Ali S A

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.

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Correspondence: Syed Atif Ali
Email: ali2@amdi.usm.edu.my

A Preliminary Study on the Anti-Proliferative Activity of Hydroquinone (benzene-1,4-diol) Against Promastigotes of *Leishmania major* and *Leishmania tropica in vitro*

Hydroquinone, a natural ingredient of many plant-derived products and an important metabolite of benzene is found to possess strong antiproliferative activity against promastigote forms of *Leishmania major* and *Leishmania tropica in vitro.* Leishmanial promastigotes grown in RPMI-1640 medium supplemented with 5% fetal calf serum, 1 % adult human urine and buffered with 25 *mM* HEPES were challenged with varying concentrations of hydroquinone for 72 hours at 25°C. All promastigotes were found dead at as little as 0.65 µg/ml of hydroquinone. Whereas IC_{50} value calculated for hydroquinone was below 0.15 µg per ml. IC_{100} and IC_{50} values obtained for pentamidine, a standard antileishmanial drug were around 12 and 5 µg respectively.

Keywords: *Leishmania* Promastigotes, *In vitro* Assay, Hydroquinone, Antileishmanial Activity.

INTRODUCTION

Leishmaniases is a group of a vector borne parasitic disease with three major clinical syndromes *i.e.* cutaneous, muco-cutaneous and visceral leishmaniasis (Tuon *et al*., 2008). Leishmaniases is caused by some 20 species belonging to the genus *Leishmania,* a flagellated protozoan which is transmitted by the bite of the infected female sandfly (Tuon *et al*., 2008, Nussbaum *et al*., 2010). An estimated two million new cases of human leishmaniases (half a million visceral) are considered to occur every year in the endemic zones of Latin America, Africa, the Indian subcontinent, the Middle East and the Mediterranean region (World Health Organization). Mucocutaneous leishmaniasis is however not very common and restricted to South America (Tuon *et al*., 2008).

The treatment of all types of leishmaniases depends on a small number of drugs *i.e.* pentavalent antimonials, amphotericin B and pentamidine (Sharlow *et al*., 2010). Besides having serious side effects, pentavalent antimonials are still the drugs of choice in the treatment of all three types of leishmaniases (Neghina and Neghina 2010). Although these drugs have a useful therapeutic index, inadequate dosages and discontinuous use has resulted in the resistance to these drugs in many countries especially in poor ones(Palumbo 2009, Frézard *et al*., 2009). In cases of unresponsiveness and/or resistance to the pentavalent antimonials, amphotericin B is recommended as, a second line drug, however owing to its toxicity, it is used under strict medical supervision (Baginski and Czub 2010). At-least three lipid associated amphotericin B formulations are marketed which are much safer and less toxic (Baginski and Czub 2010). Pentamidine is more toxic than above mentioned two drugs and is only choice in cases of unresponsiveness to pentavalent, antimonials and amphotericin B (Frézard *et al*., 2009). Several other compounds are used in treating leishmaniases with varying degree of success (Richard and Werbovetz 2010); there is an urgent need of developing new antileishmanial drugs (Polonio and Efferth 2008).

In present article we are reporting potent antileishmanial activity of hydroquinone, a compound naturally present in various plants, against the promastigote forms of *Leishmania major,* and *Leishmania tropica* the etiological agent of cutaneous leishmaniasis.

MATERIALS AND METHODS

Initial Isolation and Propagation

Leishmania parasites were originally isolated from infected patients in Quetta valley and later characterized as *L. major* and *L. tropica* on the basis of their isoenzyme pattern. Parasites (promastigotes) were maintained in NNN medium overlaid with incomplete RPMI-1640 medium. For bulk cultivation and subsequent assay, the promastigotes were cultured in RPMI-1640 medium supplemented with 5% fetal calf serum, 1 % adult human urine and buffered with 25 mM HEPES (supplemented or complete medium).

Drug Preparation

0.1 mg of hydrouinone (Sigma H 9003) and 0.1 mg of pentamidine (generously gifted by Dr. S.L. Croft of London School of Hygiene and Tropical Medicine) were dissolved separately in 50 ul sterile DMSO (dimethyl sulfoxide) and volume was made up to 1. 0 ml with complete RPMI-1640 medium (composed of RPMI-1640 buffered with 25*mM* TES buffer and supplemented with 5% fetal calf serum and 1%

adult human urine) and kept frozen below -20°C until use.

