In-vitro effect of Artemisinin on L. tropica promastigotes

Ghuffran M. Hassan¹, Dr. Hayder Z. Ali²
¹²(Department of Biology, College of Science / University of Baghdad, Baghdad, Iraq)
Corresponding Author:

Abstract : Leishmaniasis is a widespread parasitic disease caused by Leishmania parasite, this disease considers a major health problem among worldwide. Treatments available are expensive or with cytotoxic side effect. This study was aimed to investigate the effect of an herbal new compound, called artemisinin, derived from a Chinese plant called Artemisia annua. Various concentrations were studied in vitro against L. tropica amastigotes by chamber counting to investigate its effect on the proliferation of promastigotes. Three incubation periods were adopted (24, 48, 72) hours. The results showed a significant decrease in surviving promastigotes, in parallel with the normal parasite count of untreated promastigotes, along the periods studied. This study revealed a major growth inhibition effect of artemisinin against L. tropica promastigotes, in vitro. It is recommended for future studies of artemisinin effects on amastigotes forms and in vivo study.

Keywords: Cutaneous leishmaniasis, Artemisinin, promastigote.

I. Introduction

Leishmaniasis is one of the most serious epizootic diseases according to the World Health Organization (WHO) [1]. Leishmaniasis is a crucial public health problem caused by Leishmania spp., class of Kinetoplastida. Leishmaniasis affects 98 countries worldwide, in which an estimated 1.3 million new cases and 20000 to 30000 deaths are reported annually; around 310 million people are at risk of an infection[2]. Cutaneous leishmaniasis (CL) is an endemic disease in Middle Eastern, such as Syria, Iraq, Kingdom Saudi Arabia, and Jordan and it is still considered as an essential health problem which requires international awareness [3]. The drug favorite for therapy of CL is Glucantime and Pentostam. Both of them possess toxic side effects. Therefore, the pharmaceutic study appears the main approach for finding new drugs with minimal toxicity[4,5]. Miltefosine and paromomycin are two medications that have been entered more newly for the therapy of leishmaniasis disease [6]. However, long-term medication with miltefosine’s long half-life (about 152 h) can enhance early starter of drug resistance, and potential teratogenic and abortifacient effects limit its prescription through gestation [7, 8]. In-vitro tests have led to the emergence of paromomycin-resistant, miltefosine resistant [9] meglumineantimoniate-resistant [10] and pentamidine-resistant [11]. Artemisinin and its derivatives represent a very important new class of antimalarials [12]. Artemisinin and aqueous extract of Artemisia sieberi are of plant origin. Artemisinin is derived from a medicinal herb called qinghao (sweet wormwood) or Artemisia annua and is still obtained from this plant. Artemisinin is relatively facilely purified after extraction from plants [13]. Following their discovery and development of antimalarial drugs by TuYouyou’s group in the 1970s [14]. Artemisinin (ART) and its derivatives have been investigated in treating parasitic diseases or parasitic infections caused by protozoan parasites including Leishmania spp., Trypanosoma spp., Toxoplasma gondii, Neosporacanium, Eimeriatenella, Cryptosporidium parvum, Giardia lamblia, and Babesia spp. [15]. They are efficient in inhibiting the parasite metabolism while showing limited adverse effects on the host, indicating a higher safety index of the drugs. A large number of in-vitro or ex-vivo studies have shown that ART and its derivatives have activities in controlling the parasites, and the drugs shown effective against the protozoan [16].

The aim of this study was to examine the inhibitory activity of Artemisinin on the promastigote of L. tropica proliferation, in vitro.

II. Material And Methods

2.1 Preparation of Artemisinin
Artemisinin (C₁₅H₂₉O₅) was purchased from TOCRIC biotechno (England), compound structure showed in figure (1). For preparation stock solution, 3 mg of artemisinin was dissolved in 500 μL of Dimethyl sulfoxide (DMSO), according to the manufacturer’s protocol. From the stock solution, different concentrations of Artemisinin were prepared as following (500, 400, 300, 200, 100, 50, 10) μM.
In-vitro effects of the Artemisinin on L. tropica promastigotes

2.2 Cultivation of parasites:

*L. tropica* was isolated from a patient in AL-Karamahospital (Baghdad city), a patient diagnosed with cutaneous leishmaniasis and a sample was taken from hand lesion. Procyclic promastigotes of *L. tropica* were cultured in M199 medium (Sigma Aldrich St. Louis, MO, USA). The medium was prepared according to manufacturer’s procedure at pH 7.4 supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml of penicillin and 100 μg/ml of streptomycin, culture was incubated at 26 °C For three days to enable proliferation of promastigotes into log phase [18].

