Department of the Central Drug Research Institute, Lucknow, India.

Extraction fractionation and isolation of compounds

The air dried leaves (500 g.) were powdered and percolated in 95% ethanol at room temperature for 24 hours, filtered and the process was repeated four times. All the extracts were mixed and filtered. Mixed ethanolic extract was concentrated under reduced pressure below 50^0 C in a rotavapour to a green viscous mass, which was dried under high vacuum for 2 hours to remove the last traces of the solvent. Weight of the dried ethanolic extract 12.8 g. which was used for the screening of antifilarial activity against *B. malayi in vitro* and *in vivo* models.

A. Antifilarial activity: in vitro assays

(i)Sample preparation

1 mM stock solution of the ethanol extract was prepared in dimethylsulfoxide.

(ii) Parasite isolation

The live adult *B. malayi* worms were isolated from the peritoneal cavity of jird (Meriones unguiculatus) infected 100-150 days earlier by intraperitoneal inoculation of 150-200 infective larvae (L3) of *B. malayi* recovered from experimentally infected mosquitoes, *Aedes aegypti*[4]. After isolating the adult parasites, the peritoneal washing was passed through a membrane filter (pore size 5.0 mm) and the microfilariae were pelleted by centrifugation[5]. All the animals and experimental procedures were duly approved by the Animal Ethics Committee of CDRI, duly constituted under the provisions of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India. This study bears the approval no. IAEC/2011/120/Renew 01/dated 14/08/2012.

(iii) Primary in vitro screening

The actively motile female worms were placed individually in the wells of 48 well culture plate containing RPMI 1640 medium fortified with antibiotics (penicillin 100 units/mL, streptomycin sulfate 100 mg/mL, and neomycin mixture; Sigma, USA). Each well contained one female worm in 1 mL of the medium. Simultaneously,10 microfilariae were suspended in 200 mL medium in each well of a 96 well culture plate (NUNC). The parasites were incubated at 37^0 C in 5% CO2 in air for 5 continuous days in the presence of 10 mM concentration of the ethanol extract of leaves and the motility of parasites was monitored microscopically at regular time intervals. At the end of the experiment, adult parasites were transferred to fresh drug free medium for one hour at 37^0 C to observe reversal, if any, in the worm motility. The worms were later processed individually for MTT [3-(4,5 dimethylthiazol- 2-yl)-2,5 diphenyl tetrazolium bromide] dye reduction assay as published earlier[6] for checking their metabolic viability. The experiments were carried out in duplicate and the degree of loss in the motility as well as percent inhibition in MTT reduction in treated parasites over the untreated controls was assessed. The compounds which consistently demonstrated their lethal effects on the parasites at 10 mM (highest concentration tested) with 50% inhibition in MTT reduction in duplicate experiments as compared to untreated respective controls were considered as active compounds[6] while those bringing about 100% irreversible inhibition in motility of microfilariae were considered microfilaricidal.

(iv) Evaluation of IC50 and CC50

The test samples found active in primary in vitro screen were followed for IC50 using four serial two fold dilutions of each sample starting from MIC in

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the same way as mentioned above. IC50 values were determined by Excel based line graphic template after plotting concentration values of each sample versus percent motility inhibition of parasite on x- and y-axis. In vitro CC50 assay on Verocells (monkey kidney cell line) was performed as mentioned earlier[7]. In brief, Vero cells (104/well/100 ml) in 96 well plate were exposed to seven three-fold serial dilutions of active test samples starting from 100 mM at 37^0 C in a CO2 incubator. After 72 h, resazurin dye was added and the plate was re-incubated for 3-4 h. The fluorometric reaction was measured at an excitation wavelength of 536 nm and emission at 588 nm in a fluorometric plate reader.

(v) Scoring and activity evaluation criterion

The motility of the adult worms and microfilariae was scored as 0% motility reduction (4þ); 1-49%motility reduction (3þ); 50-74% motility reduction (2þ); 75-99% motility reduction (1þ) and 100% motility reduction (Dead)[7].

(vi) Determination of selectivity index (SI)

The safety of the active samples was determined by assessing SI values (CC50/IC50) t select test sample/s worth following in vivo. The compounds with SI values of 10 were considered safe and therefore further followed in vivo.

B. Antifilarial activity in vivo model

(i) In vivo screening model

The samples identified as active in the *in vitro* screen were tested in the primary screening model (adult *B. malayi* intraperitoneally transplanted jird). Primary screening provides the quantitative antifilarial activity on adult parasites as known numbers of worms are transplanted into the peritoneal cavity. Intraperitoneal (i.p) transplantations of 10 females and five male adult worms of B. malayi were carried out in 6-8 week old male jirds. The jirds were anaesthetized by ketamine (50 mg/kg, i.p), animals were quickly shaved and a small incision was made on latero-ventral region of abdomen to introduce worms into the peritoneal cavity. The success of transplantation was affirmed by the presence of live microfilaria in a drop of peritoneal fluid aspired on day 4 and these jirds were selected to screen in vitro hits.

(ii) Treatment schedule

The ethanol extract and the standard drug, diethylcarbamazine were administered subcutaneously at a dose of 100 mg/kg for five consecutive days to seven groups of transplanted jirds and each group had three animals. Three jirds received vehicle only and served as control group.

(iii) Assessment of antifilarial activity in the primary jird model

The treatment was initiated from day 5 post worm transplantation and the observations were continued till day 45. On day 45, the jirds were euthanized along with the untreated controls to recover worms by peritoneal washings. The recovered parasites were counted and examined for motility, death or calcification. Live female worms were teased in a drop of phosphate buffered saline (PBS, pH 7.2) to dissect out the uterus for observing the uterine contents and observed microscopically to assess the embryostatic effect of test samples, if any[8]. The peritoneal washing collected on autopsy was microscopically observed to assess the effect of test sample on released microfilariae.

(iv) Statistical analysis

The statistical analysis of the data was carried out by PRISM 3.0 using one way ANNOVA (non-parametric). Dunnett's multiple[7] comparison test was applied to assess the statistical significance of the values between treated and control group. Values were expressed as mean ± SE. $P < 0.05$ was considered as of low significance (*) while $P < 0.01$/ 0.001 were considered as highly significant (**/***).

RESULTS

The ethanolic extract of the leaves of the *S. suaveolens* was tested in vitro on adult worms and microfilariae (mf) of *B. malayi* and the active sample was further evaluated *in vivo* in *B. malayi* (intraperitoneally) i.p. transplanted in the jird model (Meriones unguiculatus) and Mastomys coucha subcutaneously infected with infective larvae (L3). The ethanolic extract was active in vitro (IC50: adult =

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CONCLUSION

The findings reveal that the ethanol extract from the leaves of *S. suaveolens* contains promising *in vitro* and *in vivo* antifilarial activity against human lymphatic filarial parasite *B. malayi* which can be further explored to evaluate the antifilarial activity by evaluating its bioactive fractions and pure compounds from the active fractions, which may provide anew antifilarial agent.

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