The triterpenoid fraction from *Trichosanthes dioica* root exhibits *in vitro* antileishmanial effect against *Leishmania donovani* promastigotes

Sanjib Bhattacharya, Moulisna Biswas¹, Pallab K. Haldar²

*Division of Pharmacognosy, Bengal School of Technology (A College of Pharmacy), Hooghly, ¹Bengal Institute of Pharmaceutical Sciences, Nadia, ²Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India*

Submitted: 03-09-2012 Revised: 27-09-2012 Published: 15-04-2013

**ABSTRACT**

**Background:** *Trichosanthes dioica* Roxb. (Cucurbitaceae), called pointed gourd in English is a dioecious climber found wild throughout the plains of the Indian subcontinent and traditionally used in India for several medicinal purposes. **Objective:** The present study was aimed at the evaluation of *in vitro* antileishmanial effect of triterpenoid fraction from *T. dioica* root (CETD).

**Materials and Methods:** The antileishmanial activity of CETD was evaluated against *Leishmania donovani* (strain MHOM/IN/83/AG83) promastigotes by *in vitro* promastigote cell toxicity assay by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide). Potassium antimonyl tartrate was used as reference. **Results:** Here, CETD markedly inhibited the growth of *L. donovani* promastigotes *in vitro* in a concentration dependent manner and demonstrated IC₅₀ value of 18.75 µg/ml. The reference drug potassium antimonyl tartrate exhibited IC₅₀ of 7.52 µg/ml. **Conclusion:** From the present study it can be inferred that the triterpenoid fraction of *T. dioica* root exhibited remarkable antileishmanial activity against *Leishmania donovani* promastigotes *in vitro*.

**Key words:** Antileishmanial, cucurbitacins, *Leishmania donovani*, promastigotes, root, *Trichosanthes dioica*

**INTRODUCTION**

Leishmaniasis is a wide spread life-threatening disease caused by protozoa of genus *Leishmania* transmitted by female sandflies of the genera of Phlebotominae subfamily. According to available estimates of World Health Organization (WHO), the disease is spread across 88 countries causing serious health problems especially in developing countries with 350 million at risk of contracting the disease and with approximately 2 million new cases being reported each year. The three main manifestations of disease are visceral, cutaneous and muco-cutaneous leishmaniasis. Visceral leishmaniasis (VL), also commonly known as *kala-azar* is caused by *L. donovani*. More than 90% of world’s cases of VL are reported in India, Bangladesh, Nepal, Sudan, Brazil and Ethiopia. In India, most of the leishmaniasis cases have been reported in Bihar, Orissa and Uttar Pradesh states. Cutaneous and muco-cutaneous leishmanisases are more prevalent in Afghanistan, Saudi Arabia and some Latin American countries.[1–4]

Proven therapies against human leishmaniasis include pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, and paromomycin.[5,6] The mentioned drugs have the disadvantages of high cost, lack of oral formulations (e.g., amphotericin B can be used only intravenously), or serious side effects that require close monitoring of the patients.[6] Also, rapid development of resistance by the parasites has been reported,[7–9] so that new therapies are needed to supplement or replace currently available therapies. More recently, emergence of coinfection of leishmaniasis with human immunodeficiency virus (HIV) has made the treatment even more challenging.[10]

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.
In our previous course of studies, we have reported anthelmintic, antibacterial, antimitotic, antiproliferative, antitumor, analgesic, laxative, chemopreventive and arsenic toxicity ameliorative activities of the root of *T. dioica*.\cite{15–27} As there are no experimental reports on antileishmanial activity on *T. dioica*, in the present study we found it necessary to evaluate the *in vitro* antileishmanial effect of triterpenoid enriched extract from *T. dioica* root extract against *Leishmania donovani* promastigotes.

**MATERIALS AND METHODS**

**Collection and authentication of plant material**

The mature tuberous roots of *T. dioica* were collected during December 2009 from Majdia, Nadia district, West Bengal, India. The species was identified by Dr. M. S. Mondal, at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India, and a voucher specimen (CNH/I-I/57/2009/Tech.II/493) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology, Delhi Road, Hooghly 712102, India for future reference.

**Preparation of triterpenoid fraction (CETD)**

Just after collection, the fresh roots were washed thoroughly with water, cut into moderate pieces and immediately crushed thoroughly in tepid water (~50°C) using a mechanical grinder. After cooling to room temperature (23 ± 2°C), the extract was separated from the remaining vegetable debris by pressing the material through muslin cloth. The resulting liquid is filtered and the remaining vegetable debris by pressing the material using a mechanical grinder. After cooling to room immediately crushed thoroughly in tepid water (~50°C) and subcultured every 72 h.

