Antiparasitic activity of Artemether and combination Artemether with Artemisinin against Leishmaniasis, *in vitro*.

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Abstract

The ***Leishmania donovani*** parasite causes visceral leishmaniasis (VL), an acute and fatal form of leishmaniasis. Because traditional therapy alternatives, such as glucantime and other pentavalent medicines, are toxic and have side effects, new treatments with fewer negative effects are needed. Only a handful of drugs are clinically beneficial to treatments of the disease, but considerable limitations threaten their very usage. Novel, safe, and efficient drugs, including those against anti-malaria and leishmaniasis co-infections, are so essential. Artemether (ATM) is an Artemisinin derivative that has been demonstrated to be useful in the treatment of malaria and, more recently, leishmaniasis. The current research was carried out to evaluate the anti-leishmanial effects of Artemether (ART) and combination of Artemether- Artemisinin (ART- ATM) against procyclic promastigotes of ***Leishmania donovani***. In this fundamental-applied research, we compared the effect of (ATM) and combination of (ART- ATM) on ***Leishmania donovani*** procyclic promastigotes, at different concentrations by using the MTT assay method after 24 ,48 and 72 h of treatment. The results prove ATM and combination (ART- ATM) efficiency against the procyclic promastigotes viability with IC50 measured after 24, 48- and 72hours treatment. The combination of (ART- ATM) could be used in the treatment of leishmaniasis to improve the therapeutic outcome for *Leishmania* species.

Key words: (VL) visceral leishmaniasis, (ATM) Artemether, ART (Artemisinin), (DMSO) Dimethyl sulfoxide.

Introduction

Leishmaniasis is one of the most an emerging disease parasitic infection of the world and current curative options show several limitations. In the search for more efficient medication, plant compounds represent a powerful natural source (1). ***Leishmania*** is an obligate intracellular protozoon. It's a protozoan parasite spread by sandflies, with promastigotes spread via the bite of infected female phlebotomine. It infects macrophages in vertebrate hosts' livers, spleens, and bone marrow. There are around 30 distinct species of ***Leishmania***. With three distinct clinical illnesses based on the parasite's species: Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), and Visceral Leishmaniasis (VL) (2). Chemotherapy, which comprises polyene antibiotics (amphotericin B), pentavalent antimonate glucantime, pentostam, allopurinol, and meltifosine, is still used to treat leishmaniasis. However, using these treatment procedures causes a slew of issues, including medication drug resistance, negative drug responses, recurrence, secondary bacterial infection, considerable toxicity, and exorbitant

treatment costs (3). It is a significant new family of anti-malarial medications that is becoming increasingly popular around the world. Derivatives that are semi-synthetic and synthetic are also being developed. Artemisinin derivatives act swiftly and are soon removed (4).

Artemether, a highly effective antimalarial medication, has the potential to cure Plasmodium falciparum infections. Artemether, on the other hand, is rapidly destroyed by stomach acids and removed from the body following oral administration, resulting in weak therapeutic effects and a severe impediment to the clinical cure of malaria (5). To address this issue, we developed this drug by combination of (ART- ATM) they might be an excellent candidate for visceral leishmaniasis treatment (VL).

Material and methods

Culture of *Leishmania donovani*

L. donovani (MHOM/IQ/2005/MRU15) isolate, from a patient clinically diagnosed with VL, was kindly provided from Medical Research Unit, College of Medicine, AL-Nahrain University (6). Procyclic Promastigotes of ***L. donovani*** was cultured in RPMI-1640 medium (Roswell Park Memorial Institute) (HiMedia Laboratories, India). The medium was prepared according to the manufacturer's instructions at pH 7.2, supplemented 10% heat-inactivated fetal calf serum was added to the mix (HIFCS),100 IU/ml penicillin, and 100 g/ml streptomycin, and cultured for three days at 25 ± 1°C to allow promastigotes to enter log phase (7).

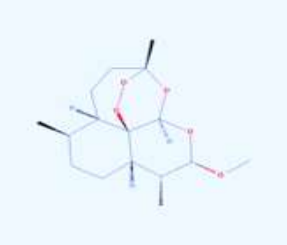

Figure 1. Chemical structure of Artemether

Artemether Preparation

ATM ($C_{16}H_{26}O_5$) was obtained from TOCRIS biotechne, UK and prepared according to manufacturer's, in which 3 mg was dissolved in 500 l of DMSO (99.9%). For this study, six 2-fold serial dilutions of the ATM stock (200 to 6.25g/mL) were made.

Artemisinin Preparation

ART ($C_{15}H_{22}O_5$) was purchased from TOCRIS biotechne, UK and prepared according to manufacturer's, in which 3 mg was dissolved in 500 l of DMSO (99.9%). For this study, six consecutive dilutions of the ART stock at a factor of two (200 to 6.25g/mL) were made.

