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Original paper

In vivo comparative study of the efficacy of β-sitosterol, ketoconazole 2% and mupirocin for the treatment cutaneous leishmaniosis

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ABSTRACT. Cutaneous leishmaniosis (CL) is an important parasitic disease characterized by specific skin lesion, includes the vector that cause the CL and treatment in general. The study aimed to identify the effect of three different drugs which are β-sitosterol, ketoconazole 2% and mupirocin in the treatment of cutaneous leishmaniosis. The study was conducted at Dermatological Unit of Al Hussein Teaching Hospital/Thi-Qar/Iraq from October to November 2021. The patients presented with a lesion will be involved in this study and its involved isolation of parasites from the lesion of patients and the parasite was replicated in (NNN) media then the inoculum was concentered. A total of 40 male of mice *(Mus musculus*) of BALB/c strain injected by parasite suspension, after the appearance of lesion, β-sitosterol, ketoconazole 2% and mupirocin was applied on a lesions daily for 2 weeks, the statistical analysis was done by SPSS program. In the current study, the β-sitosterol was most effective in the treatment of skin leishmanial lesion than the other drugs with mean is (11.9±1.449 mm) in compared with the other drugs under *P*-value < 0.046 with the complete recovery. β-sitosterol was highly effect on the *L. tropica* infections with complete recovery and no scar appearance than ketoconazole and mupirocin and can be used for treatment of the disease lesion.

Keywords: cutaneous leishmaniosis, β-sitosterol, ketoconazole, mupirocin

Introduction

Leishmaniosis is classified under neglected tropical disease and remains a public health problem in many poor countries because of the absence of effective and safe anti-leishmanial drugs for treatment and the presence of resistance to the anti-leishmanial drugs in certain areas [1]. There are three main types of leishmaniosis including: cutaneous, visceral and muco-cutaneous. Cutaneous leishmaniosis is still major public health problem particularly in low middle countries which the clinical manifestations are varied from small cutaneous nodule's to overall mucosal tissue destruction [2,3]. No a viable vaccine of leishmaniosis are available for human use and the recommended therapy depends on anti-leishmanial drugs such as pentavalent, antimonials or amphotericin B, and pentamidine. These common drugs presents with several limitations, including high cost, parasite résistance and toxicity [4]. There is no sufficient therapy for cutaneous leishmaniosis, the World Health Organization suggested the topical treatment regime can be applied for uncomplicated ulcerative skin lesions, while the patients who have acute form of the disease should use parenteral therapy [5–7].

Parenteral administration of pentavalent antimony stay the first choice therapy for CL although a high frequency of side effects in including anorexia, arthralgias, myalgias, chemical pancreatitis and cardiotoxicity [8–10].

Over the last few decades, major confirmation, including formulations for both oral and topical treatment were developed by scientists to treatment leishmaniosis [10]. Topical treatment has several advantages in comparison with parenteral administration represented by lower adverse reaction incidence and easy administer [11]. The newer anti-leishmanial drugs are miltefosine

Table 1. The effects of drugs expressed by the size of lesions (mm)

Groups	Cona. Intb.	Con. Finc	β-sitol Int.	β-sitol Fin.	Keto. Int.	Keto. Fin.	Mup. Int.	Mup. Fin.
Mean (mm)	33.50	21.70	35.10	11.90	34.40	18.50	35.10	18.30
SD	1.080	0.948	1.100	1.449	1.173	1.354	0.875	2.110
Minimum	32.00	20.00	34.00	10.00	33.00	16.00	34.00	14.00
Maximum	35.00	23.00	37.00	14.00	36.00	20.00	36.00	21.00

Explanations: a: control; b: initial; c: final

(hexadecyl-phosphocholine) and ketoconazole, β-sitosterol is well documented as anti-leishmanial against *Leishmania donovani* and the researchers were used it against *Leishmania tropica* and gave an excellent theraptic results [5,8].

The present study aimed to examine the efficacy of β-sitosterol, ketoconazole 2% and mupirocin for the treatment of cutaneous leishmaniosis.

Materials and Methods

Parasite isolation

The isolated parasite was obtained from the patients presented with the Baghdad boil in Dermatological Unit of Al Hussein Teaching Hospital/Thi-Qar/Iraq from October to November 2021. The parasite was isolated on semi-solid medium which prepared according to method conducted by Roger [12].

Multiplication of parasites

To enhance the growth of parasites in the inoculum, the specimen were transferred onto the semi-solid (NNN) medium that prepared according to the method of Kang and Norman [13] and Meredith et al. [14].

Preparation of the parasite dose

Parasites grown on NNN medium were collected on the fifth or sixth day of inoculation where the parasite is at the high peak of growth. The liquid medium containing promastigote forms was distributed in test tubes and centrifuged at 1500 rpm for 10 minutes to concentrate the parasites. After the centrifugation procedure, the promastigote was observed in the bottom of the tubes and the supernatant of the liquid was removed using a sterile pipette. Then add an appropriate volume of the locks solution, mixed well and the number of parasites was calculated using Neubauer chamber [13,14].

