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Antiparasitic Effect of Artemisinin Drug on Alanine Transaminase, Aspartate Transaminase, Cholesterol and Triglyceride in Infected Mice with *Leishmania donovani*

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Abstract

Background The pathogenic risk caused by the *Leishmania donovani* (*L. donovani*) parasite has long been known, and it has become necessary to find a suitable treatment other than traditional medications and their many side effects, among the best medicines in China, Artemisinin (ART) is not only an anti-inflammatory medicine, but also an anti-allergy medicine and and antibiotics.

Objective To investigate the effect of the ART in inhibiting the activity of the *L. donovani* parasite through studying its impact on alanine transaminase (ALT), aspartate transaminase (AST), cholesterol (CL) and triglyceride (TG).

Methods The ART drug was compared with Pentostam drug (the traditional drug used in the treatment of leishmaniasis), a concentration of 20 mg/ml (0.01 ml/ day) of the ART was prepared and was tested in vivo by observation the levels of liver enzyme ALT, AST, CL and TG in serum of infected mice and comparing with control positive group after 7, 14 and 21 days of treatment.

Results The results showed that there were statistically significant differences between the groups treated with ART drug and Pentostam compared to the positive control group, in addition to the statistical differences between these groups over the three weeks. The AST and ALT values increased in positive control mice over the three weeks, while they returned to normal levels in mice treated with ART drug and Pentostam. As for the CL value, it decreased significantly and statistically significantly in the group of positive control mice. As for the treated groups, there were also statistical differences during the three weeks. While the TG value had statistical differences between the different groups for one week, the TG value for the same group was not affected much.

Conclusion The results indicate the 20 mg/ml of ART had the approximately same positive effects of Pentostam in returning ALT, AST, CL and TG to normal level in mice infested with L. donovani promastigotes parasite.

Keywords *Leishmania donovani*, Artemisinine, ALT, AST, CL, TG

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List of abbreviations: ALT = Alanine transaminase, ART = Artemisinin, AST = Aspartate transaminase, cholesterol (CL), L = Leishmania, TG = Triglyceride, VL = Visceral leishmaniasis

Introduction

Leishmania donovani (*L. donovani*) is obligate protozoan parasites, cause leishmaniasis, a vector-borne disease. They are called flagellate amastigotes, which is transmitted by various species of Phlebotomus sandflies, and develop as intracellular parasites

(flagellate amastigotes) in the mononuclear cells of mammalian hosts [1]. Visceral leishmaniasis VL, formerly known as kala-azar, is an important public health problem, especially in developing countries [2]. Despite the development that has occurred in the field of medicine but the mortality rate of cancer, malaria, leishmaniasis remains high around the world [3].

L. donovani can cause chronic liver and spleen damage, secondary pancytopenia, fever and immunosuppression [4]. The parasite enters the spleen and the series of immunological reaction is started that lead to disorder of it and create a disease-promoting phenotype, while in liver. Another organ seriously affected by parasitic diseases is the skeletal system, bone marrow aspirates from chronic VL patients with concomitant hypergammaglobulinemia show proliferation of erythroid and myeloid-monocytic cells, differential inhibition of megakaryocyte infection, and diffuse plasma cell infiltration [5]. Infection with the *L. donovani* parasite causes swelling and necrosis in the liver, which causes a noticeable increase in the level of liver enzymes [6]. As for cholesterol (CL), its level decreases during parasite infection because it is consumed in building eukaryotic cell membranes [7]. Likewise, for triglycerides (TG), their percentage decreases during the transition between promastigotes to amastigotes [8].

There are many treatments for leishmaniasis, most of which are chemical in composition. They have many side effects and contraindications, such as their high toxicity and the need to be hospitalized in order to administer treatment. These medications are considered chemical anti-leishmaniasis drugs, such as meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam). They were initially used for treatment, but they were discovered that they produce harmful effects such as pancreatitis, cardiotoxicity, and nephrotoxicity [9]. Therefore, it is necessary to find new treatments that have fewer side effects and are easy to use. The researchers at the Chinese Academy of Traditional Chinese Medicine isolated Artemisinin (ART) from *Artemisia annua L*. in a timely manner, making it the second natural remedy for the treatment of malaria after quinine [10]. Research has gradually discovered that ART exhibited a variety of pharmacological effects against viral infections, cell and tumor proliferation, inflammation, and other diseases. Additionally, it has shown a comparatively low toxicity profile [11]. On the other hand, ART exhibited reduction of pathological changes in tissues of colon in experimentally induced colitis of rats [12]. Also, research in Iraq demonstrated the positive effects of flavonoids fraction derived from *Artemisia annua L* on atopic dermatitis (AD) [13].