Combination of (ART- ATM)

When utilizing the ATM combination, each drug was combined in a same concentration (for example, 200 µg/mL **ART** + 200 µg/mL **ATM**, 100 µg/mL **ART** + 100 µg/mL **ATM**, and so on).

Promastigote Assay

A microtitration plate was used to determine the 50% inhibitory concentration (IC50) of Artemether and the combination of (ART- ATM) on ***L. donovani.*** Each well had a total volume of 200 µl and included parasite culture medium (10^6 promastigotes/mL), as well as antibiotic. Artemether and a combination of (ART- ATM) were also used in the wells at concentrations of (200, 100, 50, 25, 12.5, and 6.25) µg /ml (each one was in triplicate). The plate was incubated at 26°C for 24 hours, 48 hours, and 72 hours before being examined under a microscope. The IC50 was calculated based on the results of the first 24 hours. The IC50 was calculated using a linear regression of plots.

Promastigote Viability by MTT Assay

The MTT test was used to assess whether Artemether and a mixture of and combinations (ART- ATM) were cytotoxic to ***L. donovani.*** Briefly,10^6/mL of log-phase procyclic promastigotes were treated to various concentrations of Artemether and a mixture of Artemether and other chemicals (ART- ATM). MTT solution (20 µL) was added to each well after 24, 48, and 72 hours of incubation at 26°C, and the culture were then incubated in a dark environment at 37°C for 4 hours. The pellets were put to (50 µL) Dimethyl sulfoxide (DMSO) after centrifugation at 300 rpm for 10 minutes (8). The optical densities (OD) at 620 nm were measured after 10 minutes, and the viability % was calculated in the following manner: Viability percentage= (Absorbance of treated cells/Absorbance of control cells) ×100.

Analyze the Data

The findings were evaluated using the SPSS program version 28 by comparing the mean parasite count in each medicine and its corresponding concentrations, as well as discovering significant differences between the control and test groups. ($P< 0.05$) was used to determine whether the difference was significant. Non-linear regression was used to calculate the IC50.

Results

***In vitro* Experiments Procyclic Promastigote screening**

The current research looked at the impact of (200,100 ,50 ,25 ,12.5 ,6.25) µg/mL concentrations of Artemether (ATM) and (ART- ATM) combination under in vitro circumstances, they were assessed on procyclic promastigotes of ***L. donovani.*** The study's findings showed that the combination of Artemether (ATM) and (ART- ATM) inhibited the proliferation of procyclic promastigotes. In this case, however, Artemether (ATM) was more effective than the (ART- ATM) combination figures 2 and 3 demonstrate the growth curves of promastigotes. At 24-, 48-, and 72-hours following incubation, a dose-

dependent growth suppression was seen in this figure. The IC50 is a measure of how effective a drug is (50 percent inhibition concentration of cell growth) the concentrations of Artemether (ATM) and (ATR- ATM) were determined to be 100 g/mL and 200 g/mL, respectively. Artemether (ATM) has a greater growth-inhibitory impact on promastigotes when compared to the other drug combination. MTT techniques validated the effect of these medicines in the promastigote assay.

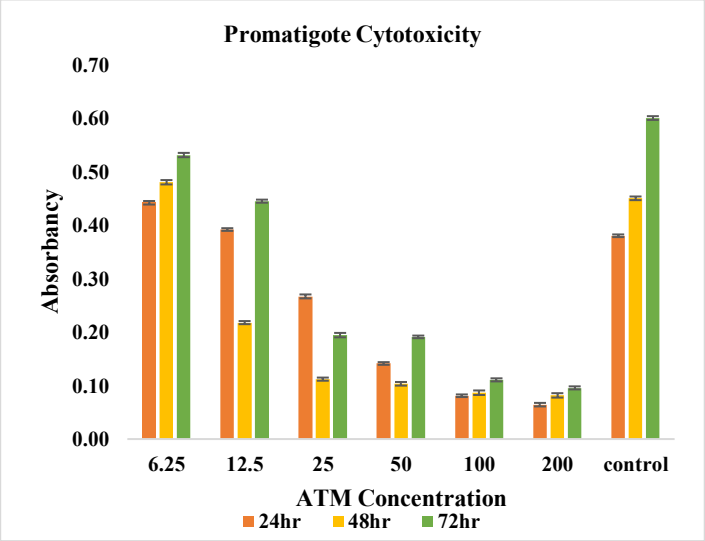

Figure 2. Absorbency with different concentrations of **ATM** against promastigotes through 24, 48, and 72 hours.

