Antileishmanial activity of alcoholic extract of \((Trigonella\ foenum-graecum)\) seeds against ulceration of cutaneous leishmaniasis \textit{in vivo}

Hind Mahdi Jarallah*, Dawood Salman Mehdi**

*Marine Science Center, University of Basrah, Iraq
**Dept. of Pharmacy, The Technical Institute, Basrah, Iraq

Key words: Cutaneous leishmaniasis, parasites, \textit{Trigonella foenum-graecum} seeds

Abstract:

The effect of alcoholic extract from \textit{Trigonella foenum-graecum} seeds was studied on the ulcerative lesions of cutaneous leishmaniasis caused by \textit{Leishmania major} in BALB/c mice experimentally infected with \textit{L. major} promastigotes. The \textit{Leishmania major} strain was isolated from Iraqi patient suffering from cutaneous leishmaniasis, the patient lives in rural village belong to Basrah marshland. The alcoholic extract of this plant was administrated by intralesion injection. The alcoholic extract from \textit{Trigonella foenum-graecum} seeds showed good results. The plant extract decrease the growth and development the cutaneous ulcers and its size of treated mice when compared to the control mice which were worsening and more increased in ulcers size.

Introduction

Cutaneous leishmaniasis (CL) was an endemic disease in Iraq (Pringle, 1957). Leishmaniasis was consider as the tenth parasitic infection in the world (WHO, 1990). In Iraq, the disease was found to be more prevalent in central region and mostly in the rural area and semi-desert region (Sukkar, 1978). The incidence of leishmaniasis is increasing, with many endemic areas reporting a 50% increase over the past seven years. Leishmaniasis are now endemic in 88 countries with a total of 350 million people at risk (WHO, 2000). In Iraq, especially in Basrah governorate, cutaneous leishmaniasis cases were reported and caused mainly by \textit{Leishmania major} species (Jarallah, 2003). Tradition medicine has been practiced to some degree in all cultures and other terms based on culture include Africa, Asia or Chinese medicine (Al-Rahbi, 2000). Many alternative treatment modalities have been proposed and screening of medicinal plants for antileishmanial activities is a very effective way to find new active substances (Iwu, et. al., 1994).\textit{Trigonella foenum-graecum} seeds contains fatty oils, mucilage protein, mannogalactan, resin, trigonellin, cholin, saponin, tannin, essential oils, phosphorus, iron; contains no starch (Al-Rawi and Chakravarty, 1988). The aqueous and alcoholic extracts of \textit{Trigonella foenum-graecum} seeds have antileishmanial effect on \textit{Leishmania major} parasites \textit{in vitro} at different concentrations when compared to the control (Jarallah, 2005) and antimicrobial (Alkofahi, et. al., 1996) and antibacterial (Bhatti, et. al., 1996). In recent study, demonstrate that the aqueous extract fenugreek \textit{T. foenum-graecum} has amply good effect on the weight on visceral organs which are second to none in importance in both taste and popularity among the people (Khan, et. al., 2009). That is mean \textit{T. foenum-graecum} seeds contains lecithin and choline that help to dissolve cholesterol and fatty substances.
Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have antimicrobial properties in vitro (Cowan, 1999).

**Materials and Methods:**

**Leishmania strain** The Iraq strain of *L. major* was used in this study, the strain was isolated from skin lesion of male child patient have 11 years old by specialized clinic of dermatology, he live in rural village belong to Basrah marshland suffering from cutaneous leishmaniasis. The patient has ulcerative lesions on right forearm Figure (1). Aspirate material from the margin of the lesion was spread on slide to made smears. The prepared smears were stained by Leishman’s stain to detect the Leishman bodies and examine under the oil immersion objective of the light microscope Figure (2).

**Culture media:**

Cultures were carried out using Nicolle-Novy-McNeal (NNN) medium. The *Leishmania* strain was isolated on diphasic (NNN) medium, it is made of two phases, a solid and liquid phase (Kagan and Norman, 1970; Meredith, et. al., 1995). The promastigote parasites were harvested on the 6th day. The number of parasites were adjusted to 1x10^7/0.1 ml for inoculation by counting in hemocytometer.

**Plant extraction procedure:**

The aqueous and alcoholic extracts of *T. foenum-graecum* seeds were prepared according to Harborne (1984) and WHO (1998). 9 mg/ml concentration of alcoholic extract were prepared and used in this study.

**Animals and experimental design:**

Fifteen laboratory female BALB/c mice 6-10 weeks old were used in this study, mice were divided into three groups (A,B,C), each group contain 5 mice. Each mouse in all groups (A,B,C) were injected in a shaved area above the tail with 0.05ml of 1x10^7/0.1 ml of promastigotes intradermaly. Groups (A,B) daily injected intradermaly (subcutaneous) in the same area with 0.05 ml of 9mg/ml of alcoholic extract of *T. foenum-graecum* seeds for two weeks as infected treated group while group (C) was kept as infected control. The effect of extract on mice ulcerative lesions was monitored assessed at second week post infected and continuous every two weeks. Clinically by measuring the morphology and size of the lesions and parasitologically by smears were made from ulcer stained with Leishman’s stain and examined under oil immersion of light microscope to determine the density of parasites by counted the number of amastigote form of parasite (Schnur, et. al., 1973).
Figure (1): The leishmanial case see lesion on the right forearm

Figure (2): Smear from cutaneous leishmaniasis showing amastigotes forms (Leishman’s stain, oil immersion)

Results

Clinically: The infection started on mice at the site of inoculation as swelling, thickening and redness were noticed and observed at second weeks post infection on infected control group while the lesion in infected treated group was late to last weeks post infection, addition the lesion was more decrease in size, cellular infiltration, swelling and redness. Table (1)

Parasitology:

The density of *L. major* amastigotes was detected in both infected treated group and infected control group after (2, 4, 6, 8, 10, 12) weeks post infection Table (1).

