International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.3, pp 1181-1184, July-Sept 2012

Antifilarial Potential of Butea monosperma L. against microfilaria in vitro.

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Abstract: Currently available antifilarial drugs DEC, albandazole and Ivermactin and other combination of drugs are not able to control the filariasis. Therefore better antifilarial drug is required for control and management of the disease. Usage of herbal drugs in traditional medicine is well known but largely observed. In the present study, the antifilarial activity was screened for aqueous extracts of *Butea monosperma* L. leaves and roots. In *in vitro* experiment, extracts were tested from 20-100 ng/ml for 24 hours incubation, against microfilaria stage of *Brugia malayi* worm. *Butea monosperma* roots extracts showed significant inhibition of motility of microfilariae in dose dependent manner then leaves extract, in such nano concentration, contributes to the development of database for novel drug candidates for human lymphatic filariasis.
Key words : Antifilarials, microfilariae, *Brugia malayi*, *Butea monosperma* L.

Introduction

The human lymphatic filariasis is a tropical disease, prevalent in all over India. The World Health organization (WHO) has precisely recognized human lymphatic filariasis as one of the ten diseases in its tropical disease research (TDR) scheme highlighting the enormous disease burden leading to 5.8 million disability adjusted life years (DALYs) and therefore launched a global programme for elimination of filariasis (GPELF)[1]. However, there are some serious limitations, such as lack of potent vaccines, potential risk of insecticide resistance for vector control and the absence of effective drugs. Diethylcarbamazine (DEC) is the most widely popular drug for treatment of filariasis has becomes since couple of years[2]. Though the existing drug DEC is a good microfilaricide, it

might have side effects[3]. Due to the lack of patient's fulfillment with failure in achieving targeted coverage (85%), use of this drug in mass drug administration approach might not succeed in serving its purpose[4]. Specifically because of these reasons, it is reasonably essential to discover novel antifilarial drugs. World Health Organization has already outlined the nature of traditional medicine and herbal therapy[5].India has Ayurveda, Unani and Siddha systems of medicine, having a rich tradition of keen traditional herbal therapy[6]. Thus, it is very much necessary to develop a large database of antifilarials. Experimental evidences of antifilarial activities of various such phytochemicals are coming up with the constant effort made by the workers from various parts of the world. Few of them the filaricidal property of the plant, *Plumbago indica/rosea* was investigated *in vitro* against *Setaria digitata*, a filarial parasite[7]. More interestingly such antifilarial effect has also been reported against human lymphatic filarial worm, *Brugia malayi.* Twelve extracts of 11 Guatemalan medicinal plants were screened *in vitro*. The ethanol extract of leaves of *Neurolaena lobata* showed significant antifilarial activity against *Brugia pahangi* filarial worm[8]. In the present study one plant, *Butea monosperma* L. (Family: *Fabaceae*), local name *palas* or *dhak*, which is found throughout India which reportedly have antihelminthic/antifilarial effect in traditional usage, was screened *in vitro* for their antimicrofilarial activity against *Brugia malayi*.

Materials and Methods

Plant materials: *Butea monosperma* L. (leaves and roots) were collected from the local areas of Sausar, Chhindwara, Madhya Pradesh (India) and identified in the P.G. Dept. of Botany, RTM, Nagpur University, Nagpur (Voucher specimen numbers 9024 is allotted).

Preparation of herbal extracts: The leaves and roots of medicinal plants were washed, shade dried and powdered. The powdered form of *Butea monosperma* L. leaves and roots were extracted with double distilled water[9] and further filtered and concentrated by keeping in hot air oven (40^{o}C) to get semi solid residue which yielded 6.7 and 5.8 per cent (w/w) respectively. Extracts were stored in refrigerator for *in vitro* evaluation against *Brugia malayi* microfilaria using standard method[10].

Parasites: The *Brugia malayi* life cycle was established and maintained in jirds (*Meriones unguiculatus*) and mastomys (*Mastomys natelansis*) using mosquitoes (*Aedes aegypti*) as a vector by standard methods[11,12]. Microfilariae (mf) were obtained by lavage of the peritoneal cavities of jirds with intraperitoneal filarial infection of 3 months or more duration. The mf were washed with RPMI 1640 medium (GIBCO laboratories, USA) (containing 20mg/ml gentamycin, 100mg/ml penicillin, 100mg/ml streptomycin) plated on sterile plastic petri-dishes and incubated at 37°C for 1 h to remove jirds peritoneal exudate cells. The mf were collected from petri-dishes, washed with RPMI 1640 medium and used for *in vitro* maintenance[10]. The use of animals for the study was approved by the Institutional animal ethical committee of Mahatama Gandhi Institute of Medical Sciences, Sevagram (Wardha) India.

