The effect of Sodium chloride and Cephalexin antibiotic on the growth of
Leishmania tropica and Leishmania donovani
promastigote

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Abstract
To examine the tested drugs (sodium chloride and Cephalexin), we tested the drugs in vitro
against promastigote stage of parasite with multi concentrations of the drugs. The viability of the
parasite was counted daily to promastigote for 5 days. Hypertonic sodium chloride solution LD
value against Leishmania donovani and Leishmania tropica promastigote was (0.021) and (0.04) mg/ml
respectively, while Cephalexin LD value against L. donovani and L. tropica promastigote was (1.4)
and (2.55) mg/ml respectively. Hypertonic sodium chloride solution and Cephalexin a wide-spectrum
antibiotic have effective leishmaniacidal agents against L. tropica and L. donovani promastigote in vitro.

Key words : L. donovani, L. tropica, sodium chloride, Cephalexin

Introduction
Leishmaniasis is a parasitic disease caused by the hemoflagellate Leishmania spp. The
parasite is transmitted by the bite of an infected female Phlebotomine sand fly (1). According to
the geographic region in which different Leishmania species are found and the host
response, this disease can affect the skin, viscera, or mucocutaneous areas. According to
WHO, it is estimated that approximately 400,000 new cases of leishmaniasis occur
annually, with almost 400 million people at risk of the disease. The overall prevalence of
leishmaniasis is estimated at 12 million cases with 0.5 million new visceral leishmaniasis (VL)
cases per year and 1.0-1.5 million new cutaneous leishmaniasis (CL) cases per year (2).
Both VL and CL have been reported in Iraq caused by L. donovani, L. major and L. tropica
respectively (1).

Pentaivalent antimonial drugs (pentostam and glucantime) is the first choice drug for the
treatment of leishmaniasis, the current drug treatment of leishmaniasis is unsatisfactory due
to drug resistance, lack of efficacy, toxicity and route of administration. New drugs are required
primarily for treatment visceral form of leishmaniasis and secondarily for cutaneous
form of the disease (3). Many treatment studies have been proposed and screening drugs for
antileishmanial activities are very effective way to find new active substances such as
bleomycin, Co-trimoxazole, sodium chloride and zinc sulfate (4,5,6,7).

Recently, it was found that Miltefosine, chloroquine and clindamycin were found to be
effective leishmaniacidal agents against L. donovani, L. major and L. tropica in vitro (8). In
an experimental study on BALB/c mice infected with L. major, Jarallah obtained good results
after topical treatment with ciprofloxacin and co-trimoxazole antibiotics (5). The aim of this
study was to test leishmaniacidal agent of sodium chloride and cephalexin antimicrobial
drug against L. tropica and L. donovani promastigotes.
Materials and Methods

a- Source of parasites and culture media:

The Leishmania strains of cutaneous and visceral leishmaniasis L.tropica (MHOM/IQ/93/MRC2) and L. donovani (MHOM/IQ/1982/BCR1/AA3) were provided from the Leishmania unit at medical research center, Al-Nahrain University, Iraq. These parasites were maintained in vitro by sub-cultured on diphasic Nicolle-Nove-MacNeal (NNN) medium at (23-26)°C. The diphasic medium is made of two phases, a solid and liquid phases (9,10).

b- The effect of Sodium chloride and Cephalexin on L. tropica and L. donovani Promastigotes:

The antibiotic Cephalexin was obtained from pharmacy (tablet, 500 mg/ml). Two drugs (Sodium chloride and Cephalexin) were sterilized by filtration through a sterile filter paper (0.45 micron) in diameter with sterile filter unit and this was made by using vacum pump. 0.9ml of the liquid phase medium (Lock’s solution) which contains different concentration of two drugs were added to screw-capped vials containing 5ml of solid phase medium. Each concentration was done in triplicate, vials of diphasic medium were kept as the vehicle control without drug it was done also in triplicate. The promastigotes were adjusted to 1x10^6 cell / 0.1ml of Lock’s solution and added to each vial. All these vials were then incubated at 25°C. The parasites were counted once daily for the following 5 days. 1:20 dilution in saline (PBS) together with the 0.4% trypan blue stain (promastigote permeable to the blue dye are dead while viable ones exclude the dye). The number of alive and death cells was estimated in each of the experimental vials, compared to the number of alive cells of control vials.

The chamber of Neubauer haemacytometer is charged and the number of organisms in 16 small corner square is counted. The total number per ml = N x 10 x 1000 x 20 (N = No. of cell counted, 10 = No. of cell in lmm^3, 1000= No. of cell in ml, 20= dilution factor).

Percentage of growth index (GI %) was calculated (11) as follows :

\[
GI \% = \frac{\text{Number of treated parasites (experimental)}}{\text{Number of untreated parasites (control)}} \times 100
\]

The LD_{50} values after 3 days from drug addition was calculated according to the method of Healy (12). In this study probability (p) was less than 0.05 (P<0.05) which was considered to be significant (13).

Results

a-Effect of hypertonic sodium chloride solution on L. tropica and L. donovani promastigotes

Figures (1,2) showed the efficacy of hypertonic sodium chloride solution against L. donovani and L. tropica promastigotes, that increased in its applied concentrations (0.005, 0.01, 0.03, 0.05 mg/ml). Sodium chloride inhibits the growth of the promastigotes from the first day of incubation Figures (1,2) show a decrease in the percentage (GI %) from 100% at zero time and zero concentration to reach at (10%), (15.5%) on 5 days at high concentration for each L. donovani and L. tropica respectively. The LD_{50} for sodium chloride after 3 days of exposure was (0.021), (0.04) mg/ml for L. donovani and L. tropica respectively. Statistically there are significant differences (P<0.05) between L. donovani and L. tropica Growth Index percent (GI%) at high concentration after 5 days of sodium chloride exposure.

b- Effect of cephalxin on L. tropica and L. donovani promastigotes

Cephalexin showed high efficacy against L. donovani and L. tropica promastigotes, that increased in its applied concentrations (0.05, 0.1, 1, 3 mg/ml). The results in Figures (3,4) show that (GI %) was decreased with increased Cephalxin concentrations from 100% at zero time and zero concentration to reach at (17.5%), (37.7%) on 5 days at high concentration for each L. donovani and L. tropica respectively.