Table (1): Ulcer’s diameter in infected treated and control groups after treated with plant extract

<table>
<thead>
<tr>
<th>Weeks post infection</th>
<th>Ulcer’s diameter (mm)</th>
<th>Infected treated group</th>
<th>Infected control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>0.5 x 0.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1 x 1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>1.5 x 2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.5 x 0.5</td>
<td>2.5 x 4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.5 x 1</td>
<td>4 x 7</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1 x 1.5</td>
<td>5 x 9</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): The density of *Leishmania major* amastigotes in stained smears in infected treated and control groups after treated with plant extract

<table>
<thead>
<tr>
<th>Weeks post infection</th>
<th>Density of amastigote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected treated group</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>±</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>
1: No amastigote seen after 10 minutes search  
±: Very few amastigotes after 5-10 minutes search  
1: 1-20 amastigotes in some fields (50 fields were scanned)  
2: about 50 amastigotes in most fields (at least 25 fields were scanned)  
3: More than 150 amastigotes in every fields (at least 25 fields were scanned)

**Discussion:**

Due to resistance of *Leishmania* strain against the drug first choice pentavalent antimonials, there is a great need for the development of new, effective and safe drug for the treatment of leishmaniasis. This present study demonstrated that *T. foenum-graecum* seeds possessed a potent antiprotozoal activity. Clinical examination show the infected treated mice with *T. foenum-graecum* seeds has mild improvement and decrease in the ulcer size. In contrast, the infected control mice developed worsening and large boils ulcer. The result of this study was agreement with other study (Jarallah, 2003), who indicated that aqueous and alcoholic extracts of *Nigella sativa* seeds have antileishmanial effects against *L. major* promastigote *in vitro* and amastigote *in vivo*. Signs of ulcer healing and clinical response were more clear in infected treated mice than control, few number of amastigotes were detected by cutaneous smear.

Seeds of *T. foenum-graecum* is used as a demulcent and emollient, usually in combination with other remedies and especially in veterinary medicine (Al-Rawi and Chakravarty, 1988). It was choice the alcoholic extract of *T. foenum-graecum* seeds to effect against boils ulcer of CL on infected mice because the alcoholic extract of *T. foenum-graecum* has high activity than aqueous extract in inhibition of Growth Index (GI)% of *L. major* promastigote, that is related to the natural of the active compounds (volatile oils) and also the solvents used in the extraction (Jarallah, 2005). Oils are arise non-polar compound, not easy to be dissolved in water but it is dissolved in nonpolar organic solvent such as ethanol (Hussein, 1981). The mechanism of action of *T. foenum-graecum* seeds against *Leishmania* parasite in both forms amastigote and promastigote is not known, the antileishmanial effect may be due to inhibition of protein synthesis or DNA synthesis. The activity of the antileishmanial plant extracts has been attributed to compounds belong to diverse chemical groups. Among the most promising chemotypes are the benzylisoquinolines, the β-caroline alkaloids, the iridoid and steroidal glycosides, and the quinines (Iwu, *et al.*, 1994). The leaf extract of *Kalanchoe pinnata* used as oral treatment to decrease the ulceration of CL caused by *Leishmania amazonensis* in BALB/c mice (Da Silva, *et al.*, 1995), in other study, Chen, *et al.* (1994) demonstrated the An oxygenated chalcone, *licochalcone A*, isolated from the roots of Chinese licorice plant have potent antileishmanial activity against *L. major* in mice and *Leishmania donovani* in hamsters. Certainly in this study pretreatment of mice with alcoholic extract of *T. foenum-graecum* seeds, reduced the size of the lesion and the parasite numbers burdens compared with control. It was concluded from this study that *T. foenum-
The seeds of *Trigonella foenum-graecum* possess a potent antileishmanial effect. Further work should be progress to discover the active substance for *T. foenum-graecum* seeds.

**References:**


الخلاصة

تم دراسة تأثير المستخلص الكحولي لبذور نبات الحلبة على الآفات المتشرقة لداء اللبامية الجلدي المتسبب عن Leishmania major نوع BALB/c في الفئران المختبرية نوع Leishmania major للطفيلي. تم عزل سلالة في قرية ريفية تعود إلى أهوار البصرة ثم إعطاء المستخلص الكحولي لهذا النبات عن طريق الحقن داخل الأفه. اظهر المستخلص الكحولي لبذور نبات الحلبة نتائج جيدة حيث قلل من تطور ونمو الفرح الجلدي وأحجامها للفران المعالجة عندما تم مقارنتها مع فنان السيطرة التي كانت ذات زيادة في أحجام الفرح وأكثر سوءاً.