ISSN: 2574-1241
DOI: 10.26717/BJSTR.2018.07.001519
Vijai Lakshmi. Biomed J Sci & Tech Res

Research Article Open Access

Antifilarial Activity of *Gymnema Sylvestre* R. Br Leaves Against *Brugia Malayi*

Vijai Lakshmi*[1] and Shailja Misra Bhattacharya[2]

[1]*Division of Medicinal and Process Chemistry, CSIR-Central Drug Research Institute, India*

[2]*Division of parasitology, CSIR-Central Drug Research Institute, India*

Received: July 4, 2018; **Published:** August 01, 2018

***Corresponding author:** Vijai Lakshmi, Division of Medicinal and Process Chemistry, CSIR-Central Drug Research Institute, Uttar Pradesh, India

Abstract

The present study is aimed to evaluate the anti-filarial activity of *Gymnema sylvestre* (Asclepiadaceae), against human lymphatic filarial parasite *Brugia malayi in vitro* and *in vivo*. The ethanolic extract of the leaves 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 of the leaves of the *G. sylvestre* 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. The ethanolic extract was active *in vitro* (IC50: adult= 65.0 µg/ml; mf = 32.5. µg/ml) where it demonstrated 65.0% adulticidal and embryostatic effect on *B malayi* in Mastomys at a dose of 5 × 100mg/kg by oral route. The antifilarial test conducted was at 5×100mg/kg by subcutaneous route revealed excellent adulticidal efficacy resulting in to the death of 65.0% transplanted adult *B. Malayi* in the peritoneal cavity of jirds in addition to noticeable microfilaricidal action on the day of autopsy. The findings revealed that the extract from the leaves of *G. Sylvestre* contains promising *in vitro* and *in vivo* antifilarial activity against human lymphatic filarial parasite *B. malayi* which may be further explored to new antifilarial agents.

Keywords: *G. Sylvestre*; Antifilarial Activity; *In Vitro*; *In Vivo*; *B Malayi*

Abbreviations: IP: microfilariae; MF: Microfilariae; CSIR: Central Drug Research Institute; PBS: Phosphate Buffer Saline

Introduction

Over the past few years, the interest in natural medicine has been increasing in industrialized societies because of the ever-growing problem of side effects and high cost of synthetic medicines. gymnema is a native to central and western India, tropical Africa and Australia. Other names Sanskrit: Meshashringi, madhunashini, Hindi: Gur-mar, merasingi [1]. *G. Sylvestre* (Asclepiadaceae), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India (Figure 1). It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite. In addition, it possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. It also acts as feeding deterrents to caterpillar, Prodenia eridania; prevent dental caries caused by Streptococcus mutans and in skin cosmetics [2].

Figure 1 : *Gymnema Sylvestre* Leaves.

G. Sylvestre is a large, more or less pubescent, woody climber. It is occasionally cultivated as medicinal plant. Leaves are opposite, usually elliptic or ovate (1.25-2.0inch × 0.5-1.25inch). Flowers are small, yellow, in umbellate cymes. Follicles are terete, lanceolate, up to 3 inches in length. *G. Sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarane classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarane ponies are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentriacontane, pentatriacontane, α and β-chlorophylls, phytic, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives [3]. Gymnemic acids have antidiabetic, antisweetener and anti-inflammatory activities. The antidiabetic array of molecules has been identified as a group of closely related gymnemic acids after it was successfully isolated and purified from the leaves of *G. Sylvestre* [4,5-14]. Later, the phytoconstituents of *G. sylvestre* were isolated, and their chemistry and structures were studied and elucidated [6-8,9].

Materials and Methods

Plant Material

Leaves of the *G. Sylvestre* (500g) were purchased from the local market of Uttar Pradesh, India and was authenticated at the Botany Department of the Central Drug Research Institute, Lucknow, India. where voucher specimen have been preserved with the code number 895.

Extraction

The air-dried leaves of the *G. Sylvestre* (500g) 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 50ºC in a rota vapour 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 16.25 g which was used for the screening of antifilarial activity against B. malayi.

Antifilarial Activity: *In Vitro* Assays

Sample Preparation

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

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 Mc Call et al. [10]. After isolating the adult parasites, the peritoneal washing was passed through a membrane filter (pore size 5.0mm) and the microfilariae were pelleted by centrifugation [11]. 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.

Primary *In Vitro* Screening

The actively motile female worms were placed individually wells of 48 well culture plate containing RPMI 1640 medium fortified with antibiotics (penicillin 100units/mL, streptomycin sulfate 100mg/mL, and neomycin mixture; Sigma, USA). Each well contained one female worm in 1mL of the medium. Simultaneously, 10 microfilariae were suspended in 200mL medium in each well of a 96 well culture plate (NUNC). The parasites were incubated at 37 ^{0}C in 5% CO_2 in air for 5 days in the presence of 10mM 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-diphenyltetrazolium bromide] dye reduction assay as published earlier [12] 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 extract which consistently demonstrated their lethal effects on the parasites at 10mM (highest concentration tested) with 50% inhibition in MTT reduction as compared to untreated respective controls were considered as active extract [12], while those bringing about 100% irreversible inhibition in motility of microfilariae were considered microfilaricidal.

Evaluation of IC50 and CC50

The test samples found active in primary in vitro screen were followed for IC50 using four serials two fold dilutions of each sample starting from MIC in 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 [13]. In brief, Vero cells (104/well/100ml) in 96 well plate were exposed to seven three-fold serial dilutions of active test samples starting from 100mM at 37 ^{0}C in a CO_2 incubator. After 72h, resazurin dye was added and the plate was re-incubated for 3-4h.