The IC50 values of Artemether (ATM) and (ART- ATM) combination were determined as 100 and 200 µg/mL, respectively for three periods show in Table (1). The Artemether (ATM) was found to be effective in eliminating ***L. donovani*** procyclic promastigote, and the results were validated using the MTT method. Figures 4 and 5 indicate that as the medicine dose was raised, the parasite number decreased. The (ART- ATM) combination had a lower effect on procyclic promastigotes than either Artemether (ART) ($P< 0.05$), and both combinations exhibited modest cytotoxic effects on procyclic promastigotes. Artemether (ART) was effective against promastigotes more than (ART- ATM) combination.

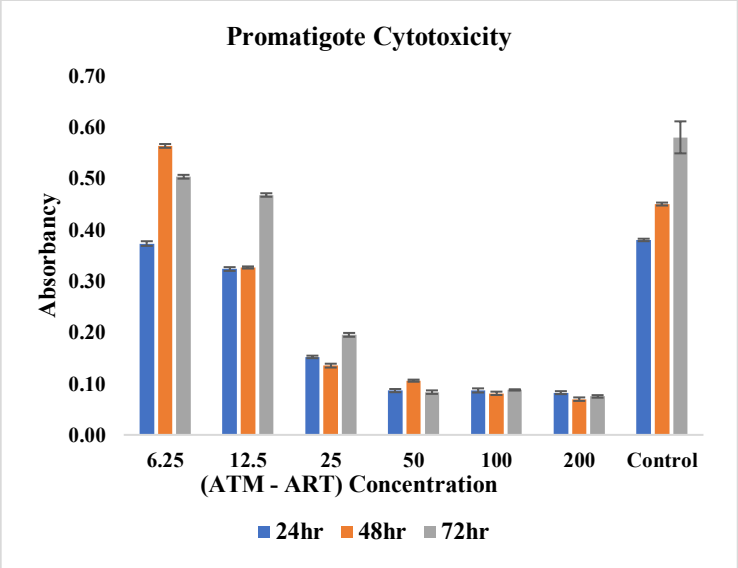

Figure 3. Absorbency with different concentrations combination of **(ATM - ART)** against promastigotes through 24, 48, and 72 hours.

Table 1. IC_{50} values of promastigotes for **(ATM)** and Combination of **(ART- ATM)**

IC50 mg	24hr	48hr	72hr
Artemether (ATM)	3.55	2.06	6.40
Combination of (ART-ATM)	1.96	4.87	5.86

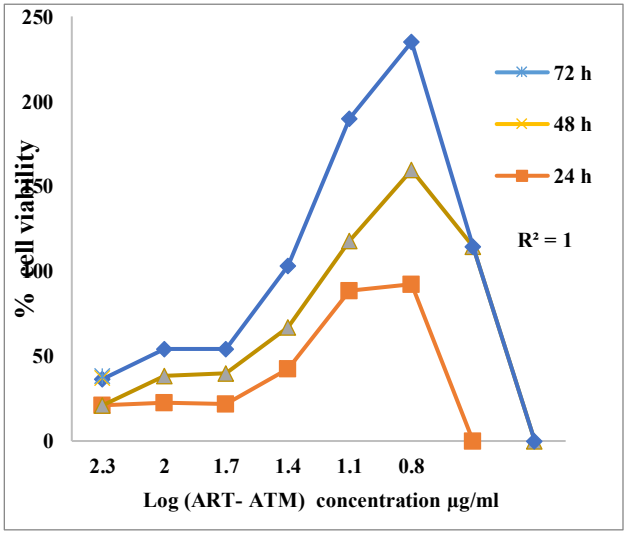

Figure 4. Cell viability of promastigotes after 24 ,48 and 72 hours treatments with **(ART- ATM) combination.**

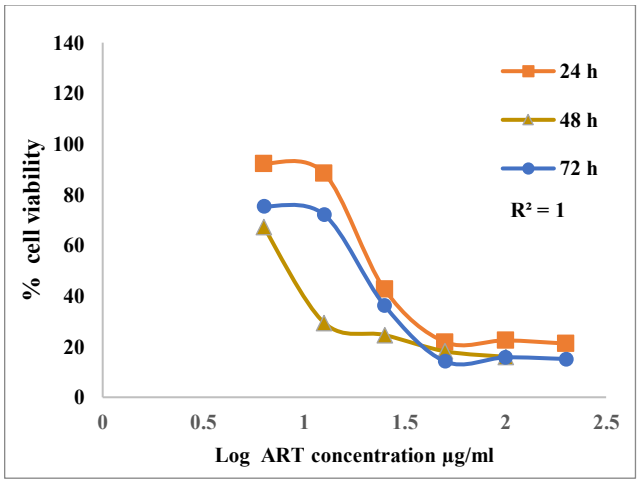

Figure 5. Cell viability of promastigotes after 24 ,48 and 72 hours treatments with (**ATM).**

Discussion

Because there is currently no viable vaccine for visceral leishmaniasis (VL), it is vital to discover new, safe therapeutic options. In this context, existing medications for visceral leishmaniasis are linked to a slew of negative consequences. As a result, new approaches to leishmaniasis treatment are necessary (9). For the present being, the most important drugs used to treat the condition are pentavalent antimonial compounds.

