Effect of Ethanolic Extract of *Mespilus germanica* on Cutaneous Leishmaniasis in BALB/c Mice

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Abstract

Background: Leishmaniasis is a vector-borne disease caused by flagellated protozoan parasites of the genus *Leishmania*, which affects both humans and other mammals. Most of the available drugs against the disease are toxic and some are parasite resistance to them.

Objective: The purpose of this study was to examine the effect of *Mespilus germanica* extract on cutaneous leishmaniasis (CL) in BALB/c Mice.

Methods: Ethanolic extract of *Mespilus germanica* with 40, 60 and 80% concentrations were prepared. Then, the BALB/c mice were inoculated subcutaneously by 0.1 ml liquid phase culture containing promastigotes of *Leishmania major*. Ethanolic extract of the leaves of *Mespilus* germanica in different concentrations, were used topically on CL lesions.

Results: The mean diameter of the lesions were decreased, and also the number of parasites in the lesions had declined with complete healing by ending the period time of treatment in 4 mice (26.7%), ($p< 0.05$) and in 9 animals (82%), ($p<0.05$) respectively, by using the 40% concentration of the extract. Also in a concentration of 60%, mean ulcer diameter decreased, with complete healing in 3 mice (20%), ($p<0.001$). In this concentration, the mean number of parasites in lesions had declined (66.4%), with total elimination in 8 animals ($p< 0.001$).

Conclusion: We showed that the extract of *Mespilus germanica* has the highest effectiveness in concentration of 40%, causing greater reductions in both ulcer diameter and the number of parasites in the lesions compared with other prepared concentrations. Therefore, we suggest the use of 40% extract for the treatment of human cases.

Keywords: Cutaneous leishmaniasis, *Mespilus germanica*, Extract, BALB/c mice

Introduction

Leishmaniasis is a group of infectious diseases caused by organisms of the genus *Leishmania* and is a significant cause of morbidity and mortality in several countries. leishmaniasis has been identified as a major public health problem, particularly in Africa, Asia and Latin America. As no available vaccine is exist, therefore the drug indication for treatment is the main approach against leishmaniasis. At present, Leishmaniasis is endemic in 88 countries in the world and 350 million people are considered be at risk. An estimated 14 million people are infected and each year about two million more new cases are occurred [1]. The disease contributes significantly to the propagation of poverty, because treatment is expensive and hence either unaffordable or of a substantial economic burden. The basic treatment for the disease consists of the administration of pentavalent antimonials that were developed more than 50 years ago; however, serious toxic effects and the emergence of resistance are limiting for the drugs' usefulness [2, 3, 4]. Amphotericin B and pentamidine, as traditional alternatives to antimonials used for the treatment of unresponded cases, which more and less cause serious toxic effects [3, 5]. Moreover, antifungal agents such as imidazole and triazole derivatives inhibit ergosterol biosynthesis and are effective against only some species of *Leishmaniasis* [6, 7, 2, 8, 9]. The lack of an effective antileishmania drug has caused a renewed interest in the study of natural compounds as sources of new chemotherapeutic compounds with better activities and fewer side effects. Many people who live in areas where leishmaniasis is endemic rely on traditional medication for treatment. In most cases, the therapy consists of oral administration of plant extracts for the systemic forms of the disease and of topical preparations for the cutaneous forms of infection [10]. Although the idea that herbal drugs are totally safe and free from side effects is erroneous, adverse effects of phytotherapeutic agents are less common compared with synthetic drugs. Over the last 15 years, interest in herbal medicines has increased worldwide in both developed and developing countries [11]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [12]. Natural extract of different plants is directly used on skin lesions as well as on the parasite in NNN medium. Nakanishi et al (2005) reported the efficacy of lucidine a-beta prime and rocide (lup) found in the roots of *Rubia tinctorum* on the immune system [13]. Fata et al (2006) reported alcoholic extract of both stems, leaves, and roots of *Berbris vulgaris* have therapeutic effect on experimental CL ulcers of BALB/c mice [14]. Yousefi et al (2009) showed inhibition of intracellular and extracellular growth of *L. major* and suggested that, some plant extracts which are efficient and safe can be applied as new treatment agents for cutaneous leishmaniasis [15]. Shariatifar et al (2004) showed the inhibition growth of promastigotes by fruit extracts of *Cassia Fistula* in NNN medium [16]. However there are some proofs for cutaneous application of *Mespilus germanica* extracts in Iranian traditional medicine as well as folk medicine in some central-east parts of Iran such as Kerman and Esfahan regions since long time ago. Due to the national historic background of the applications, the main purpose of this study was set to evaluate the effect of *Mespilus germanica* extracts on cutaneous leishmaniasis (CL) in BALB/c Mice.

Materials and Methods

The leaves of *Mespilus germanica* (collected from Mahmood abad city in north of Iran and south of Caspian Sea) were prepared, washed, shade-dried and powdered. Then were placed in a glass percolator with ethanol 85% (Merck) and were allowed to stand at room temperature for about 72 h (in dark place). The percolate was filtered through N.1 Whatman filter paper. The ethanolic extracts were concentrated under vacuum using rotavaporator at 40ºC. Different concentrations (40, 60 and 80%) of the alcoholic extract of leaves were prepared in vaseline base. Sixty BALB/c mice divided into 4 groups: Control group without receiving any extract and three experimental groups receiving 40, 60 and 80% concentration extracts of, *Mespilus germanica*. Each group was inoculated subcutaneously by 0.1 ml liquid phase culture containing at least 2×10^6 promastigotes of *L. major*. After 2-3 weeks, nodules and ulcers appeared. Different concentrations of the ethanolic extract in vaseline base rubbed topically on CL lesions of 3 groups of mice two times a day for two month and vaseline alone was applied on the lesions of control mice. The greater and lesser diameters of each lesion were measured by metric caliber. A direct stained smear by Geimsa was prepared from the clinically healed and non-healed lesions of experiment and control groups.

