IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 6 Ver. III (Nov. – Dec. 2017), PP 82-86
www.iosrjournals.org

In-vitro effect of Artemisinin on *L. tropica*promastigotes

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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.*

Date of Submission: 18-11-2017 Date of acceptance: 30-11-2017

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 *Artemisiasieberi* are of plant origin. Artemisinin is derived from a medicinal herb called qinghao (sweet wormwood) or *Artemisiaannua* 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., *Toxoplasmagondii*, *Neosporacaninum*, *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 studywas 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 (C_{15} H_{22} O_5) was purchased from TOCRIC biotechne (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.

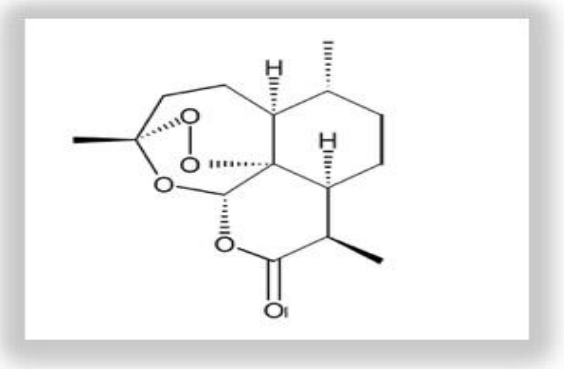

Figure 1: Artemisinin structure [17].

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 (1×10^5 promastigotes/ml) was harvested in complete M199 medium, 10% fetal bovine serum at 26°C, then, 1×10^5 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]:

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

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

2.4Statistical 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 ($p \leq 0.05$) 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).

A B

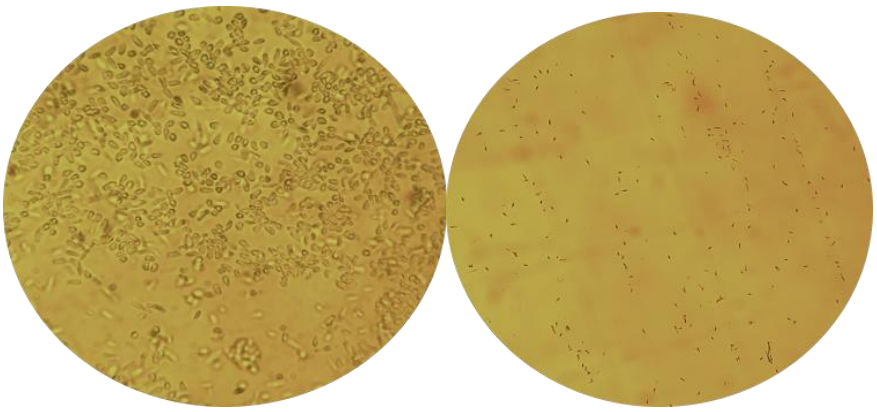

Figure 2: Microscopic examination of promastigote.
A: Axenic culture of control group. (40x) **B:** After 24 h. of ART treatment. (10x) In with M199 medium pH 7 at 26ºC.

The results showed dose-dependent anti-leishmanial activity significant differences ($p \geq 0.05$) 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.

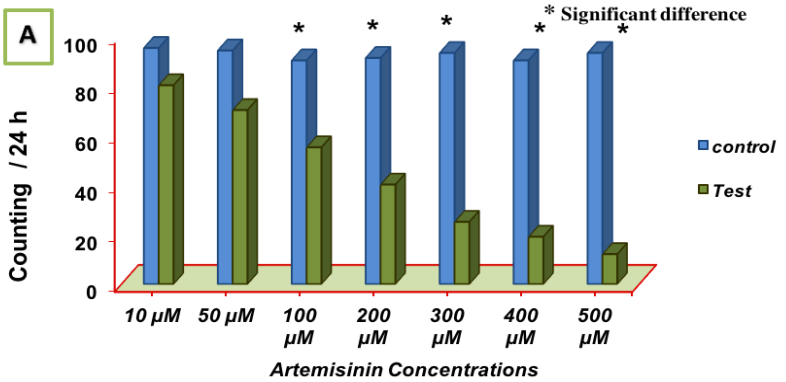

Figure 3: Proliferation percentage of promastigotes after artemisinin treatment, 24 hours.

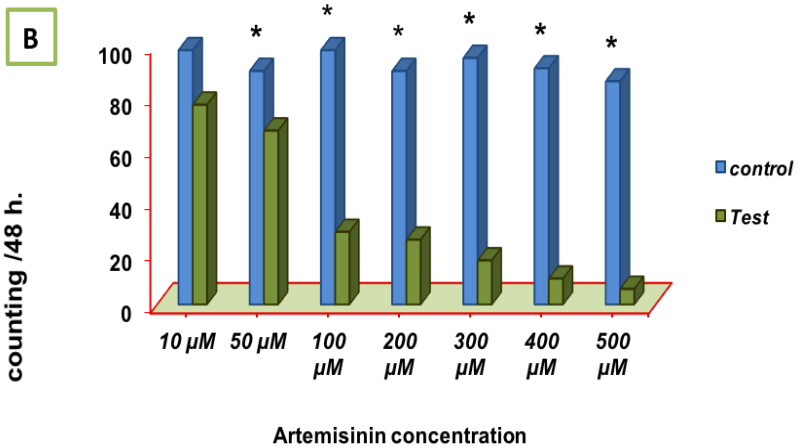

Figure 4: Proliferation percentage of promastigotes after artemisinin treatment, 48 hours.

