Antileishmanial Effects of Traditional Herbal Extracts Against Cutaneous Leishmaniosis In Vivo

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ABSTRACT

Ilam province, is located in southwest of Iran close to border with Iraq where had been engaged severely in war for more than 8 years continuously. leishmaniasis has become an endemic disease in this area which strikes both native and soldiers. Out of 552 referral positive patients to diagnostic centers, 150 cases of leishmaniasis were selected for this study, group I: 110 patient under treatment with glucantime 110 patient and group II: 40 patients treated with a combination of Euphorbia milli, Aloe vera juice, animal fat and turmeric. The study was carried out with assistance of urban and rural health center. Results indicated a significant clinical improvement and parasite reduction in the patient treated with combination of plant extraction in comparison with those treated with glucantime., The majority of patients were recovered of lesion less than 100 days after appearance of disease, however 7.5% of these patient their wound remain untreated during same period of treatment. The results of those patient who received glucantime revealed 32.7% wound healing was observed within 63-76 days, 61.8% within 90-120 days, however only 5.5% wounds of this patients did not heel during this period. The application of current plant combination represented more efficient therapy leading to tissue softening, pinkish color and lesion healing.

Key word: Cutaneous Leishmaniasis, Amistogotes, Glucantime, Euphorbia milli, Aloe vera.

Introduction

The study area, Ilam province, is located in western and southwestern of Iran in the vicinity of Iraq. The length of common Iran-Iraq borderline is 465 Km. This region had been engaged severely in war for more than 8 years. Lieshmaniasis has become an endemic disease in the area which strikes both natives and military service men. The Leishmaniasis are caused by parasitic protozoa of genus leishmania, by a female sand fly belonging to genus phlebotomies in the old world [12,34,40]. The incidence of leishmaniasis is increasing in the world; between one million and one-half million cases of cutaneous leishmaniasis are reported annually worldwide [32]. In World War II, there was a high incidence of leishmaniasis in troops deployed to the Persian Gulf region [40,7,18]. Almost 150 cases of leishmaniasis have reportedly been diagnosed in US soldiers serving in Iraq in 2003, and more are expected [22]. Preliminary data on 22 cases of cutaneous leishmaniasis contracted by American troops in Afghanistan, Kuwait, and Iraq who treated at Walter Reed Army Medical Center between August 2002 and September 2003 [40]. Leishmaniasis is now an emerging zoonosis in the United States [13,21,33] and US soldiers and peacekeeping corps currently in the middle East are experiencing a large outbreak of leishmaniasis with more than 500 parasitological confirmed cases [30]. Most antileishmanial drugs are expensive, toxic and have unacceptable side effects; dosin in also complicated by the fact that they are given parenterally [26,24], moreover, cases of drug resistance are on the rise [24,10]. This has caused a renewed interest in the study of medical plant or traditional medicine as a source for new antiparasitic leads. Furthermore, understanding the mode of action and scar remain after healing of the wound with traditional medicinal plant of these natural products to specific target sites may be used to design potent, novel, selective, and less toxic antileishmanial analogous of this compound on the structural basis [24]. Plant extracted or plant-derived compounds are likely to provide a valuable source of new medicinal agent [6,14] and the urgent need for potential use in the therapy of leishmaniasis and fungi infections.

Many human cases of leishmaniotic cutaneous ulcers were reported from different rural health centers in all over Ilam province, it make us to undertaken this study to collect more knowledge regarding this disease and effectiveness of Euphorbia millii, Aloe vera plant extracts which combined with animal fat and turmeric to introduce a new local
manufactured homeopathy drug against these chemical drug which are expensive, unavoidable the other aim of this research were to see the acting effect of novel local manufactured homeopathy medicine. The classic chemical treatment are expensive, painful with some drug resistant therefore there is a need to investigate other targets for therapy and control, and compare it to current synthetic glucantime [30].

Traditional Medicine:

Traditional medicine is a comprehensive term used to refer either to systems such as Chinese medicine, Indian ayurveda and Arabic unani medicine or to various forms used in indigenous and traditional communities [5]. Traditional medicine is characterized by the use of plants, animals and mineral substances by archaic communities for therapeutically purposes to treat various diseases [4]. Traditional medicines are widely disseminated and it is estimated that 80% of the world’s population depends on them as a primary health source [5]. In Iran and other countries of the world’s population of rural areas and forests rely on popular and traditional medicine for treatment of many infectious diseases. Some species are included in prescriptions for therapeutic purposes such as the healing of wounds, inflammation due to microbial or parasitic infections, skin lesions, and ulcers [20]. In some cases, the same plant may be used for different purposes [5].