These chemicals have several drawbacks, including side effects, painful injections, a protracted treatment course, expensive costs, medication resistance, and therapeutic failure. As a result, researchers are increasingly concentrating their efforts on developing cheaper and more effective medications with fewer or no adverse effects (10). Plant extracts having a high anti-leishmania effect and minimal cytotoxicity for human cells are thus beneficial. In the current investigation, the medications tested were found to have effective characteristics against the parasite in vitro. Artemether is a new anti-malarial medicine that has been demonstrated to have anti- leishmanial effects. It is a derivative of Artemisinin, which derived from the Artemisia annua plant. Artemether, which has a high efficiency and low toxicity, could be a good choice for leishmaniasis treatment. Scientists have confirmed that the powerful antiparasitic action of Artemether is attributable to the presence of an endoperoxide bridge. It is used to treat the erythrocytic phases of malaria caused by Plasmodium species (9). Artemether is used to treat parasite illnesses such as Fasciola (11). *Schistosoma (japonicum, mansoni,* and *hematobiuom*) were treated alternately for 2, 3, and 4 weeks (12) and Clonorchis (13).

Artemether is a potent medication with a short half-life (three hours). We employed artemisinin in conjunction with artemether to manage drug dosage in oral and injection forms due to artemether's short half-life (14). The antiparasitic effect of Artemisinin in ***Leishmania major*** was estimated in a study by Ghaffarifar F *et al.,* who discovered the cytokine pattern as well as the percentage of apoptosis generated by Artemisinin.It was discovered that Artemisinin causes cytotoxicity in ***L.major*** cells through an apoptosis- related mechanism (15). To our knowledge, no research has investigated on ***Leishmania donovani*** promastigotes, the effects of combining (ART- ATM). The (ART- ATM) combination demonstrated an inhibitory effect on

promastigotes at all time points, according to our findings; however, the mechanism of action is unknown. In addition, Artemisinin-glucantime or Artemisinin-Shark cartilage combinations were found to be effective inhibitors of ***L. major*** in a study conducted by Ghaffarifar F *et al*. It's also discovered that the Art-ShCE combination inhibits ***L. major*** promastigotes and amastigotes more effectively than other medication combinations, and that it's not hazardous to un-infected macrophages (16).

Artemether (ART) has been shown in several studies to be an effective and safe treatment option for localized cutaneous leishmaniasis (CL). Although the intralesional meglumine antimoniate medicines had a higher cure rate, intralesional Artemether is considerably cheaper, more commonly available, and efficacious and safe (17). Artemisinin's anti-leishmanial properties are connected to the rupture of its endoperoxidase bridge, which leads in the generation of oxygen radicals. Oxygen radicals can easily block ***Leishmania*** mitochondrial complexes due to the parasite's relatively low antioxidant system (18). An experimental ***Leishmania donovani*** research on mice, verified the survival of animals after treatment with Artemisinin alone or in combination with diminazene with IC50 of 4.64 µg/ml and 2.28 µg/ml, respectively [19].

According to some research, *A. sativum* extract enhanced *apoptosis in* ***Leishmania major*** promastigotes as a plant product. The proportion of apoptosis in their study using 37 µg /mL as IC50 was 86.11 percent, but the proportion of apoptosis in the other study using 25 µg /mL as IC50 was 33.19 percent (20,21). Artemether, particularly in oral therapy, is an effective and straightforward technique that could be used to treat visceral leishmaniasis (21). According to the findings, it is a very promising medicine that can be recommended as an effective, convenient, and even alternative treatment option for ***leishmania*** species. The current study has several advantages. For starters, there have been few studies on visceral leishmaniasis in the country, and our work is unquestionably valuable in this regard. Second, two curative medicines, Artemether and a combination of (ART- ATM), were studied for management. There is a need to more further comparative test on Artemether and Combination of (ART- ATM), so that the current study's findings may be confirmed.

Conclusion

Finding a novel treatment for Visceral leishmaniasis appears to be more feasible today than it has ever been. On *L. donovani* promastigotes, the data demonstrated a potential lethal effect. This shows that these substances could be regarded as a future herbal therapeutic candidate for the treatment of Visceral leishmaniasis (VL).

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