This study aimed to investigate the effect of the ART in inhibiting the activity of the *L. donovani* parasite and study its impact on some liver enzymes (alanine transaminase (ALT) and aspartate transaminase (AST)), and on some lipid profile markers (CL and TG).

Methods

Culture of *L. donovani* in RPMI-1640 medium was obtained from College of Science, University of Baghdad, cultured and maintained by Novy-MCNeal-Nicolle medium (NNN) every eight days in manufacturer's environment at 27°C [14].

Preparation the concentration of 20 mg/ml of ART drug

The semisynthetic derivatives ART was used in the experiment is a jar containing a capsule containing 200 mg ART material, manufactured in USA, Nutricost Company of production, concentration of ART drug (20 mg/ml) was prepared by opening the capsule, measuring the quantity of ART powder (20 mg) on a sensitive balance, then dissolving in injection water (1 ml) and consider stock solution [15].

Animal grouping

Eighty albino mice were obtained from the National Center for Drug Control and Research weighing 28-30 g. The experiment extended for the period from October 2022 to April 2023.

Following the procedures of the Department of Biology, Animal House, Faculty of Science, Al-Mustansiriayah University, Baghdad, 10 out of 80 mice were healthy and considered negative control (G1), while 70 mice were infected with *L. donovani* (promastigotes) by intraperitoneal injection ($1x10^7$ parasites/ml) [16]. After 14 days, 7 infected mice were sacrificed to determine the presence of infection. Once the liver was extracted, the smear was applied to the slide, stained with Giemsa, and observed under a microscope with oil lenses to confirm the presence of amastigotes in the cells (phagocytes). The infected mice were divided into three groups of 21 animals. After that, each group received an inoculation as follow:

Group 1 (G1): consumed normal saline (0.1 ml/day) orally via stomach tube for 21 days, which is regarded as negative control group, uninfected group and without any treatment during the experiment (healthy group).

Group 2 (G2): consumed normal saline (0.1 ml/day) orally via stomach tube for 21 days, which is regarded as a positive control group without any treatment during the experiment (infected).

Group 3 (G3): After diluting the ART with water and preparing a concentration of 20 mg/ml, 0.1 ml of it was withdrawn daily during the treatment period, take orally via stomach tube for a period of 21 days, regarded as ART treatment group, (infected).

Group 4 (G4): injected with (0.1 ml/day) from Pentostam (0.041 mg/ml) intramuscularly and considered as Pentostam treated group, (infected).

Blood collection for animal Group

After the 7^{th}, 14^{th}, and 21^{st} days, mice were anesthetized by diethyl-ether, 1-2 ml of blood was drawn from the ocular vein of each mouse, then the blood of each mouse was placed separately into gel tube, with the tubes labeled, after which it was kept at room temperature for a period of 35 minutes before being centrifuged. In order to separate the serum for the biochemical tests, blood was centrifuged at 3000 rpm for 15 min, a clear serum was obtained and stored at -20°C until used.

Biochemical analyses

In this study, all biochemical analyses were carried out in the automatic Cobas 6000c (501) apparatus, the company manufactures all tools for the equipment that operated according to an automatic system, where liver enzyme (ALT, AST), CL and TG were measured in serum.

A day before conducting the laboratory tests, the serum was extracted from the freezer and thawed at 2-8°C in the refrigerator, then serum samples were taken on the working day and placed in special tubes of the Cobas equipment, which drew (25 µL) of each serum sample for each test, and treats it with special reagents for the test automatically under the temperature 25-28°C.

Statistical analysis

Version 21 of the statistical package for social sciences (SPSS) has been employed for statistical analysis of the data. The information is given in the form of a mean and standard deviation. Paired ttest was used to compare the level of parameters between G1 and G2. Unpaired ttest was used to compared between the effects of drugs on each parameter in each interval. While analysis of variance (ANOVA) was used to compare the level of study parameters among three intervals and among the all study groups except control negative (G1). P values considered as significant when $P < 0.05$.

Results

In the current study, the effect of ART on experimental mice was evaluated by measuring some biochemical indicators, and compared

with the approved drug, which is Pentostam, in the treatment of VL.

