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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_{50} value against *Leishmania donovani* and *Leishmania tropica* promastigote was (0.021) and (0.04) mg/ml respectively, while Cephalexin LD_{50} 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 4,00,000 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).

Pentavalent 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 cephalaxin 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 lml, 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

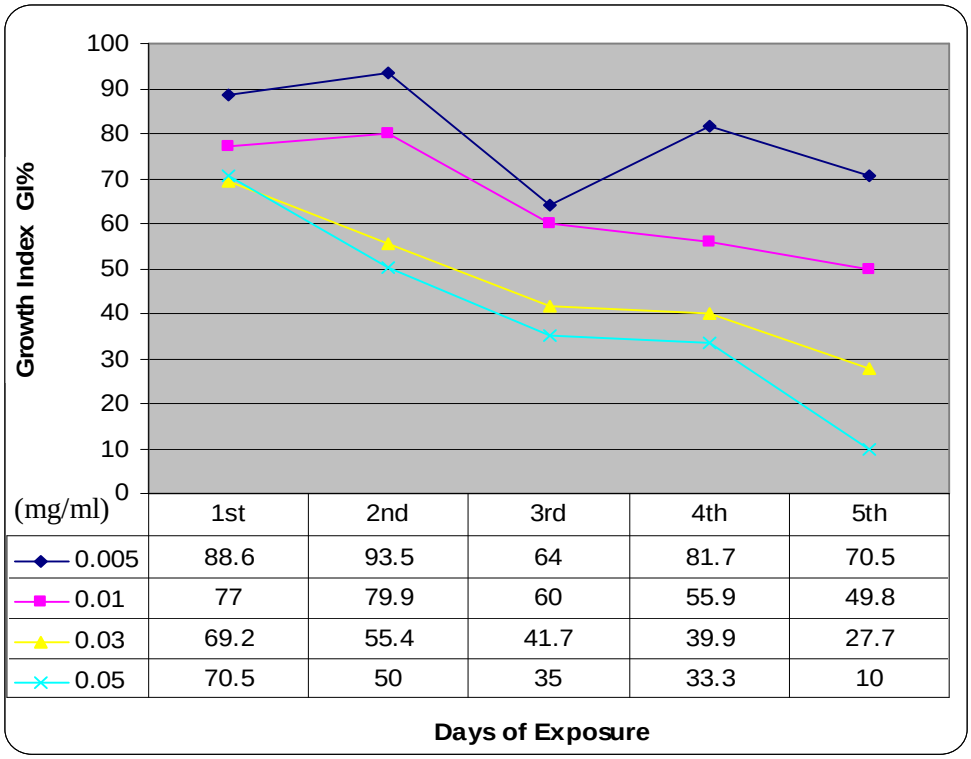
Figures (1,2) showed the efficacy of hypertonic sodium chloride solution against *L. donovani* and *L. tropica* promastigotes, that increased in its applied concentrations (0.005, 0.01, 0.03, 0.05 mg/ml). Sodium chloride inhibits the growth of the promastigotes from the first day of incubation Figures (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 cephalexin 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 Cephalexin 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.

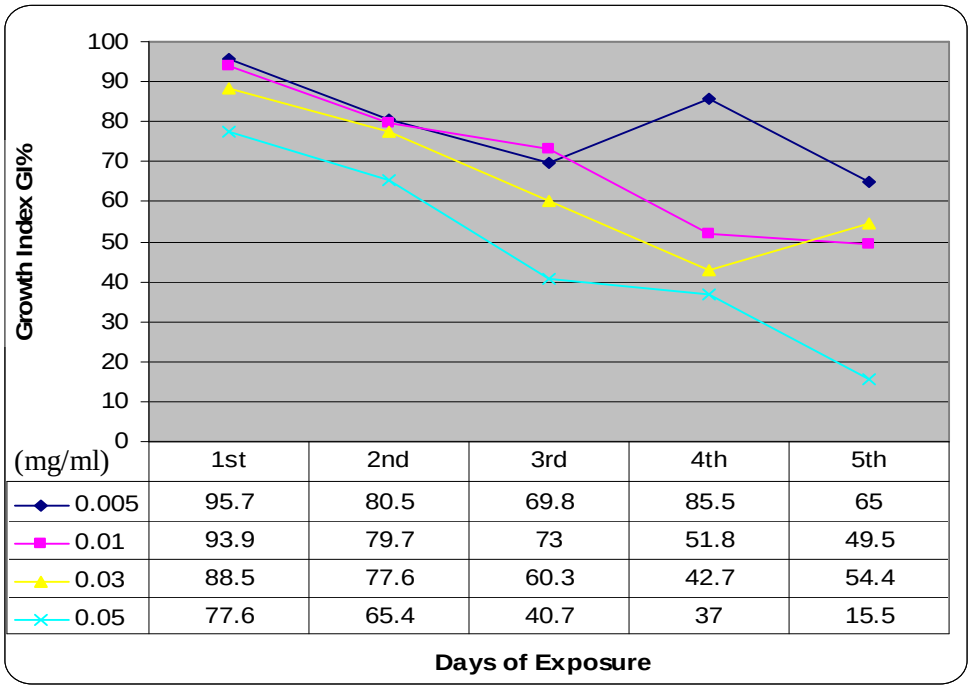
In selected concentration, the LD_{50} for Cephalexin was (1.4), (2.55) mg/ml for *L.*

donovani and *L. tropica* respectivelyafter 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.