**Evaluation of antileishmanial activity**

*In vitro* promastigote cell toxicity assay using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) cell proliferation assay was used to assess the antileishmanial activity *in vitro* as per reported methods.\cite{30} Briefly, the exponential phases of promastigotes (2 × 10^6 cells/ml) were incubated with or without the test agents along with M-199 medium at 22°C. The test extract (CETD) was dissolved in 0.2% dimethyl sulfoxide (DMSO), and added to the culture in graded concentrations of 2.5, 5, 10, 20, 40 and 80 µg/ml. Similarly the reference drug potassium antimony tartrate was employed at the concentrations of 5, 10, 20 and 40 µg/ml. After 2 h of treatment, the tubes were centrifuged at 8000 g for about 10 min. The supernatant was decanted and the pellets were washed with 20 mM phosphate buffer saline (PBS). Each pellet was dissolved in 100 µl (2 mg/ml) of MTT (3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide) solution, and the tubes were incubated at 22°C for 4 h and then centrifuged at 8000 g for 10 min. The resulting pellets were dissolved in 500 µl of 0.2% DMSO and the absorbance was measured spectrophotometrically at 570 nm. Lysis of promastigotes (%) by the CETD was calculated by the formula as shown below:

Lysis % = 100 – [(test – positive control)/(control – positive control)] × 100

All the tests were carried out in triplicate and the results averaged. The IC_{50} value (50% inhibitory concentration) was
determined by plotting percentage lyses of promastigotes with respect to control against treatment concentrations.

RESULTS AND DISCUSSION

The in vivo efficiencies of drugs have been reported to be under the control of different parameters, such as pharmacokinetic parameters,[33] so that for various reasons, including simplicity in in vitro culture maintenance, routine screenings of antileishmanial chemotherapeutic agents are often based on promastigote susceptibility assays.[32] In the present study, a relevant cell viability test (MTT assay) was used to investigate the inhibitory effect of CETD on the in vitro growth of Leishmania donovani promastigotes and the effects was compared with a trivalent antimonial reference drug. Here, the test extract CETD significantly and concentration dependentily inhibited the growth of the promastigote forms of L. donovani (strain no. MHOM/IN/83/AG83) in vitro and demonstrated IC\textsubscript{50} value of 18.75 \(\mu g/ml\). Potassium antimonyl tartrate was used as reference which also concentration dependentily inhibited the growth of the L. donovani promastigotes and exhibited IC\textsubscript{50} value of 7.52 \(\mu g/ml\) [Table 1]. Here, the reference trivalent antimonial agent was found to be more active than CETD. It was quite obvious. However, CETD was also quite toxic and effective against L. donovani promastigotes.

Parasites of the genus Leishmania are transmitted by the female sandflies that ingest the parasite in the amastigote stage resident within macrophages, and then inoculate the promastigote stage into other hosts. There is a general lack of effective and inexpensive chemotherapeutic agents for the treatment of leishmaniasis. Although trivalent antimonials (Sb (III)) like potassium antimonyl tartrate or emetic tartar and pentavalent antimonial drugs are the first-line treatment for this disease, with amphotericin B and pentamidine being used as alternative drugs, all of these have serious adverse effects and resistance has become a severe problem. Therefore, new drugs are urgently required. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans.[38] T. dioica root is a potent herbal drug demonstrating several important pharmacological properties (see the introduction section). Its main bioactive constituents were found to be triterpenoids and its antimicrobial (bacterial) antiparasitic (anthelminthic), cytotoxic (antimitotic) and antiproliferative effects were found prominent in the triterpenoid enriched extracts.[15–18] This is the reason why the triterpenoid fraction of T. dioica root (CETD) was selected for the present study.

Being triterpenoid enriched extract, the abundance of triterpenoids especially cucurbitacin type triterpenoid aglycones was affirmed in CETD by qualitative phytochemical analysis and thin layer chromatography (HPTLC). Cucurbitacins are known to possess several biological activities including antimicrobial property.[34,33] The presence of putative cucurbitacin aglycones could provide the chemical basis of its antileishmanial efficacy in vitro. Previously the present authors have reported in vitro antibacterial potential of T. dioica root.[40] This notable property may be responsible for the promising antileishmanial activity of CETD. However, studying the exact mechanism of CETD behind its antileishmanial effect is beyond the scope of the present investigation.