ALT

Table (1) shows that ALT level was significantly higher in G2 compared with G1 during the three measurements at day 7, 14 and 21 days (P <0.001) which reflect occurrence of the disease in G2. The ALT level was significantly increased during the three intervals in G2 (54.15±5.2, 65.5±7.2, 83.55±8.3 U/L) respectively (P <0.001), while in G3 and G4, the ALT level at 7th day was (50.8±5.3, 48.1±3.6 U/L) respectively and significantly decreased until 21st day (35.02±8.2, 34.12±2.3 U/L) respectively, (p =0.001 and P <0.001) respectively. No significant difference in ALT level among the three groups (G2, G3 and G4) was noticed at day 7 (P = 0.085), and between G3 and G4 (P = 0.287), however, at days 14 and 21, there was significant difference in ALT level among the three groups (G2, G3 and G4), (P <0.001) in both interval, but comparison between two drugs show insignificant difference at both intervals (P = 0.664, P = 0.785) respectively.

Table 1. Effect of ART and Pentostam drugs on ALT levels (U/l) over 7, 14 and 21 days among four study groups on in the four study groups

Study groups	7 days Mean±SD	14 days Mean±SD	21 days Mean±SD	P value [a]
Control -ve (G1) N=10	33.14±2.9	33.14±2.9	33.14±2.9	1.000
Control +ve (G2) N=7	54.15±5.2	65.5±7.2	83.55±8.3	<0.001
ART drug (G3) N=7	50.8±5.3	42.41±6.1	35.02±8.2	0.001
Pentostam drug (G4) N=7	48.1±3.6	41.3±2.5	34.12±2.3	<0.001
P value [b]	<0.001	<0.001	<0.001	
P value [c]	0.085	<0.001	<0.001	
P value [d]	0.287	0.664	0.785	

[a] P value represents comparison among three intervals for each group using ANOVA
[b] P value represents comparison between control -ve and control +ve for each interval by paired ttest
[c] P value represents comparison among control positive, ART drug and Pentostam groups by ANOVA
[d] P value represents comparison between ART drug and Pentostam drug for each interval by unpaired ttest

AST

Like ALT, the AST level also was significantly higher in G2 than in G1 during the three intervals (P <0.001), which reflect occurrence of the disease in G2. The study shows a significant increment in AST level during the three intervals in G2 (56.15±6.1, 66.72±4.9, 85.97±7.3 U/L) respectively (P ≤0.001), the opposite occurred in G3 and G4, were the AST level significantly decreased with time (52.92±11.1, 45.3±9.1, 39.71±3.2 U/L) respectively (P = 0.03) in G3 and (51.72±5.4, 46.14±3.7, 38.75±2.1 U/L) respectively (P <0.001) in G4. Likewise in ALT, there was no significant difference in AST level among the three groups (G2, G3 and G4) was noticed at day 7 (P = 0.569), and between G3 and G4 (P = 0.801), however, at days 14 and 21, there was significant difference in AST level among the three groups (G2, G3 and G4), (P <0.001) in both interval, but comparison between two drugs show insignificant difference at both intervals (P = 0.825, P = 0.52) respectively (Table 2).

Table 2. Effect of ART and Pentostam drugs on AST levels (U/l) over 7, 14 and 21 days among four study groups on in the four study groups

Study groups	7 days Mean±SD	14 days Mean±SD	21 days Mean±SD	P value [a]
Control -ve (G1) N=10	37.44±3.1	37.44±3.1	37.44±3.1	1.000
Control +ve (G2) N=7	56.15±6.1	66.72±4.9	85.97±7.3	<0.001
ART drug (G3) N=7	52.92±11.1	45.3±9.1	39.71±3.2	0.03
Pentostam drug (G4) N=7	51.72±5.4	46.14±3.7	38.75±2.1	<0.001
P value [b]	<0.001	<0.001	<0.001	
P value [c]	0.569	<0.001	<0.001	
P value [d]	0.801	0.825	0.52	

[a] P value represents comparison among three intervals for each group using ANOVA
[b] P value represents comparison between control -ve and control +ve for each interval by paired ttest
[c] P value represents comparison among control positive, ART drug and Pentostam groups by ANOVA
[d] P value represents comparison between ART drug and Pentostam drug for each interval by unpaired ttest

Cholesterol

There was no significant difference in CL level at day 7, but significant at day 14 and 21 between G1 and G2 (P = 0.358, P =0.013, P <0.001) which illustrate change in CL level delayed until became more obvious at day 21. CL level significantly during the three intervals in G2. However, in G3, there was significant increment in CL level till day 21 (P = 0.024). Still CL level did not change significantly during the three intervals in G4 (P = 0.132). Regarding comparison of CL level among G2, G3 and G4, no significant difference was obvious at day 7 (P = 0.358) and between G3 and G4 (P = 0.031) but significant at day 14 and 21 (P <0.001 in both days). Moreover, the comparison of the effect of two drugs on CL level was significantly different at day 14 and 21 (P <0.001 for both days (Table 3).