In selected concentration, the LD_{50} for Cephalxin was (1.4), (2.55) mg/ml for L.
*L. donovani* and *L. tropica* respectively after 3 days. There are significant differences (P<0.05) between *L. donovani* and *L. tropica* growth index percent (GI%) at high concentration after 5 days of Cephalexin exposure.

**Figure (1): Effects of various sodium chloride concentrations against *L. donovani* promastigote**

**Figure (2): Effects of various sodium chloride concentrations against *L. tropica* promastigote**
Figure (3): Effects of various cephalexin concentrations against *L. donovani* promastigote

![Graph showing growth index (GI%) over days of exposure for different concentrations of cephalexin. The graph includes data points for concentrations of 0.05, 0.1, 1, and 3 mg/ml. The x-axis represents days of exposure from 1st to 5th, and the y-axis represents growth index (GI%) from 0 to 100.]

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Figure (4): Effects of various cephalexin concentrations against *L. tropica* promastigote
**Discussion**

Infections by *Leishmania* causes a wide spectrum of disease ranging from the asymptomatic to the severely clinically symptomatic. The efficacy of drugs used for the treatment of visceral and cutaneous leishmaniasis is influenced by many factors such as host factors and pharmacokinetics as well as variation in the sensitivity of *Leishmania* species and acquired drug resistance due to selection (14).

Hypertonic sodium chloride solution had more influence on promastigotes of *L. donovani* and *L. tropica* with LD$_{50}$ (0.021 , (0.04) mg/ml than antibiotic with LD$_{50}$ (1.4), (2.55)mg/ml. In the present study, both the antibiotics and sodium chloride were effective on *L. donovani* and *L. tropica* promastigotes. The parasites density decreased with increasing the applied concentrations of antibiotic and hypertonic sodium chloride solution. In an experimental study *in vitro* effect of hypertonic sodium chloride solution on *L. tropica* promastigotes were investigated, the destruction of promastigotes was observed at five minutes with 7% Nacl solution (6), these observations were similar with our results at five days and high concentrations.

In Iraq, cutaneous leishmaniasis (CL) Baghdad boil caused by two species *L. major* zoonotic disease and *L. tropica* anthroponotic disease (15). Co-trimoxazol antibiotics had antileishmanial activity drug against *L. major* promastigotes with LD$_{50}$ (4.21) mg/ml (5) while in this study cephalaxin antibiotic had antileishmanial activity against *L. tropica* promastigote with LD$_{50}$ (2.55) mg/ml this difference between LD$_{50}$ values may be due to many factors, these include difference of strains causing CL, or to difference in virulence of two strains *L. major* and *L. tropica* as well as biochemical and molecular differences between species are reflected in the variation of the sensitivity of *Leishmania* species (16).

The lack of an effective drug for the treatment of leishmaniasis has led to development of new antileishmanial agents with better activity and low the toxicity (3). Many studies confirmed that antibiotics have leishmanicidal activity with the potential to treat all clinical leishmaniasis. Jarallah demonstrated that both Co-trimoxazol and ciprofloxacin antibiotics have a reduction in lesion size and a duration of ulceration caused by *L. major* after treatment with two antibiotics as a topical application paste (5). The antimicrobial miltefosine was efficient *in vitro* leishmanicidal activity against *L. donovani, L. major* and *L. tropica* (17, 18).

Cephalexin is in a group of drugs called cephalosporin antibiotics and is used to fight bacteria in the body. It works by interfering with the bacteria's cell wall formation, causing it to rupture, and killing the bacteria (19).

In the present study when comparing between LD$_{50}$ values for *L. tropica* and *L. donovani*, it’s found that LD$_{50}$ value for *L. tropica* was higher than *L. donovani* for two selected drugs that means the promastigote of CL strain is more resistant to the tested drugs than promastigote of VL strain. Antileishmanial activity of cephalaxin *in vitro* against *L. tropica* and *L. donovani* in this study was in agreement with Mohammed who demonstrated that *in vitro* potent leishmanicidal activity of some antimicrobials (8). The mechanism of the action of antileishmanial drugs is still poorly understood, the mode action of these drugs as an antileishmanial drugs may be due to inhibit the virulence enzymes acidophosphatase ACP and protease for *Leishmania* parasite cell.

In conclusion, hypertonic sodium chloride solution and cephalexin antibiotic show antileishmanial activity towards *L. tropica* and *L. donovani* promastigotes *in vitro*. Nacl and antibiotic hold a promise as sources of chemical leads for the development of novel therapeutic agents in the fight against leishmaniasis. Further studies must use these drugs *in vivo*.

**References**


Leishmania tropica و Leishmania donovani

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الخلاصة

لفحص الآداب المختارة (كولوريد الصوديوم والسيفالكسين)، أجري الاختبار خارج جسم الكائن الحي تجاه الطور المـنـحـب للطفل غير المكثف. تم حساب جسم الآداب المختارة لطور المـنـحـب لمدة خمسة أيام. كانت قيمة الجرعة النصفية قياسية لـً institutions متحركة يـاً لـً LEISHMANIA DONOVANI AND LEISHMANIA TROPIKA (0.004) و (0.021) مـلـمـل مـل مـل مـمـل مـل مـمـل مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم مـم م~