Experimental Parasitology 138 (2014) 9–17

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Selective inhibition of *Leishmania donovani* by active extracts of wild mushrooms used by the tribal population of India: An *in vitro* exploration for new leads against parasitic protozoans

Suvadip Mallick [a,1], Aritri Dutta [a,1], Somaditya Dey [a,1], Joydip Ghosh [a], Debarati Mukherjee [a], Sirin Salma Sultana [a], Supratim Mandal [a,2], Soumitra Paloi [b], Somanjana Khatua [b], Krishnendu Acharya [b], Chiranjib Pal [a,*]

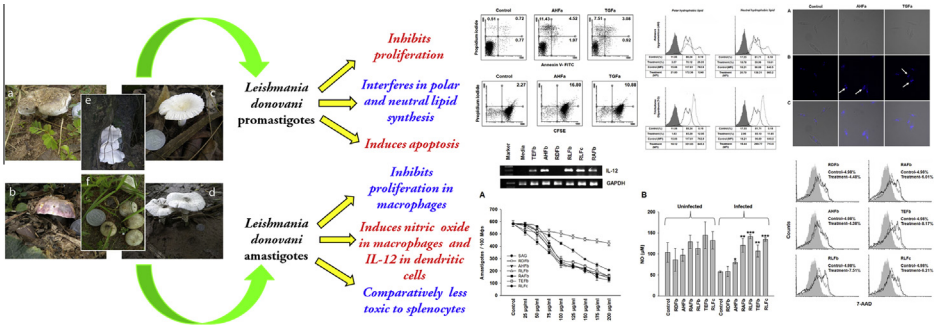
[a] Cellular Immunology and Experimental Therapeutics Laboratory, Department of Zoology, West Bengal State University, Barasat, West Bengal, India
[b] Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, Kolkata, West Bengal, India

HIGHLIGHTS

- We assessed 18 mushroom extracts against *L. donovani* promastigote and amastigote.
- *A. hygrometricus* and *T. giganteum* were found efficient against promastigotes.
- *A. hygrometricus* and *R. laurocerasi* were found effective against amastigotes.
- The active extracts were found to induce IL-12 and nitric oxide.
- The active extracts were found considerably non-toxic to murine splenocytes.

GRAPHICAL ABSTRACT

Identified mushrooms used in folklore of Santal tribal populations in West Bengal, India. (a) *Russula albonigra*, (b) *Russula laurocerasi*, (c) *Russula delica*, (d) *Termitomyces eurhizus*, (e) *Tricholoma giganteum*, (f) *Astraeus hygrometricus*.



ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 21 September 2013
Accepted 7 January 2014
Available online 17 January 2014

Keywords:
Anti-leishmanial
Mushroom extract and fraction
IL-12
Nitric oxide
Lipid synthesis

ABSTRACT

The study was intended at evaluating the anti-proliferating effect of mushrooms used in traditional folklore of Santal tribal population in India against *Leishmania donovani* (MHOM/IN/83/AG83). A total of eighteen extracts, three estracts from each mushroom [(80% ethanol extracted; Fa), (water-soluble polysaccharide fraction; Fb), (polyphenolic fraction; Fc)], from six wild mushrooms were obtained. These extracts were tested against the promastigotes and amastigotes for their antileishmanial capacity. Fa fractions (250 μg/mL) of *Astraeus hygrometricus* and *Tricholoma giganteum* significantly inhibited the growth of *L. donovani* promastigotes and interfered in lipid biosynthesis. Moreover, both fractions induced apoptosis in promastigotes. Water soluble Fb fractions of *A. hygrometricus*, *Russula laurocerasi*, *Russula albonigra*, *Termitomyces eurhizus*, *Russula delica* and polyphenolic Fc fraction of *R. laurocerasi* were found to inhibit the replication of intracellular amastigotes in macrophages dose dependently. Significantly, 50% inhibitory concentration of the active extracts against intracellular amastigotes induced release of nitric oxide and IL-12 in murine macrophages and dendritic cells assay and also found

* Corresponding author. Address: Cellular Immunology and Experimental Therapeutic Laboratory, Department of Zoology, West Bengal State University, Barasat, DT: 24 PGS (N), Kolkata 700126, West Bengal, India. Fax: +91 033 2524 1977.
E-mail address: cpcu.immunology@gmail.com (C. Pal).
[1] These authors contributed equally to this work.
[2] Present address: PG Department of Microbiology, APC College, New Barrackpore, West Bengal, India.

0014-4894/$ - see front matter
http://dx.doi.org/10.1016/j.exppara.2014.01.002

considerably non-toxic on murine splenocytes. Results of this study can be used as a basis for further phytochemical and pharmacological investigations in the effort for search of novel anti-leishmanial leads.

1. Introduction

Leishmania donovani is the causative agent of visceral leishmaniasis (VL) or kala azar (KA) in Indian subcontinent. The reported case-fatality of VL rates ranged from 1.5% in Bangladesh, 2.4% in India and 6.2% in Nepal as estimated during 2004–2008. However, community-based studies that included active searches for deaths due to kala-azar estimate case-fatality rates of more than 10%, while data from a village-based study in India suggest that as many as 20% of VL patients, disproportionately poor and female, died before their disease was recognized (Alvar et al., 2012). New drugs are desperately needed to treat visceral leishmaniasis (VL), and this in turn requires new approaches to discover novel lead compounds that might populate a pipeline of new therapeutics for patients with VL. Current therapies for the leishmaniasis are toxic, difficult to deliver, expensive, and their efficacy is hindered by parasite resistance (reviewed in Croft and Olliaro, 2011). One of the main sources for new anti-leishmanial molecules is the isolation of secondary metabolites from plants and herbs (Fournet and Munoz, 2002; Dua et al., 2011). Very recently people around the world have started exploration of anti-leishmanial leads from mushrooms and mushroom metabolites (Valadares et al., 2012; Lai et al., 2012). The purpose of the present study is to develop a depository for active anti-leishmanial and immunomodulatory constituents extracted and fractionated from some mushrooms of folklore of tribal populations of Lateritic zones and Gangetic plane of West Bengal, India (See Graphical abstract).