Triglyceride

Table (4) shows that TG level was significantly different in G2 compared to G1 (P <0.001) at all three days (7, 14 21). The level of TG was not changed significantly in G2 and G3 among the three intervals of the study (P = 0.156, P = 0.135) respectively but the change was significant at G4 (P = 0.026). When TG level compared among the study groups except G1, no significant difference was noticed at day 7, and significant at days 14 and 21 (P = 0.033 and P <0.001) respectively. However, no significant difference between the effect of two drugs of TG was observed in all three intervals (P = 0.559, P = 0.254, P = 0.117) respectively.

Table 3. Effect of ART and Pentostam drugs on cholesterol levels (mg/dl) over 7, 14 and 21 days among four study groups

Study groups	7 days Mean±SD	14 days Mean±SD	21 days Mean±SD	P value [a]
Control -ve (G1) N=10	159.62±11.3	159.62±11.3	159.62±11.3	1.000
Control +ve (G2) N=7	164.92±9.33	143.8±8.9	122.97±7.2	<0.001
ART drug (G3) N=7	160.38±11.3	166.92±9.5	175.5±6.6	0.024
Pentostam drug (G4) N=7	163.2±12.5	170.45±13.7	177.54±11.5	0.132
P value [b]	0.358	0.013	<0.001	
P value [c]	0.746	<0.001	<0.001	
P value [d]	0.666	0.031	0.691	

[a] P value represents comparison among three intervals for each group using ANOVA
[b] P value represents comparison between control -ve and control +ve for each interval by paired ttest
[c] P value represents comparison among control positive, ART drug and Pentostam groups by ANOVA
[d] P value represents comparison between ART drug and Pentostam drug for each interval by unpaired ttest

Table 4. Effect of ART and Pentostam drugs on triglyceride levels (mg/dl) over 7, 14 and 21 days among four study groups

Study groups	7 days Mean±SD	14 days Mean±SD	21 days Mean±SD	P value [a]
Control -ve (G1) N=10	92.82±6.1	92.82±6.1	92.82±6.1	1.000
Control +ve (G2) N=7	123.45±4.8	126.92±9.3	131.82±8.4	0.156
ART drug (G3) N=7	120.57±9.3	116.92±7.2	111.19±8.4	0.135
Pentostam drug (G4) N=7	117.42±10.3	114.6±8.5	102.31±11.1	0.026
P value [b]	<0.001	<0.001	<0.001	
P value [c]	0.430	0.033	<0.001	
P value [d]	0.559	0.254	0.117	

[a] P value represents comparison among three intervals for each group using ANOVA
[b] P value represents comparison between control -ve and control +ve for each interval by paired ttest
[c] P value represents comparison among control positive, ART drug and Pentostam groups by ANOVA
[d] P value represents comparison between ART drug and Pentostam drug for each interval by unpaired ttest

Discussion

Liver enzymes ALT and AST

The results of this study seem to indicate that the serum levels of ALT and AST increased within a period of three weeks after infection. These results matched with Tesfanchal et al. [6] who mentioned that VL affects liver function and that, on rare occasions, VL patients may

also present with serious infections that are life-threatening, also mentioned that in VL patients, an enlargement of the liver and the emergence of an immune complex may cause an alteration in liver function. On the other hand, after mice were intravenously injected with *L. donovani*, the majority of parasites were harbored by liver-resident (Kupffer) macrophages, these cells have limited inherent potential to destroy intracellular Leishmania and hepatic parasite load grows quickly in the first few weeks [17]. The pathogenic parasite's amastigote stage undergoes proliferation in the bone marrow, liver, and spleen mononuclear phagocytic system (MPS), which causes an anomaly in those active organs, Furthermore, fibrosis of liver was discovered during the histological analysis of patients with VL, liver damage results from the invasion of parasites to macrophages and Kupffer cells [18].