The reaction was monitored fluorometrically at an excitation wavelength of 536nm and emission at 588nm in a fluorometric plate reader. 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) [13]. Determination of selectivity index (SI): The safety of the active samples was determined by assessing SI values (CC50/IC50) The extract with SI values of 10 were considered safe and therefore further followed *in vivo*.

In Vivo Ant filarial Activity

In Vivo Screening Model

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 (50mg/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 vivo* hits.

Treatment Schedule

The ethanol extract of the leaves of *G. Sylvestre* and the standard drug, diethylcarbamazine were administered subcutaneously at a dose of 100mg/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.

Assessment of Ant Filarial Activity in The Primary Jird Model

The treatment was initiated from day 5 post worm transplantation and the observations were continued till 45 days. On 45th day, 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 buffer saline (PBS, p^{H} 7.2) to dissect out the uterus for observing the uterine contents microscopically to assess the embryostatic effect of test samples, if any [14]. The peritoneal washing collected on autopsy was microscopically observed to assess the effect of test sample on released microfilariae.

Statistical Analysis

The statistical analysis of the data was carried out by PRISM 3.0 using one-way ANNOVA (nonparametric). Dunnett's multiple [13-14] 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 *G. Sylvestre* was tested in vitro on adult worms and microfilariae (mf) of *B. Malayi* and the active sample was further valuated *in vivo* in *B. Malayi* intraperitoneally transplanted in the jird model and Mastomys coucha subcutaneously infected with infective larvae (L3). The ethanolic extract was found to be active *in vitro* (IC50: adult = 65.0µg/ml; mf = 32.5µg/ml) and demonstrated 65.0% adulticidal and embryostatic effect on B. malayi, respectively, in Mastomys at a dose of 5 × 100mg/kg. The antifilarial activity at 5 × 100mg/kg by subcutaneous route revealed excellent adulticidal efficacy resulting in the death of 65.0% transplanted adult *B. Malayi* in the peritoneal cavity of jirds in addition to noticeable microfilaricidal action on the day of autopsy.

Conclusion

The present findings revealed that the ethanol extract of the leaves of *G. Sylvestre* contains promising in vitro and *in vivo* antifilarial activity against human lymphatic filarial parasite B. malayi which can be further explored to isolate and characterize the active molecules to provide new antifilarial agents.

Acknowledgment

The authors are grateful to the Director of CSIR (Central Drug Research Institute), Lucknow, India for providing necessary research facilities to carry out the above work and also excellent library facility. The one of us (VL) emeritus scientist grateful to acknowledge UGC Government of India, New Delhi for providing financial support which helped in compiling this work for publication.

References

1. The Wealth of India Raw materials (1956) vol IV Council of Scientific and Industrial Research, New Delhi: A Dictionary of Indian Raw materials and Industrial products, pp. 276–277.
2. Komalavalli N, Rao MV(2000) In vitro micropropagation of Gymnema sylvestre: multipurpose medicinal plant. Plant Cell Tissue and Organ Culture 61: 97-105.
3. Quattrocchi U (1999-11-23) CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology Taylor & Francis US ISBN 9780849326769.
4. Tiwari P, Mishra BN, Sangwan NS (2014) "Phytochemical and pharmacological properties of Gymnema sylvestre: an important medicinal plant" BioMed Research International 830285.
5. Ulbricht C, Abrams TR, Basch E, Davies Heerema T, Foppa I, et al. (2011) An evidence-based systematic review of gymnema (Gymnema sylvestre R Br) by the Natural Standard Research Collaboration. Journal of Dietary Supplements 8 (3): 311-30.
6. Gardner Z, Mc Guffin M (2013) American Herbal Products Association's Botanical Safety Handbook. (2nd Edition.). CRC Press ISBN 9781466516946.
7. Gent JF, Hettinger TP, Frank ME, Marks LE (1999) Taste confusions following gymnemic acid rinse. Chemical Senses 24(4): 393-403.
8. Kurihara Y (1969) Antisweet activity of gymnemic acid A1 and its derivatives. Life Sciences 8(9): 537-43.
9. Sanematsu K, Kusakabe Y, Shigemura N, Hirokawa T, Nakamura S, et al. (2014) Molecular mechanisms for sweet-suppressing effect of gymnemic acids. The Journal of Biological Chemistry 289(37): 25711-25120.
10. Mc Call JW, Malove JB, Hyong Sun A, Thompson PE (1973) Mongolian jirds (Meriones unguiculatus) infected with Brugia malayi by the intraperitoneal route: a rich source of developing larvae adult filariae and microfilariae. Journal Parasitology 59: 436.
11. Singh DP, Rathore S, Misra S, Chatterjee RK, Ghatak S, et al. (1985) Studies on causation of adverse reactions following diethylcarbamazine therapy (Dipetalonema viteae) in Mastomys natalensis. Tropical Medicinal Parasitology 36(1): 21-24.
12. Mukherjee M, Misra S, Chatterjee RK (1998) Development of in vitro screening system for assessment of antifilarial activity of compounds. Acta Tropica 70(3): 251-255.
13. Misra S, Verma M, Mishra SK ,Srivastava S, Lakshmi V, et al. (2011) Gedunin and Photogedunin of Xylocarpus granatum possess antifilarial activity against human lymphatic filarial parasite Brugia malayi in experimental rodent host. Parasitology Research 109(5): 1351-1360.

14. The Wealth of India Raw materials (1956) vol IV Council of Scientific and Industrial Research, New Delhi: A Dictionary of Indian Raw materials and Industrial products, pp. 276–277.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2018.07.001519

Vijai Lakshmi. Biomed J Sci & Tech Res