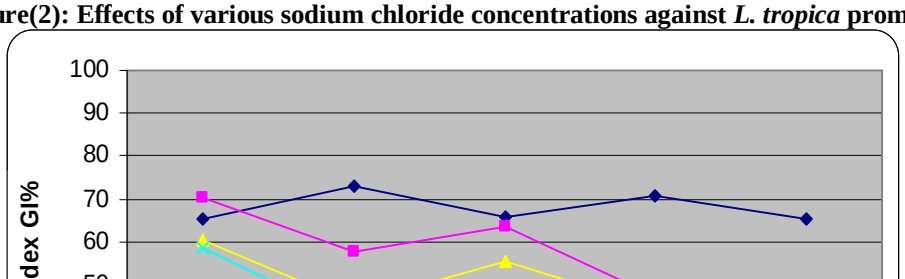
(mg/ml)	1st	2nd	3rd	4th	5th
0.005	88.6	93.5	64	81.7	70.5
0.01	77	79.9	60	55.9	49.8
0.03	69.2	55.4	41.7	39.9	27.7
0.05	70.5	50	35	33.3	10

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

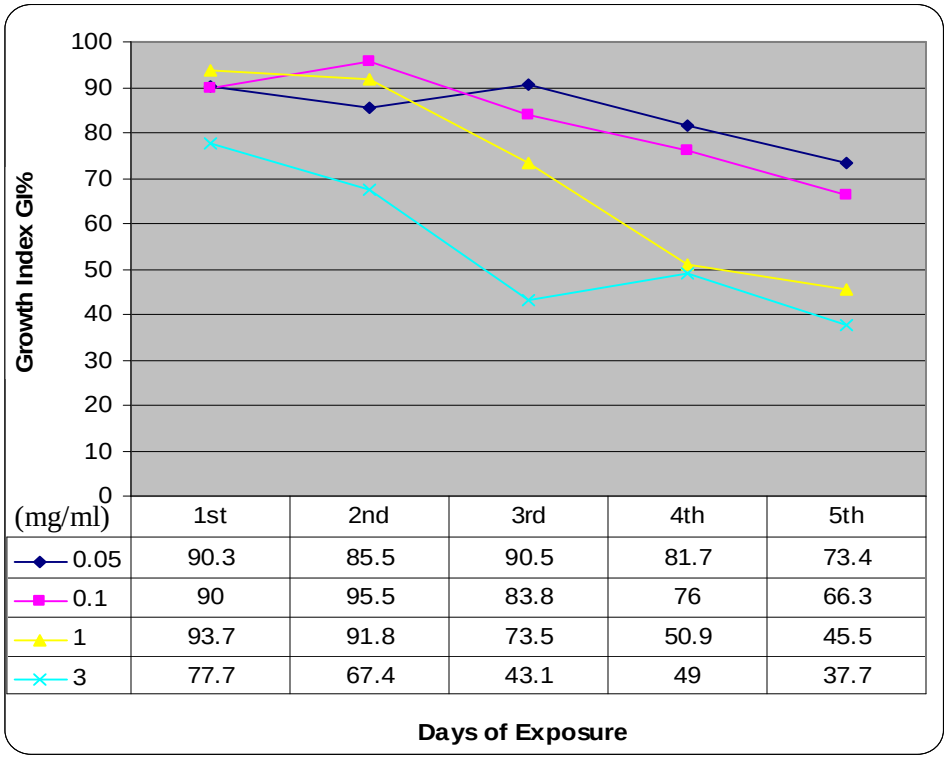


(mg/ml)	1st	2nd	3rd	4th	5th
0.005	95.7	80.5	69.8	85.5	65
0.01	93.9	79.7	73	51.8	49.5
0.03	88.5	77.6	60.3	42.7	54.4
0.05	77.6	65.4	40.7	37	15.5

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



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



(mg/ml)	1st	2nd	3rd	4th	5th
0.05	90.3	85.5	90.5	81.7	73.4
0.1	90	95.5	83.8	76	66.3
1	93.7	91.8	73.5	50.9	45.5
3	77.7	67.4	43.1	49	37.7

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 anti-leishmanial activity drug against *L. major* promastigotes with LD_{50} (4.21) mg/ml (5) while in this study cephalexin 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 ciprophloxacin 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 sourses of chemical leads for the development of novel therapeutic agents in the fight against leishmaniasis. Further studies must use these drugs *in vivo*.

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تأثير كلوريد الصوديوم والسيفالكسين على نمو الطور المسوط للشمانيا *Leishmania tropica* و *Leishmania donovani*

هند مهدي جارا لله

مركز علوم البحار/جامعة البصرة/البصره/العراق

الخلاصة

لفحص الادويه المختبرة (كلوريد الصوديوم والسيفالكسين)، اجري الاختبار خارج جسم الكائن الحي تجاه الطـور المـسوط للطفيلي بتراكيز متعددة من الادويه. تم حساب حيوية الطفيلي يوميا" لطور المسوط لمدة خمسة أيام. كانت قيمة الجرعة النصف قاتله لمحلول كلوريد الصوديوم المفرط التوتر تجاه الطور المسوط للشمانيا دونوفاني واللشمانيا تروبكا (0.021) و(0.04) ملغم/مل على التوالي، بينما كانت قيمة الجرعة النصف قاتله للسيفالكسين تجاه الطور المسوط للشمانيا دونوفاني واللشمانيا تروبكا (1.4) و (2.55) ملغم/مل على التوالي. وجد بان محلول كلوريد الصوديوم المفرط التوتر والسيفالكسين عوامل تملك فعاليه قاتله تجاه الطور المسوط للشمانيا دونوفاني واللشمانيا تروبكا خارج جسم الكائن الحي.