Mushroom and its metabolites have multi beneficial effects for human welfare. Wild mushrooms are widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems. Mushroom research has focused on discovery of compounds that can modulate positively or negatively the biological response of immune cells and it attracts an enormous international attention as a valuable herb due to the wide variety of its biological activities, such as antimicrobial, anticancer, antidiabetes, and hepatoprotective activities (Chang and Mshigeni, 2001; Lindequist et al., 2005; Acharya, 2007). There are approximately 20,000 described species of mushroom which mostly correspond to basidiomycetes. The total number of mushroom forming species has been estimated at between 53,000 and 110,000 (Mueller et al., 2007). This would suggest that only 18% to 38% of all the mushrooms have been documented. More than 778 species of macrofungi (Swapna et al., 2008) are available in India and it anticipates further investigation for its pharmacological importance. The Lateritic region of West Bengal, India has been colonized by many ethnic races like Bhumij, Lodha, Kol, Vil, Munda, Sabar, Santals, etc. in heavily forested areas (Pradhan et al., 2010). In this communication we have translated the traditional knowledge into technical understanding in respect to anti-leishmanial aptitude of these mushrooms.

2. Materials and methods

2.1. Preparation of extracts

Astraeus hygrometricus (Pers.) Morgan (Local name – Putko/Kurkure Chatu); *Russula laurocerasi* Melzer (Local name – Jhaal Patra); *Russula albonigra* (Krombh.) Fr. (Local name – Kalo/Kend Patra); *Termitomyces eurhizus* (Berk.) Heim (Local name – Parab Chatu) and *Russula delica* Fr. (Local name – Sada Patra) are native to Lateritic zones of five districts of Birbhum, Murshidabad, Burdwan, Bankura and West Midnapore and *Tricholoma giganteum* Massee (Local name – *Dhoodh Chatu*) from Gangetic plane of West Bengal, India and are used as food and also for a broad variety of medicinal uses by local Santal tribes of these regions (Pradhan et al., 2010, 2013). Mature fruitbodies (Basidiocarps) were collected and the voucher specimens were deposited at the Mycological Herbarium of Department of Botany, University of Calcutta, Kolkata, West Bengal, India. Specific voucher numbers were assigned for the each mushroom specimen, *A. hygrometricus* (AMFH- 583); *R. laurocerasi* (AMFH- 602); *R. albonigra* (AMFH-598); *T. eurhizus* AMFH- 604); *R. delica* (AMFH- 600); *T. giganteum* (AMFH- 510). Freeze dried basidiocarps were prepared for extraction by milling with a ball mill (Retsch) to pass through a 40 m screen (Cui et al., 2005). Powdered samples (100 g) were dissolved in 80% ethanol at room temperature and shaken overnight. The sample was then filtered and ethanol filtrate fraction was freeze-dried (Fa fraction). The residual fraction was then dissolved in distilled water and placed in a boiling water bath for 4 h until the aqueous phase was evaporated to half its volume. It was then filtered and the aqueous filtrate was mixed with 95% ethanol (1:4, v/v), centrifuged at 10,000 rpm and the precipitate was collected, washed with ethanol and freeze-dried (Fb fraction). The aqueous phase was then evaporated to remove the ethanol and mixed with ethyl acetate (2:1, v/v). The upper phase was then evaporated and lyophilized (Fc fraction). The freeze-dried fractions Fa and Fc were reconstituted in DMSO and Fb was reconstituted in distilled water at a concentration of 10 mg/mL. These solutions were sterilized by filtration through a 0.45 m pore membrane and kept in the dark at −80 °C (Fig. 1). At least three batches of each isolate were extracted and verified their similarity by thin-layer chromatography (TLC). The Fa, Fb and Fc fractions of *A. hygrometricus* were designated as AHFa, AHFb and AHFc; *R. laurocerasi* were designated as RLFa, RLFb and RLFc; *R. albonigra* were designated as RAFa, RAFb and RAFc; *T. eurhizus* were designated as TEFa, TEFb and TEFc; *R. delica* were

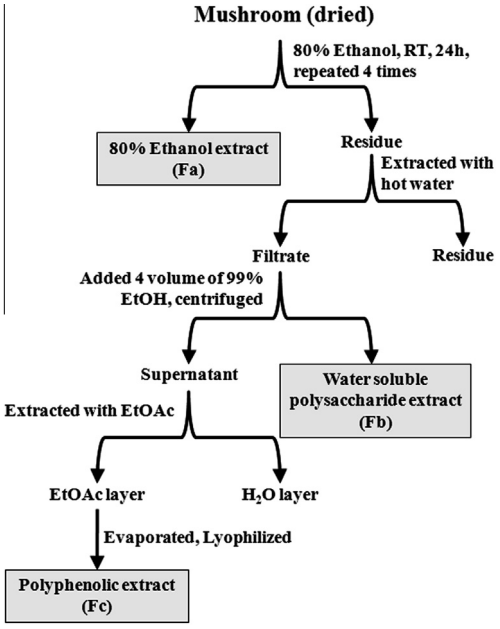


Fig. 1. Preparation of mushroom fractions.