Materials and Methods

Study Area:

Thy nature of this study was because of the biogeographically highly epidemic outbreak of leishmaniasis disease in the study area. Several patients from different localities were referred for treatment in local hospital. The area, Ilam province, gone under study having common border line 465 Km with Iraq and this zone were involved continuously in war for more then 8 years. There are several military comp in the area, population 65% are running their life with traditional husbandry. This people grazes their animal by migrating within the province, with two different tropical and subtropical environment, High mountain Zagros with forest, shepherd along with their family living in black tents (made of goat hair), Lived and a slipped beside their herds, dog living within a less than 50-60 m radius from tent or house, every herdsman kept 4-6 local breed (Kurdish) dog as a shepherd guard dogs. Animal product kept for several hours within the living place tent before supply to market. These environment situation mention above were collectively helping to this diseases be endemic in this province.

Human Sampling:

Totally 552 suspicious patients to cutaneous leishmaniasis (CL) were undergone study (2004-2008) with the help of city and rural health center (No 72) located in all over region of Ilam province, southwest Iran. Data were collected through questionnaires distributed within all covering area a detailed clinical history of each patient was compiled. They were clinically examined, and photographs of lesions were taken, the staffs of these centers were trained to detect cases and send suspected CL infected individual directly to trained physicians in local hospital, biopsy obtained from the lesions, all the data were shown that no one of this patient received previous treatment against leishmaniasis. To confirm the presence of leishmaniasis, all suspicious patients were undergone Smear and culture which explain below. Out of 552 patients, 150 positive persons to leishmaniasis were selected for this study with comparison of treatment with two different chemical (Glucantim) and local (extraction of Euphorbia milii) drugs. Forty patients (19 Female and 21 Male), group 1 were treated with local drug and group 2 (53 Female and 57 Male) were treated with chemical drug.

Euphorbia Milli, Aloe Vera Animal Fat and Turmeric Preparation:

*Euphorbia* belongs to family *Euphorbiaceae* and order *Euphorbiiales* with annual and perennial plants which have cyathium in florescence and laticifer. The total plant milky color extract was was collected directly by pressing stem and leaves. The same procedure for *Aloe vera* extract was applied than 10 ml of both extract were mixed properly with 20 gr of animal fat and 1 gr Turmeric (before animal fat were dissolved in +70° by keeping it over night in oven) cream type medicine were ready and apply directly on wound twice daily at morning and evening. *Aloe vera*, also known as the true or medicinal aloe, is a species of succulent plant in the genus Aloe that is believed to have originated in the Sudan *Aloe vera* grows in arid climates and is widely distributed in Africa, India, and other arid areas. The species is frequently cited as being used in herbal medicine. Many scientific studies of the use of *Aloe vera* have been undertaken, some of them conflicting. Despite these limitations, there is some preliminary evidence that *Aloe vera* extracts may be useful in the treatment of wound and burn healing, minor skin infections, sebaceous cyst, diabetes, and elevated blood lipids in humans. These positive effects are thought to be due to the presence of compounds such as polysaccharides, manna’s, anthraquinones and lectins [3]. *Aloe vera* is now widely used on face tissues, where it is promoted as a moisturized and/or anti-irritant to reduce chafing of the nose of users suffering hay-fever or cold *Aloe*
repeatedly pressing or rubbing the button of a deep lesion. Tissue impression smear are done by incision made at the active border and the ulcer of dermal scrapings with a scalpel obtained from an needle aspiration of a saline-injected lesion. Also, by performed on Giemsa-stained smear preparation, remedy for gastrointestinal discomfort associated has is used as an anti-inflammatory agent and clinical disorders. In Ayurvedic practices, turmeric is used as an anti-inflammatory agent and remedy for gastrointestinal discomfort associated with irritable bowel syndrome and other digestive disorders. Some may use turmeric in skin creams as an antiseptic agent for cuts, burns and bruises.