Therapeutic evaluations for medicinal plants are essential because of the growing interest in alternative therapies and the therapeutic use of natural products. Natural products can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development, and the discovery of new therapeutic properties not yet attributed to known compounds.[36] Natural products have made, and are continuing to make, an important contribution to this area of therapeutics. Perhaps their future potential will be even greater. In this study we report the inhibitory effect of CETD on the in vitro growth of Leishmania donovani promastigotes. The observed activity represents an exciting advance in the search for novel antileishmanial agents from natural sources, since a significant and important effect against the promastigote form of the protozoan was demonstrated in the present study.

From the present investigation, it can be concluded that the triterpenoid fraction from T. dioica root demonstrated remarkable in vitro antileishmanial activity compared with potassium antimonyl tartrate, a trivalent antimonial against Leishmania donovani promastigotes. To the best of our knowledge, this is the first experimental report of the antileishmanial activity of Trichosanthes dioica. However, further definitive phytochemical and in vivo studies are

---

**Table 1: Effect of CETD against L. donovani promastigotes (2x10⁶ cells/ml)**

| Test agents | Concentration (µg/ml) | Percentage lysis of promastigotes with respect to control (0.2% DMSO)* | IC\textsubscript{50} value (µg/ml) |
|-------------|-----------------------|---------------------------------------------------------------|-------------------------------|
| CETD        | 2.5                   | 19.50                                                         | 18.75                         |
|             | 5                     | 30.34                                                         |                               |
|             | 10                    | 37.27                                                         |                               |
|             | 20                    | 53.84                                                         |                               |
|             | 40                    | 68.08                                                         |                               |
|             | 80                    | 76.73                                                         |                               |
| Potassium antimonyl tartrate | 5 | 41.36 | 7.52 |
|             | 10                    | 54.71                                                         |                               |
|             | 20                    | 67.38                                                         |                               |
|             | 40                    | 92.36                                                         |                               |

*Mean of three replicates, CETD=Triterpenoid fraction of T. dioica root
necessary in this context to ascertain the mechanism of action and in pursuit of a new effective antileishmanial agent from the plant kingdom.

ACKNOWLEDGMENT

The authors would like to thank the authority of Indian Institute of Chemical Biology (IICB), Kolkata, West Bengal, India for the facilities related to the present study.