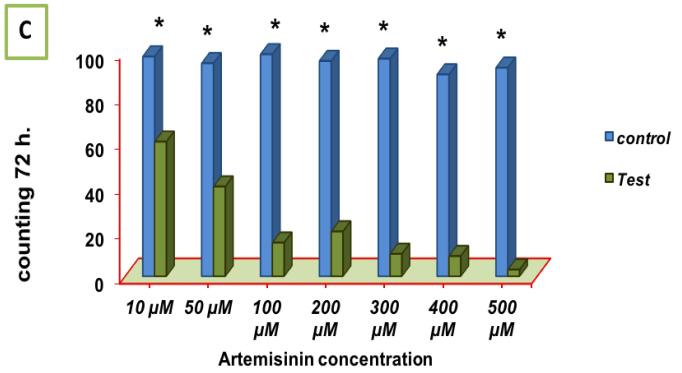

Figure 5: Proliferation percentage of promastigotes after artemisinin treatment, 72 hours.

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 mostlytoxic 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 IC_{50} values of 283 µM after 24 hours. In contrast another study of *L. donovani*, the IC_{50} 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 vivo*studies to examine the effect artemisinin on amastigotes.

References

[1]. N. Sattarahmady, A. Movahedpour, H. Heli, and G. Hatam, Gold nanoparticles-based biosensing of Leishmania major kDNA genome: visual and spectrophotometric detections, Sensors and Actuators B: Chemical, 235, 2016, 723-731.
[2]. I.F. Wulsten, T.A. Costa-Silva, J.T. Mesquita, M.L. Lima, M.K. Galuppo, N.N. Taniwaki, S.E. Borborema, F.B. Da Costa, T.J. Schmidt, and A.G. Tempone, Investigation of the Anti-Leishmania (Leishmania) infantum Activity of Some Natural Sesquiterpene Lactones, Molecules, 22(5), 2017, 685.
[3]. N. Hijjawi, K.A. Kanani, M. Rasheed, M. Atoum, M. Abdel-Dayem, and M.R. Irhimeh, Molecular diagnosis and identification of leishmania species in jordan from saved dry samples, BioMed research international, 2016, 2016.
[4]. F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, and M. Boelaert, Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?, Nature Reviews Microbiology, 5(11), 2007, 873-82.
[5]. P. Ebrahimisadr, F. Ghaffarifar, and Z.M. Hassan, In-vitro evaluation of antileishmanial activity and toxicity of artemether with focus on its apoptotic effect, Iranian journal of pharmaceutical research, 12(4), 2013, 903.
[6]. M. Den Boer and R.N. Davidson, Treatment options for visceral leishmaniasis, Expert review of anti-infective therapy, 4(2), 2006, 187-197.
[7]. N. Singh, M. Kumar, and R.K. Singh, Leishmaniasis: current status of available drugs and new potential drug targets, Asian Pacific Journal of Tropical Medicine, 5(6), 2012, 485-497.
[8]. I.A. Rodrigues, A.M. Mazotto, V. Cardoso, R.L. Alves, A.C.F. Amaral, J.R.d.A. Silva, A.S. Pinheiro, and A.B. Vermelho, Natural products: Insights into leishmaniasis inflammatory response, Mediators of inflammation, 2015, 2015.