In vitro screening for antifilarial activity against microfilaria of Brugia malayi: Crude extract of medicinal plants were diluted in suitable solvent double distilled water to obtain the desired final concentration range (20-100 ng/ml) as previously optimized in our lab so as to obtain dose dependant effects against microfilariae in sterile 24 well culture plates (Nunc, Denmark) containing 900 ml of RPMI medium. Wells without any extract but with similar solvents in 900 ml of the medium were kept as corresponding controls. Approximately 100 microfilariae in 100 ml of RPMI medium were introduced into each well for every test samples and also for corresponding control samples (each individual samples in triplicates). The plates were incubated at 37°C for 24 h in CO2 (5%) incubator. Mf motility was assessed by microscopy after 48 h of exposure (incubation for this time period was optimized during screening); the observations were recorded as the number of non motile mf out of all the 100 mf taken in each well for the study and represented as percentage (%) reduction in mf motility[10]. All these conditions of assay procedure have been standardized in our laboratory to obtain reproducible results. Each experiment (in triplicate) was repeated three times and results were represented as Mean $\pm$ SEM of per cent reduction in motility of three such observations. Entire procedure was carried out in aseptic condition.

Statistical analysis

The results compared between the plant extracts and controls, student's *t* test was used. $P < 0.05$ was considered as significant.

Table. *In vitro* effects of plant extracts on *Brugia malayi* microfilarial motility. Percentage reduction in Mf motility by plants extracts

S. No.	Concentrations (ng/ml)	*Butea monosperma* L. (Leaves)	*Butea monosperma* L. (Roots)
1.	20	3.51 ± 0. 632*	5.19 ± 0.833*
2.	40	5.05 ± 0.634*	6.68 ± 1.747*
3.	60	6.64 ± 0.597*	11.1 ± 1.027*
4.	80	9.13 ± 0.584*	15.45 ± 1.948*
5.	100	11.75 ± 0.486*	19.32 ± 0.908*
6.	Control	0.96 ± 0.326*	1.32 ± 0.319

Results shown are mean + SEM of per cent reduction in motility
*P< 0.05 when compared with respective control levels (control- RPMI + double distilled water)

Results and Discussion

Globally the disease is second to malaria in causing permanent and long term disability diseases of limbs and genitals, resulting not only physical crippling but also in serious psychosocial consequences[13]. The available antifilarial efficacies have inherent limitations. The adult filarial worm can survive in human host for several years. The drug resistance becomes crucial issue after prolonged treatment with currently available drug DEC. Therefore we need new antifilarials for replacement of existing drugs. Herbal medicines are quite popular and being used by about 80 per cent of the world population mostly in the developing countries. These are time tested for their safety, efficacy, and cultural acceptability. The chemical ingredients of these plants are believed to have better compatibility with the human body with presumably lesser side effects[14]. Hence, very aptly the WHO has referred this system of medicine as holistic approach towards health[15]. The present work is an attempt to contribute to this database by screening of crude plant extracts for antimicrofilarial activity on *Brugia malayi*. Antifilarial efficacy of two plant extracts *Butea monosperma* L. screened against microfilariae of *Brugia malayi*. The aqueous extract of two plant extracts leaves and roots were screened for antifilarial activity against microfilariae of *Brugia malayi* at concentration 20-100ng/ml for 24hrs incubation. Of them, root extract showed significant loss of motility then leaves extracts in a dose dependent manner as oppose to respective control level. A study with aqueous and alcoholic extracts of the leaves of *Mallotus philippensis (Lam.)* against *Setaria cervi* reported antifilarial effect[16]. Another study was carried out to test the antifilarial efficacy of *Cardiospermum halicacabum* against related filarial worm *Brugia pahangi*[17]. In conclusion, our findings show towards the importance of in depth study of these herbal drugs for design and development of new antifilarial therapeutic drug. This is the first ever report of antifilarial efficacy of *Butea monosperma* L. screened against microfilariae of *Brugia malayi*.

Acknowledgment

Authors thanks Dr. Alka Chaturvedi, Reader, P.G. Dept. of Botany, RTM, Nagpur University, Nagpur, India for identification and authantification of plant species. This work was supported by the research project grants from Department of Biotechnology (DBT), New Delhi.

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