REFERENCES

1. Anonymous. Report of the consultative meeting on cutaneous leishmaniasis. Geneva: World Health Organization; 2008.
2. Desjeux P. The increase in risk factors for leishmaniasis worldwide. Trans R Soc Trop Med Hyg 2001;95:239-43.
3. Handen E. Leishmaniasis: Current status of vaccine development. Clin Microbiol Rev 2001;14:229-43.
4. Chappuis F, Sundar S, Hailu A, Ghalbil H, Rijal S, Peeling R, et al. Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? Nat Rev Microbiol 2007;5:873-82.
5. Berman JD. Treatment of new world cutaneous and mucosal leishmaniasis. Clin Dermatol 1996;14:519-22.
6. Berman JD. Human leishmaniasis: Clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Cln Infect Dis 1997;24:684-703.
7. Ephros, M., Waldman E, Zilberstein D. Pentostam induces cytotoxic effect against Leishmania donovani promastigotes and axenically grown amastigotes. Antimicrob Agents Chemother 1997;41:1064-8.
8. Lira R, Sundar S, Makharia A, Kenney R, Gam A, Saravea E, et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of Leishmania donovani. J Infect Dis 1999;180:564-7.
9. Boelaert M, Leray D, Vander SP. How better drugs could change kala-azar control. Lessons from a cost-effectiveness analysis. Trop Med Int Hlth 2002;7:955-9.
10. Laguna F. Treatment of leishmaniasis in HIV-positive patients. Ann Trop Med Parasitol 2003;97:135-42.
11. Kirtikar KR, Basu BD. Indian medicinal plants. New Delhi: Bishen Singh Mahendra Pal Singh; 1935.
12. Anonymous. The wealth of India: Raw materials: New Delhi: Publication and Information Directorate, CSIR; 1976.
13. Nadkarni KM. Indian materia medica. Bombay: Popular Prakashan; 1976.
14. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. New Delhi: Central Council for Research in Ayurveda and Siddha; 2002.
15. Bhattacharya S, Haldar PK, Ghosh AK. Paralytic and lethal effects of Trichosanthes dioica root extracts in experimental worms. Pharm Biol 2010;48:960-5.
16. Bhattacharya S, Haldar PK. Antibacterial activity of Trichosanthes dioica root. Global J Pharmacol 2010;4:122-6.
17. Bhattacharya S, Haldar PK. Evaluation of in vitro cytotoxic effect of Trichosanthes dioica root. Pharmacognosy Res 2010;2:355-8.
18. Bhattacharya S, Prasanna A, Haldar PK. Evaluation of antiproliferative activity of Trichosanthes dioica root against Ehrlich ascites carcinoma cells. Acad J Cancer Res 2011;4:38-42.
19. Bhattacharya S, Prasanna A, Majumdar P, Kumar RB, Haldar PK. Antitumor efficacy and ameliorative of oxidative stress by Trichosanthes dioica root against Ehrlich ascites carcinoma in mice. Pharm Biol 2011;49:927-36.
20. Bhattacharya S, Haldar PK. Evaluation of antimitic and genotoxic effects of the triterpenoid enriched extract from Trichosanthes dioica root. Am-Euras J Toxicol Sci 2012;4:20-3.
21. Bhattacharya S, Haldar PK. The triterpenoid fraction from Trichosanthes dioica root exhibits antiproliferative activity against Ehrlich ascites carcinoma in albino mice: Involvement of possible antioxidant role. J Exp Ther Oncol 2012;9:281-90.
22. Bhattacharya S, Haldar PK. Exploration of anti-noiceptive and locomotor effects of Trichosanthes dioica root extracts in Swiss albino mice. Asian Pac J Trop Biomed 2012;2:S224-8.
23. Bhattacharya S, Haldar PK. Gastrointestinal effects of triterpenoid enriched extract of Trichosanthes dioica root in albino mice. Oriental Pharm Exp Med 2012;12:113-21.
24. Bhattacharya S, Haldar PK. Trichosanthes dioica root possesses stimulant laxative activity in mice. Nat Prod Res 2012;26:952-7.
25. Bhattacharya S, Haldar PK. Chemopreventive property of Trichosanthes dioica root against 3-methylcholanthrene-induced carcinogenesis in albino mice. J Environ Pathol Toxicol Oncol 2012;31:109-19.
26. Bhattacharya S, Haldar PK. Ameliorative effect Trichosanthes dioica root against experimentally induced arsenic toxicity in male albino rats. Environ Toxicol Pharmacol 2012;33:394-402.
27. Bhattacharya S, Haldar PK. Ameliorative effect Trichosanthes dioica root against arsenic-induced brain toxicity in albino rats. Toxicol Environ Chem 2012;94:769-78.
28. Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. New Delhi: Springer (India) Pvt Ltd; 1998.
29. Wagner H, Bladt B. Plant drug analysis, a thin layer chromatography atlas. Berlin Hiedelberg: Springer Verlag; 1996.
30. Pal D, Bhattacharya S, Baidya P, De BK, Pandey JN, Biswas M. Antileishmanial activity of Polyalthia longifolia leaf extract on the in vitro growth of Leishmania donovani promastigotes. Global J Pharmacol 2011;5:97-100.
31. Sereno D, Lemesre JL. Axenically cultured amastigote forms as an in vitro model for investigation of antileishmanial agents. Antimicrob Agents Chemother 1997;41:972-6.
32. Gupta N, Goyal N, Rastogi AK. Antileishmanial activity of Polyalthia longifolia extract on the in vitro growth of Leishmania donovani promastigotes. Asian Pac J Trop Biomed 2012;2:S224-8.
33. Wright CW, Phillipson JD. Natural products and the development of selective antiprotozoal drugs. Phytother Res 1990;4:127-39.
34. Miro M. Cucurbitacins and their pharmacological effects. Phytochem 1993;33:29-33.
35. Chen JC, Wu SH. Antileishmanial effects of the Trichosanthes dioica leaf extract against Leishmania donovani promastigotes. Phcog Res 2013;5:109-12.
36. Hamburger M, Hostettmann K. Bioactivity in plants: The possible antioxidant role. J Exp Ther Oncol 2012;9:281-90.

Cite this article as: Bhattacharya S, Biswas M, Haldar PK. The triterpenoid fraction from Trichosanthes dioica root exhibits in vitro antileishmanial activity against Leishmania donovani promastigotes. Phcog Res 2013;5:109-12.

Source of Support: Nil, Conflict of Interest: None declared.