Antipromastigote activity of an ethanolic extract of leaves of *Artemisia indica*

Leishmania is a digenetic protozoan parasite responsible for cutaneous, mucocutaneous, or visceral leishmaniasis infecting almost 12 million people worldwide, 350 million remaining at risk, and importantly, the burden of the visceral form is borne primarily by the Indian subcontinent.[1] Sodium antimony gluconate (SAG) has been the first line of treatment for leishmaniasis, but in recent years, an alarming increase in nonresponsiveness almost to epidemic proportions in Bihar, India, has led to the development of several new antileishmanial drugs that include amphotericin B (fast gaining acceptability as the primary drug of choice), miltefosine, and paromomycin.[2] Viewed against this backdrop, plant-derived products are an attractive option, and herein, we report the antileishmanial efficacy of an ethanolic extract of an indigenous medicinal plant *Artemisia indica*. *A. indica* has been used for general malaise and fevers of unknown origin,[3] whereas artemisinins, the sesquiterpine lactones isolated from *A. annua* have been used to treat multidrug-resistant malaria, analogs of which have been reported to exhibit both antimalarial and antileishmanial activity.[4]

The leaves of *A. indica* were collected from the Kumaon area near Mukteswar, Uttaranchal, India. The leaves were air-dried, crushed into powder, and extracted with 90% ethanol. The solution obtained was filtered thrice; the filtrate was pooled and evaporated in a rotary evaporator. A stock solution (10 mg/ml in 20% DMSO) was prepared and stored at 4°C until use.

Leishmania promastigotes from seven strains, as indicated in Table 1, were routinely cultured at 24°C in M-199 medium supplemented with 10% fetal calf serum and gentamicin (200 μg/ml). To study the *in vitro* effect of an ethanolic extract of *A. indica* on *Leishmania* promastigotes, exponentially growing parasites were resuspended in 96-well tissue culture plates (2×10^5/200 μl/well). The plates were incubated at 24°C for 6 h followed by the addition of an ethanolic extract of *A. indica* (0–1.0 mg/ml) and incubated for an additional 48 h. At the end of 48-h incubation, the parasite viability was checked using 3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfonyl)-2*H*-tetrazolium (MTS), inner salt, and phenazonium methosulphate (PMS). MTS (2.0 mg/ml) and PMS (0.92 mg/ml) in a ratio of 5:1 was added (20 μl per well) and the plates were incubated for 3 h at 37°C. The resultant absorbances were measured at 490 nm in an ELISA reader. Accordingly, the specific absorbance that represented formazan production was calculated by subtraction of background absorbance from total absorbance. The mean percent viability was calculated as follows:

$$\frac{\text{Mean specific absorbance of treated parasites}}{\text{Mean specific absorbance of untreated parasites}} \times 100$$

Accordingly, the IC_{50} for each drug, i.e., the concentration of drug that decreased the percent viability by 50% was graphically extrapolated by plotting percent viability against the respective drug concentration. All the experiments and protocols described in the present letter were approved by the Institutional Animal Ethical Committee and are in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

The viability of promastigotes has a proportional relationship with the formazan complex formed as the conversion of MTS to formazan by the mitochondrial dehydrogenases is only achievable by viable cells, in the presence of the electron coupler PMS. As evident from Table 1, *A. indica* showed a pronounced leishmanicidal activity in all the *Leishmania* strains studied, the IC_{50} ranging from 0.21 to 0.58 mg/ml, indicating its effectiveness in all three forms

Table 1

Effect of *A. indica* on *Leishmania* promastigotes

Strain	Designation	Species	Disease	Location	*A. indica*[a] IC_{50} (mg/ml)
CK2	–	*L. donovani*	Visceral	Old world	0.21
MON29	MHOM/FR/1996/LEM3249	*L. infantum*	Visceral	Old world	0.39
K27	MHOM/SU/74/K27	*L. tropica*	Cutaneous	Old world	0.33
L280	MHOM/PE/66/L280	*L. braziliensis*	Mucocutaneous	New world	0.58
LV4	–	*L. mexicana*	Cutaneous	New world	0.34
LV81	MORY/BR/72/M1824	*L. amazonensis*	Mucocutaneous	New world	0.29
JISH118	MHOM/SA/85/JISH118	*L. major*	Cutaneous	Old world	0.43

[a]Log-phase promastigotes were incubated with increasing concentrations of *A. indica* (0–1 mg/ml) and cell viability was measured by the MTS assay. Each experiment was repeated thrice in duplicates, cell viability was plotted against the drug concentration, and the IC_{50} was graphically extrapolated.

of leishmaniasis. To put the obtained results into perspective, the IC_{50} values of two established antileishmanial drugs, amphotericin B and miltefosine, were determined in the *Leishmania* strains used in this study. The values for amphotericin B ranged from 36 to 61 nM, whereas those for miltefosine varied between 11.5 and 27.5 μM (mentioned in a personal communication). Further confirmatory studies will be undertaken in the amastigote form as also the active principles in *A. indica* contributing to the observed antileishmanial activity will be delineated. In this regard, it is worthwhile to isolate artemisinin, a sesquiterpene lactone, analogs of which have displayed anti-leishmanial activity.[4] Such studies are ongoing.

Acknowledgments

The study received financial assistance from the Council of Scientific & Industrial Research, Life Sciences Research Board, DRDO, Govt. of India, and from the University Grants Commission—S. B. is a recipient of Senior Research Fellowship from the Indian Council of Medical Research and S. G. is a recipient of Junior Research Fellowship from the University Grants Commission.

[1]S. Ganguly, [1]S. Bandyopadhyay, [2]A. Bera, [1]M. Chatterjee

[1]Department of Pharmacology,
Institute of Postgraduate Medical Education and Research,
244B, Acharya JC Bose Road, Kolkata 700020, India.
[2]Eastern Regional Centre,
Indian Veterinary Research Institute, Kolkata, India.
E-mail: ilatim@vsnl.net

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