Cosmetics:

Turmeric paste is traditionally used by Indian women to keep them free of superfluous hair and as an antimicrobial. Turmeric paste, as part of both home remedies and Ayurveda, is also said to improve the skin and is touted as an anti-aging agent. Turmeric figures prominently in the bridal beautification ceremonies of India, Bangladesh, and Pakistan. Staining oneself with turmeric is believed to improve the skin tone and tan. Turmeric is currently used in the formulation of some sunscreens. The government of Thailand is funding a project to extract and isolate tetrahydrocurcuminoids (THC) from turmeric. THCs are colorless compounds that might have antioxidant and skin-lightening properties, and might be used to treat skin inflammations, making these compounds useful in cosmetics formulations.

Glucantime:

There are two common therapies containing antimony (known as pentavalent antimonials), meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam). The patient were under systematic treatment with this drug by specialist doctors in local hospital for at list 45 days and they received daily dose of 20 mg/kg body weight for 2 weeks, through intramuscular injection, patient were followed up and their clinical improvement were recorded.

Smear:

In brief, direct examination for amastigotes done by performed on Giemsa-stained smear preparation, needle aspiration of a saline-injected lesion. Also, dermal scrapings with a scalpel obtained from an incision made at the active border and the ulcer of the lesion. Tissue impression smear are done by repeatedly pressing or rubbing the button of a deep biopsy specimen onto a glass slide. The smears are air-dried then fixed in methyl alcohol for five minutes prior to staining. Amastigotes could be differentiated from other intracellular pathogens by visualizing the nucleus and kinetoplast (a distinctive rod-shaped structure found in the cytoplasm) surrounded by a cell wall. Oil-immersion microscopy was used for proper identification [2].

Culture:

In brief, specimens from a biopsy, smear, or tissue fluid were inoculated in a biphasic medium, such as Novy-Mac-Neal-Nicolle (NNN) in the presence of liver infusion tryptose plus 10% fetal bovine serum [19]. Culture was maintained at room temperature. Promastigotes were appear after several days or one weeks, however the culture were kept at lest up to 4 weeks.

Experimental In Vitro Study:

To conform the results of effectiveness of plant combination extracts on CL, cultivation of parasites in artificial media and than inoculation in to surie balb/c breed, including 18 divided in 3 groups, 6 mice, group: group 1 as control, group 2 as experimental infected with MHOM-75-ER Leishmania major strain collected from Pasteur Institute of Iran. Tehran Iran and group 3 were inoculated with collected promastigotes from the skin biopsy from the patients were experimental performed . collection of suspension in brief: after washing the skin biopsy with phosphate-buffered saline (PBS) containing gentamycin 2000 IU ml \(^{-1}\) and gentle maceration in a tissue grinder 0.1 ml of the suspension was seeded in tubes of blood agar base containing 10% defibrinated rabbit blood with a liquid phase of 1% glucose in PBS. Entire Balb/c mice were inoculated on base of the tail with 0.1 ml of suspension [41].

Results:

Out of the 552 suspicious patients, 150 were selected for CL, the rate of infection in female cases treated with local drug were 12.7% and in the cases of male found to be 14.0%. Among patients treated with glucantime drug 35.3% were female and 38.0% were male. The percentage of accumulation is given in Table 1. The patients used plants juice medicine 40% of them were treated within 63-76 days, 52.5% had been treated within 90-133 days, and 7.5% of these patients remain their wound untreated within this period of time. The result of those infected patient who used glucantime shows that 32.7% of their wound treated within 63-76 days, 61.8% of them within 90-120 days, and 5.5% of this group of patients remain untreated within these days and they
have been advised and supervised under a GP to continue their treatment (Table 2 and Diagram 1). Present of *Amastigote* in infected, mice were microscopically observed of stained material from lesions of both experimental groups of animal. The healing period after applying plants juice combination on wound the average time of healing development were between 68 to 78 days but there were no any sign of hair growing in the wounded place of some animals. The effects of combination plant medicine on the healing of the wounds and scar as it shows in the picture No 1-2-6 and7 in comparison to glucantime was better, the patients were using plant medicine during treatment were more relax and skin of around their wound were soft and pinkish color, they were not had feeling to scratch their wound area but those patient were receive chemical injection they were not relax and feel more like to scratching their wound, skin around their wound were dried and peeling.

**Table 1**: Comparison of the two tested groups according to sex, number, percent, accumulation percent and the type of drug.