[9]. S. Hendrickx, G. Boulet, A. Mondelaers, J. Dujardin, S. Rijal, L. Lachaud, P. Cos, P. Delputte, and L. Maes, Experimental selection of paromomycin and miltefosine resistance in intracellular amastigotes of Leishmania donovani and L. infantum, Parasitology research, 113(5), 2014, 1875-1881.
[10]. F. Faraut-Gambarelli, R. Piarroux, M. Deniau, B. Giusiano, P. Marty, G. Michel, B. Faugère, and H. Dumon, In vitro and in vivo resistance of Leishmania infantum to meglumine antimoniate: a study of 37 strains collected from patients with visceral leishmaniasis, Antimicrobial agents and chemotherapy, 41(4), 1997, 827-830.
[11]. A.C. Coelho, L.G. Gentil, J.F. da Silveira, and P.C. Cotrim, Characterization of Leishmania (Leishmania) amazonensis promastigotes resistant to pentamidine, Experimental parasitology, 120(1), 2008, 98-102.
[12]. S.R. Meshnick, Artemisinin: mechanisms of action, resistance and toxicity, International journal for parasitology, 32(13), 2002, 1655-1660.
[13]. F.E. Heydari, F. Ghaffarifar, S. Soflaei, and A. Dalimi, Comparison between in vitro effects of aqueous extract of Artemisia seiberi and artemisinin on Leishmania major, Jundishapur journal of natural pharmaceutical products, 8(2), 2013, 70.
[14]. Y.K. Wong, C. Xu, K.A. Kalesh, Y. He, Q. Lin, W. Wong, H.M. Shen, and J. Wang, Artemisinin as an anticancer drug: Recent advances in target profiling and mechanisms of action, Medicinal Research Reviews, 2017.
[15]. C.S.N. Loo, N.S.K. Lam, D. Yu, X.-z. Su, and F. Lu, Artemisinin and its derivatives in treating protozoan infections beyond malaria, Pharmacological research, 117, 2017, 192-217.
[16]. R.L. Berens, R. Brun, and S.M. Krassner, A simple monophasic medium for axenic culture of hemoflagellates, The Journal of Parasitology, 1976, 360-365.
[17]. M. Tayyab Ansari, Z. Saeed Saify, N. Sultana, I. Ahmad, S. Saeed-Ul-Hassan, I. Tariq, and M. Khanum, Malaria and artemisinin derivatives: an updated review, Mini reviews in medicinal chemistry, 13(13), 2013, 1879-1902.
[18]. T. Efferth, Cancer combination therapies with artemisinin-type drugs, Biochemical Pharmacology, 2017.
[19]. K.S. Louis and A.C. Siegel, Cell viability analysis using trypan blue: manual and automated methods, Mammalian cell viability: methods and protocols, 2011, 7-12.
[20]. G.P. Quinn and M.J. Keough, Experimental design and data analysis for biologists. 2002: Cambridge University Press.
[21]. J. O'Neill, R. Johnston, L. Halferty, R. Hanna, G. Brennan, and I. Fairweather, Disruption of spermatogenesis in the liver fluke, Fasciola hepatica by two artemisinin derivatives, artemether and artesunate, Journal of helminthology, 91(1), 2017, 55-71.
[22]. C. Thiemermann, Artemisinin and its derivatives for use in the treatment of kidney disease. 2017, Google Patents.
[23]. A. Haniloo, S. Nemati, H. Nahrevanian, A. Fazaeli, and M. Farahmand, In Vivo and in Vitro Investigation on Anti-Leishmanial Efficacy of Artemisinin on Iranian Strain of Leishmania major, 2016.
[24]. M.Y. Want, M. Islammudin, G. Chouhan, H.A. Ozbak, H.A. Hemeg, A.P. Chattopadhyay, and F. Afrin, Nanoliposomal artemisinin for the treatment of murine visceral leishmaniasis, International Journal of Nanomedicine, 12, 2017, 2189.
[25]. D.E. Goldberg, R.F. Siliciano, and W.R. Jacobs, Outwitting evolution: fighting drug-resistant TB, malaria, and HIV, Cell, 148(6), 2012, 1271-1283.
[26]. M.P. Barrett and S.L. Croft, Management of trypanosomiasis and leishmaniasis, British medical bulletin, 104(1), 2012, 175-196.
[27]. C. Ferreira, D.C. Soares, M.T.C. do Nascimento, L.H. Pinto-da-Silva, C.G. Sarzedas, L.W. Tinoco, and E.M. Saraiva, Resveratrol is active against Leishmania amazonensis: in vitro effect of its association with amphotericin B, Antimicrobial agents and chemotherapy, 58(10), 2014, 6197-6208.
[28]. L. Kedzierski, A. Sakthianandeswaren, J.M. Curtis, P.C. Andrews, P.C. Junk, and K. Kedzierska, Leishmaniasis: current treatment and prospects for new drugs and vaccines, Current medicinal chemistry, 16(5), 2009, 599-614.
[29]. D. Yang and F. Liew, Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis, Parasitology, 106(1), 1993, 7-11.
[30]. Y. Ma, D. Lu, X. Lu, L. Liao, and X. Hu, Activity of dihydroartemisinin against Leishmania donovani both in vitro and vivo, Chinese medical journal, 117(8), 2004, 1271.
[31]. S. Ganguly, S. Bandyopadhyay, A. Bera, and M. Chatterjee, Antipromastigote activity of an ethanolic extract of leaves of Artemisia indica, Indian journal of pharmacology, 38(1), 2006, 64-65.
[32]. R. Sen, P. Saha, A. Sarkar, S. Ganguly, and M. Chatterjee, Iron enhances generation of free radicals by Artemisinin causing a caspase-independent, apoptotic death in Leishmania donovani promastigotes, Free radical research, 44(11), 2010, 1289-1295.
[33]. R. Sen, S. Bandyopadhyay, A. Dutta, G. Mandal, S. Ganguly, P. Saha, and M. Chatterjee, Artemisinin triggers induction of cell-cycle arrest and apoptosis in Leishmania donovani promastigotes, Journal of medical microbiology, 56(9), 2007, 1213-1218.

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