Since there aren't any secure and efficient medications accessible right now to treat leishmaniasis. The majority of currently available antileishmanial medications have a number of drawbacks, including toxicity, drug resistance, and the need for hospitalization [3], because currently existing medications like Paromomycin, Pentamidine, and Amphotericin B have drawbacks like invasive delivery methods, extensive treatment plans, high costs, resistance, and negative side effects [19]. Anti-leishmanial treatment approaches without a vaccine have changed from single-drug formulations to combination therapy procedures, which, despite their early effectiveness, were subsequently revealed to be drug-sensitive [20].

ART drug was found to be effective in the current study for the treatment of VL; the decline in levels of ALT and AST enzymes is a sign that the ART treatment was successful and that the liver was able to resume its normal functions and repair damage from VL, with an effect similar to common drug therapy such as Pentostam.

On the other hand, when employed ART to treat mice with L. donovani, oral ART treatment is similarly connected with a considerable decrease in parasite number in BALB/c mice treated with VL. This medication was not harmful and nontoxic, according to an analysis of the liver enzymes ALT and AST [21]. A clinical study by Han et al. [22] approved that ART drug can improve liver function, plus the AST and ALT enzymes, in people with liver dysfunction at mild to moderate levels carried on by parasite infections or other reasons.

In a previous study by Tesfanchal et al. in Ethiopia [6] and Al-Abbas et al. in Iraq [20], they showed that VL patients' serum (ALT and AST) mean values were considerably higher than those of healthy controls, current result was consistent with these studies.

Cholesterol

The level of CL in the infected mice started to decline, as shown in table (3). This finding is coinciding with are search from Ethiopia and Brazil [6,23], also they noticed that hypocholesterolemia be considered the primary symptoms of VL. This could be as a result of the inverse relationship between CL levels and splenic parasite load found in patients with VL. Patients with active VL exhibit dysfunctions in the liver, which in charge of CL production, as a result of the high parasite loads in these organs [23].

On the other hand, acute-phase proteins and cytokines such as interleukin -1beta (IL-1b), IL-6, and tumor necrosis factor may also play a role in the reduction of CL levels in VL infections, the autoimmune phenomena impact on CL metabolism during an immunological process by different ways, including: generating immune complexes, accelerating enzyme degradation, and other means [24].

Also, recent studies have demonstrated that the metalloprotease GP63 released by the parasites infecting Kupffer cells in the liver is what causes the hypocholesterolemia seen in *L. donovani* infections. In hepatocytes, GP63 breaks down the enzyme dicer (endonuclease RNAse III), which lowers the expression of miR122 and lowers the synthesis of CL. A posttranscriptional regulator (miRNA) called miR122 is highly expressed in the liver and regulates a variety of processes. It makes up almost 70% of the miRNA in the liver and is

primarily in charge of lipid metabolism and homeostasis [25].

Current Study showed that the ART drug caused increased in the level of CL after administration (G3), and this is may be due to the anti-leishmanial properties of ART, which inducing apoptotic effects on amastigotes, the ability to form reactive oxygen species (ROS) and ART 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 and as a result this compound has the ability to kill of Leishmania [15], additionally ART have a therapeutic impact similar to that of the first-line medication, amphotericin B, causing a considerable decrease in hepatomegaly and parasite load in mice infected with *L. donovani* [26].

Triglyceride

The results of the current study showed that infection with VL did not affect the level of serum TG in infected mice, despite its rising, it remained within normal limits. Previous research by Tesfanchal et al. and Lal et al. showed that VL patients' mean blood TG levels were considerably higher than those of healthy controls [6,27]. this might come back to the weaken role of that enzyme which is responsible of cleavage of TG in VL patient and the result decrease of metabolism of TG, which leads to increased TG [6]. There is a study recorded hypertriglyceridemia in VL patients, also it considers a good marker of disease severity [28].

On the other hand, the usage of ART drug as shown in table (4) decreased the value of TG at the G3, although it did not increase much and this study achieved results similar to those of the drug Pentostam at the G4.

In conclusion, a concentration 20 mg/ml of ART drug leading to improvement in ALT, AST, CL and TG in mice infected with *L. donovani* promastigotes parasite approximately similar to that of Pentostam, which is considered the traditional drug for treating VL.

The authors recommend studying the effects of ART drug on the other biochemicals parameters in infected animals with VL and investigating the effects of ART drug on other parasites.

Limitation

Liver function and lipid profile involve many parameters other than those selected in this study, but due to financial limitation, only the above-mentioned parameters were studied.

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Author contribution

Ali: did all laboratory test and wrote the manuscript. Dr. Majeed: supervised this research.

Conflict of interest

None.

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