Experimental animal injection

A total of 40 male of mice *(Mus musculus*) of BALB/c strain aged 8–10 weeks and weighted between 25–35 grams were used in this experiment. The animal study was performed in the animal house of the Department of Biology, College of Sciences, University of Thi-Qar. All animals were inoculated with 1×10^6 parasites/0.2 ml of *Leishmania tropica* that identify previously by PCR at the base of the tail.

Figure 1. Cutaneous leishmaniosis lesion. A: before treatment with β-sitosterol, B: after 3 weeks of treatment

Treatment of lesion

After seven days of injection and the ulcer (Baghdad boil) was developed, the animals were divided into four groups and treated as follows: Group 1: no treated animals (control group); Group 2: the animals were treated with β-sitosterol (Rembo-Turkey) ointment 0.25% with two doses a day for 21 days; Group 3: treatment with ketoconazole (Kinazol-Turkey) ointment 2% with two doses a day for 21 days; Group 4: treatment with mupirocin ointment 2% (Turkey) with two doses a day for 21 days.

The cream is applied on the lesions 3 times daily by using sterile swabs and the size of lesions was measured each 7 days after the beginning of treatment.

Statistical analysis

The obtained data was analyzed by using analysis of variance (ANOVA) through SPSS (Statistical Package for the Social Sciences) program version 24. The results was considered significant when P-value ≤ 0.05.

Results

β-sitosterol was most effective than the other drugs (P-value < 0.046) as shown in table 1 and figure 1. The results showed significant differences between the drugs that used in this study, the β-sitosterol cream was the most effective than ketoconazol and mupirocin where the main of diameters of lesions decreased to 11.9, 18.5 and 18.3 mm for β-sitosterol, ketoconazole and mupirocin, respectively.

Discussion

Leishmaniosis is a protozoal disease that spread by the sandfly. Cutaneous disease begins typically as a papule and later developed as the ulcer with an elevated rim. Patients may develop localized or diffuse cutaneous disease according to the species of *Leishmania* that causes the disease. Untreated lesions can self-dissolve but leave scar in 2–15 months (or longer) in immune-competent persons [12]. Local intra-lesion treatments have been recommended for those who were having the small and single lesions (<5 cm) without lymph node metastasis. Mean, a systemic treatment is often indicated to reduce the risk of disease's dissemination to the mucosa or viscera, decrease the time to healing and limit the morbidity caused by large or persistent skin lesions [13]. The use of certain anti-*Leishmania* drugs requires close observation on its adverse effects such as elevated liver function tests, myalgia, arthralgia and chemical pancreatitis. Furthermore, a weak response was observed in a significant proportion of patients to some types of basic treatments in cases of primary cutaneous leishmaniosis [14–17]. Therefore, it is necessary to search for more effective alternative medicines in the treatment of primary cases, in order to prevent the development of infection and the occurrence of complications.

β-sitosterol is one of phytosterols similar in structure to that of cholesterol. Sitosterols are waxy white powders with a typical odor. In this study, we investigated the effects of three commercial local cream and ointments in treatment of CL lesions, β-sitosterol topical cream appeared to be the most effective drug in managing of local lesion caused by *Leishmania* spp. when it is applied on lesion compared with the other two drugs that have been tested in this study. It also showed a decrease in the size of lesion and aided in healing in a short period with no scar formation and complete recovery. β-sitosterol was used in the treatment of parasitic infections, Pramanik et al. [18,19] was found that β-sitosterol inhibit trypanothione reductase of *L. donovani* which is an enzyme that catalyzes the conversion of NADP to NADPH and neutralize hydrogen peroxide produced by host macrophages during infection, and it might represented a successful approach for treatment of visceral leishmaniosis and this classic plant-derived medicinal agent indicated their potential in modern medicine for their less lethal effects; hence. The researchers detected potent anti-inflammatory activities of β-sitosterol with a weak anti-leishmanial action for β-sitosterol, the study was conducted with the results that obtained by [5,20] which confirmed that β-sitosterol have a significant anti-leishmanial activity and it is necessary to perform the further studies in order to support its role as a potential drug target against *L. tropica*.

Other drugs used in this study including ketoconazole and mupirocin. Both of the drugs showed a mild effect. Ketoconazole (KTZ) cream has a broad spectrum of azole antifungal activity, which was initially used in 1981 for the treatment of systemic mycosis. Moreover, it has been revealed that it also can act as anti-inflammatory, anti-androgenic activity and antibacterial effects against

some bacterial species. In this study, ketoconazole appeared to have some anti-leishmanial activity and help in improvement by the reduction of parasitic lesions as well as recovery [21–24].

Mupirocin is an antibiotic that used as antibacterial agent against *Staphylococcus aureus* and belong to monocarboxylic acid class of antibiotics. But, in the current study mupirocin has low significant anti-parasitic activity [25].

In conclusion, β-sitosterol was highly effect on the *L. tropica* infections with complete recovery and no scar appearance than ketoconazole and mupirocin and its can be used as alternative drug in the treatment of cutaneous leishmaniosis lesion.

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