Antileishmanial Assay

Three sets of 8 sterile eppendorf tubes (capacity 0.75 ml) were added with 100 ul of mid log phase *Leishmania,* culture containing lxl0^6 parasites/ml in complete medium. Drug was added and two fold serial dilutions were made so that the highest concentration was 10 µg */ml* and lowest was 0.078 µg /ml. Negative controls received complete medium with solvent while positive controls had varying concentrations of pentamidine as standard antileishmanial drug. Cultures were incubated at 25°C in dark for 72 h after which the motile promastiotes with normal morphology were counted by the help of hemocytometer.

RESULTS AND DISCUSSION

All tests were performed in triplicate and repeated three times. 100% leishmanial promastigotes (both *L. major* and *L. tropica*) were found dead at as little as 0.65 µg /ml of Hydroquinone. At a concentration of 0.15 µg/ml, almost 50% of promastigotes were found dead. In the presence of pentamidine, a standard antileishmanial drug, 73.34 % parasites were found killed at the highest concentration tested i.e. 10 µg /ml, whereas 50% parasites were eliminated between 2.5 and 5.0 µg /ml.

Hydroquinone, the thiol reactive oxidative agent, is a natural ingredient in many plant-derived products, including vegetables, fruits, grains, coffee, tea, beer, and wine (DeCapiro A.P. 1999). It is mainly used as a reducing agent, antioxidant, polymerization inhibitor, and chemical intermediate (DeCapiro A.P. 1999). It is also used in over-the-counter (OTC) drugs as an ingredient in skin lighteners (Javel *et al*., 1996). However, there are no reports of its antileishmanial activities to the best of our knowledge. We have assayed Hydroquinone against the promastigote forms of *Leishmania major* and obtained very encouraging results. As shown in the **Table. 1A and B**., hydroquinone has simply outclassed pentamidine and proved much more potent. Other related phenols e.g. resorcinol (1,3 OH-benzene) and gallic acid (1,2,3 OH-benzene) were also tested but they failed to show any significant activity (data not shown) indicating that hydroxyl (-OH) groups attached to 1 and 4 position of benzene ring, (in case of hydroquinone) are required for the activity. These findings are in correlation with previous findings in which gallic acid and/or its derivatives were found ineffective against *Leishmania* (Koide *et al*., 1998). It is hypothesized that *L. major* possess a thiol compound, called 4, mercaptohistidine, which is involved in the detoxification of reactive oxygen species (Koide *et al*., 1998, Nose *et al*., 1998). So far, we have not studied the possible mode of action of hydroquinone by Which it kills the parasites, however based on the literature (DeCapiro A.P. 1999, Pryor W.A. 1982), we hypothesized that free radical generation might be involved in the killing process. It would be interested to investigate whether killing process involve reactive oxygen species, and if so, is there any role of 4, mercaptohistidine in the detoxification of hydroquinone. Whether hydroquinone shows the same antiproliferative potential against the intramacrophagic amastigotes (the etiological form of the parasite) of *Leishmania* is remained to be determined.

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Table I.A. Antileishmanial activity of hydroquinone and pentamidine against promastigote forms of *Leishmania major*.

Drug/Conc. (ug/ml)	**10**	**5.0**	**2.5**	**1.25**	**0.65**	**0.31**	**0.15**	**0.08**
Negative Control	100	100	100	100	100	100	100	100
Hydroquinone	0	0	0	0	0	20	46.6	93.3
Pentamidine	26.6	60	80	100	100	100	100	100

The values are (standard Error $\pm$ 5-7%) of triplicate determination from three experiments showing percentage survival of parasites vs drug concentration.

Table I.B. Antileishmanial activity of hydroquinone and pentamidine against promastigote forms of *Leishmania tropica*.

Drug/Conc. (ug/ml)	**10**	**5.0**	**2.5**	**1.25**	**0.65**	**0.31**	**0.15**	**0.08**
Negative Control	100	100	100	100	100	100	100	100
Hydroquinone	0	0	0	0	0	24	51.2	98
Pentamidine	32.2	59	82.3	100	100	100	100	100

The values are (standard Error $\pm$ 5-7%) of triplicate determination from three experiments showing percentage survival of parasites vs drug concentration.