<table>
<thead>
<tr>
<th>Title</th>
<th>Frequency</th>
<th>Percent</th>
<th>Accumulation percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants juice combination</td>
<td>♂</td>
<td>19</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>21</td>
<td>14.0</td>
</tr>
<tr>
<td>Glucantim</td>
<td>♂</td>
<td>53</td>
<td>35.3</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>57</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2**: Comparison of the two different tested groups according to treatment days, number, percentage, accumulation percentage and the types of the drug.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Treatment days</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Accumulation percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants juice combination</td>
<td>Untreated</td>
<td>3</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>63-76 days</td>
<td>16</td>
<td>40</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>90-133 days</td>
<td>21</td>
<td>52.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Glucantim</td>
<td>Untreated</td>
<td>6</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>63-76 days</td>
<td>36</td>
<td>32.7</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>90-120 days</td>
<td>68</td>
<td>61.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>110</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram 1**: Comparison of the two tested groups according to treatment days, percent, and the type of drug.

**Table 3**: Comparison of the two different tested group according to number, standard deviation, and standard error of mean.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Frequency</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>40</td>
<td>95.1</td>
<td>43.32809</td>
<td>6.850772</td>
</tr>
<tr>
<td>Group 2</td>
<td>110</td>
<td>102.8909</td>
<td>40.77491</td>
<td>3.887735</td>
</tr>
</tbody>
</table>

**Table 4**: Comparison of the two different groups according to T, degree of freedom, significance level, mean differences, and lower and upper limit of treatment days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>T</th>
<th>DF</th>
<th>Sig. (2-tailed) Significance level</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Group 1</td>
<td>13.88165</td>
<td>39</td>
<td>0.000</td>
<td>95.1</td>
<td>81.24301</td>
</tr>
<tr>
<td>Group 2</td>
<td>26.46551</td>
<td>109</td>
<td>0.000</td>
<td>95.18554</td>
<td>110.5963</td>
</tr>
</tbody>
</table>

X1 is the effect of local drug on the time of treatment.
X2 is the effect of chemical drug Glucantime on the time of treatment.
So Zero hypothesis can be explained as such: mean differences between the two groups is no significant. The obtained table shows that T and significance level (Sig = 0.000) imply that mean difference between the two groups is significant and so the local drug can be used instead of chemical one.
Fig. 1: Cutaneous leishmaniasis before treatment (left hand).

Fig. 2: *Cutaneous leishmaniasis* in the same patient picture No 1 and 2 after treatments with plants combination medicine.

Fig. 3: Amastigote in tissue.

Fig. 4 and 5: Appearance of Cutaneous leishmaniasis before and after treatment with experimental plant medicine in laboratory rats.
**T-Test and Means:**

Zero hypotheses with approach to T are: H0=MX1-MX2

Mean differences is not significant in Zero hypothesis and the difference results from chance. Rejection of Zero hypotheses shows the significant difference between tested variables.

Aim of the aforementioned test is to answer to this question that if Mean differences results from chance or from actual difference between the means of society which the sample has been chosen from. After calculating T and df (degree of freedom) and comparison with Students T-test of Table, we can propose that either mean difference between the two groups is not significant, that is the two groups are similar, or vice versa, the Zero hypothesis rejects and mean differences is significant.

**Discussion:**

In this study compound of plants juice combination has exhibited considerably good antileishmanial activity on cutaneous leishmaniasis in comparison to chemical drug Glucantim. Plant offers a vast source of molecules that present different effects in human homeostasis. These molecules which called natural products have been extensively recognized as an important source of most of the active components in therapeutically effective medicines. One of the reason for this success is, most of the time, natural products are more readily absorbed than synthetic drugs [5,20]. Before the advent of high-throughput screening and post-genomic technologies more than 80% of drug substances were natural products or inspired by a natural compound. This fact illustrates the importance of natural products for human health. The combination of evaluated traditional drug of *E. milli*, *Aloe vera* with animal fat and Turmeric which produced by the soldiers for the first time during Iran-Iraq war had extensive effect on controlling the scull and wound caused by sand fly. This medicine has been called as Sanger drug which means rifle-pit scull and wound. Research of society which the sample has been chosen from. This question that if Mean differences results from chance or from actual difference between the means of society which the sample has been chosen from. After calculating T and df (degree of freedom) and comparison with Students T-test of Table, we can propose that either mean difference between the two groups is not significant, that is the two groups are similar, or vice versa, the Zero hypothesis rejects and mean differences is significant.

**References**