Results

The effect of concentrated ethanolic extracts of the leaves of *Mespilus germanica* on viability of *L. Major* was studied and demonstrated that significant antimicrobial and anti-fungal activity [17, 18, 19]. The recommended drugs for both visceral and cutaneous leishmaniasis are the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime). Both drugs, used for over 50 years, require long courses of parenteral administration and have some toxic side effects [20]. Treatment of cutaneous leishmaniasis is directed toward the eradication of amastigotes and the reduction of the lesion size to promote healing and achieve maximum efficacy with minimal scarring and toxicity [12]. However, no treatment has yet proved to be completely satisfactory. Pharmaceutical research in natural products as a major strategy for discovering and developing new drugs has its own enough privileges [21, 22]. During period of treatment the results show that the concentration 40% has the maximum effect on cured scars diameters. However concentrations 60% and 80% show less effects as are demonstrated in Fig. 1 The results also show that the concentration 40% of methanolic herbal extract has the maximum effect on reduction of numbers of amastigotes and hence therapeutic effects reduce with increments of extract concentrations to 60% and 80% respectively as shown in Fig. 2.

Discussion

Due to obtained results in *Mespilus germanica* application for *leshmania* treatment it was concluded that the efficacy of the extract altered with increase of its concentration as it shown in Fig. 1. However the application of concentrations more than 80% had no efficacy in decreasing the population growth of parasites and even in lesion diameters too. This may be due to unwanted adverse of drug in higher concentrations than >80%. In this work the mean of lesion diameters in mice were remarkable reduced in concentrations 40% and 60% of the applied extraction compare with

control group. However this reduction in elision diameter was not accompanied so, for 80% with better efficacy than for 60% as it is seen in figure1. Hence it concluded that the mean size of the lesions in the mice that received 80% concentration of *Mespilus germanica* extracts had no statistically significant difference with control group (p>0.05) (Fig. 1). Therefore, there was a significant difference in size of lesions between the groups receiving 40%, 60% and 80% respectively. The mean number of parasites also in lesions in mice between those groups receiving 40% and with 60% and 80% was significantly different from each other ,where no significant difference was observed between two groups of 60% and 80% in mean number of parasites (p > 0.05) Fig. 2. Also statistically significant differences was observed in lesion diameter and mean number of parasites in total mice received any concentrations of *Mespilus germanica* extracts with those in control group (p>0.05), where in the end of treatment, the lesions and mean number of parasites in control group were much higher than those cured with the extract.

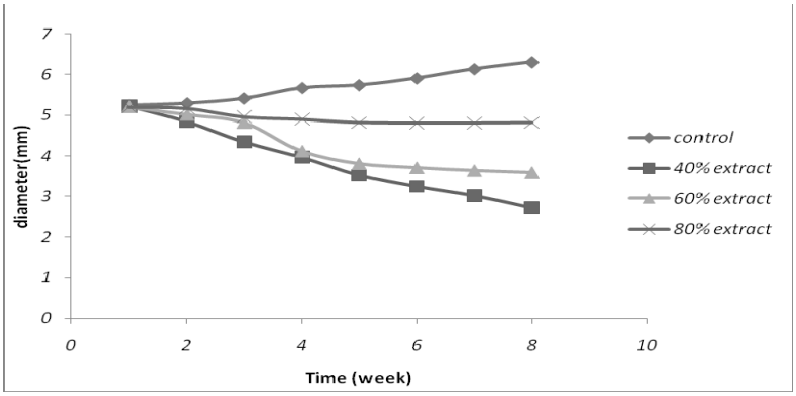

Figure 1- Ulcer diameter of cutaneous leishmaniasis in mice treated with ethanolic extract of *Mespilus germanica* during 8 weeks

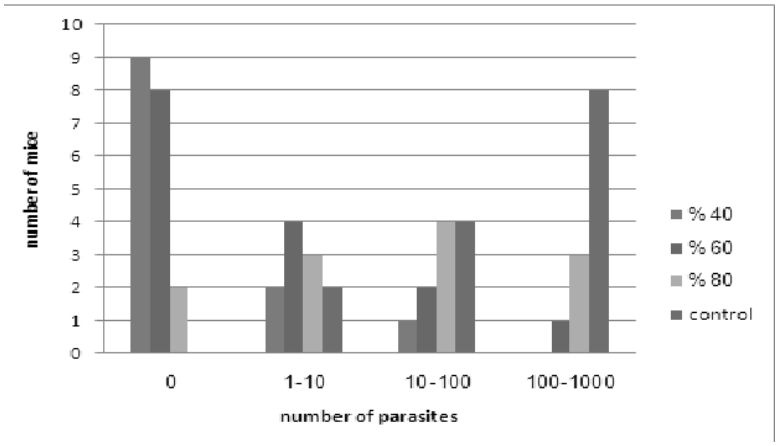

Figure 2- Frequency distribution of parasite number in ulcer after eight-week treatment with ethanolic extract of *Mespilus germanica*

Conclusion

In overall, the use of *Mespilus germanica* slowed down the lesion size increase in mice BALB/c and our results revealed a novel pharmacological activity against L. major and suggest that these ethanolic extracts have the potential of topical application in wound healing. The results presented here provide motivation for further exploration of antileishmania agents. Purification of extract to right components and determination of structural elucidation of useful components will lead to define the proper pharmacological effect of the extract on *leishmania* lesions treatment. This work is in progress with the aim of discovering a new chemical agent as an antileishmania.

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