2.3 Promastigote proliferation follow up:

Promastigote proliferation was investigated by direct counting based on growth inhibition. The stationary phase of promastigotes (1×10⁵ promastigotes/ml) was harvested in complete M199 medium, 10% fetal bovine serum at 26°C, then, 1 × 10⁷ promastigotes/ml of parasite was transferred to 8 universal vials containing 2 ml of M199 and different concentrations of Artemisinin was added (500, 400, 300, 200, 100, 50, 10) μM, respectively for each test group. Compound solvent (DMSO) was added to control group. Parasite proliferation was estimated by counting the viable motile forms on a Neubauer® chamber at different incubation period (24, 48, 72) hours; Artemisinin was added on daily basis along the 2 days follow up. Each test was performed in triplicate. Promastigotes number was calculated under light microscope via chamber and the total number of promastigotes per ml was calculated according to the following equation [19]:

\[
\text{Number of cells} = \frac{A + B + C + D}{4} \times 10^4 \times 2 \times \text{sample dilution}
\]

A, B, C, D are the 4 squares of chamber.

2.4 Statistical Analysis

To determine the significant differences between means of control and test values for each concentration after time (24, 48, and 72) h, using t-test and Different between means have analyzed at (p ≤ 0.05) and expressed as (Mean ± SEM) [20].

III. Results And Discussion:

Artemisinin cytotoxicity has been screened against promastigotes of old-world Iraqi strains of *L. tropica*, in order to examine the effect of artemisinin on the procyclic promastigote proliferation of *L. tropica*, the microscopic examination showed a significance decrease in the total number of the promastigotes along the follow-up, figure 2 (A and B).
In-vitro effects of the Artemisinin on L. tropica promastigotes

The results showed dose-dependent anti-leishmanial activity significant differences (p ≥ 0.05) between test and control groups along the follow-up (24, 48, 72). After 24 hours of incubation, there was a significant difference between test and control groups in the percentage of proliferation except the lowest concentrations of 10 and 50 µM, figure 3, while there was a significant difference between test and control groups except the concentration of 10 µM, after 48 hours of treatment, figure 4. After 72 hours of treatment, there was a significant difference between test and control groups at all concentrations, figure 5.

Figure 2: Microscopic examination of promastigote.
A: Axenic culture of control group. (40x) B: After 24 h. of ART treatment. (10x) In with M199 medium pH 7 at 26°C.

Figure 3: Proliferation percentage of promastigotes after artemisinin treatment, 24 hours.

Figure 4: Proliferation percentage of promastigotes after artemisinin treatment, 48 hours.
The Artemisinin has been widely studied in several biological state and infections including anticancer, antimalarial, antifungal, and anti Fasciolahepatica[18, 20, 21]. Artemisinin was also used in clinical trials for the treatment of different human physiological disorders like kidneys [22]. Artemisinin proved the effectiveness as anti-leishmanial activity against different strains like L. major and L. donovani, a cutaneous and visceral leishmaniasis agent in the Old World respectively [23, 24].

Drug association therapy has numerous advantages and can delay the development of resistant pathogens and increase the half-lives of the therapeutic agents, as has been shown for HIV-1, malaria, and tuberculosis[25]. Specifically, the importance of developing drug association therapy for leishmaniasis has increased over the last several years, especially for the treatment of more severe forms of this disease [26]. Drug association therapy has several important advantages, including reduced dosages and/or treatment duration, both of which result in fewer toxic side effects, increased patient compliance, lower treatment costs, and the prevention or delay of drug resistance development[27]. The classical treatment of CL is glucantime; however, it is mostly toxic and leading to prevalent side effects [28]. Although artemisinin (qinghaosu) is widely used as an anti-malarial agent, it has also demonstrated its anti promastigote activities and inhibitory effect on Leishmania proliferation [29, 30]. Analysis of data resulted from Iranian study by [23], in vitro experiment, indicated that artemisinin inhibited the growth of L. major promastigotes. Its inhibitory activity against promastigotes showed the IC50 values of 283 µM after 24 hours. In contrast another study of L. donovani, the IC50 of artemisinin was 160 µM against promastigotes [31].

Previous studies on the strategies of the compound to kill of Leishmania revealed the formation of reactive oxygen species (ROS) or reactive intermediates on promastigotes in a dose-dependent manner. This oxidative imbalance was necessary for the noted leishmanicidal activity of Artemisinin [32]. In addition, Artemisinin enables externalization of phosphatidylserine and leads to the lack mitochondrial membrane potential, cell-cycle arrest at the sub-G0/G1 phase, and programmed cell death of L. donovani promastigotes [33].

**IV. Conclusion**

In conclusion, results suggest that artemisinin proved a potential inhibitory activity, in vitro, and it is recommended for further in vivostudies to examine the effect artemisinin on amastigotes.

**References**


DOI: 10.9790/3008-1206038286 www.iosrjournals.org 85 | Page
In-vitro effects of the Artemisinin on L. tropica promastigotes


[29] E.M. Saraiva, Resveratrol is active against Leishmania amazonensis promastigotes both in vitro and vivo, Free radical research, 44(11), 2010, 1289-1295.
