Avicenna Journal of Phytomedicine (AJP)

Original Research Article

Comparison of topical *Ferula assa-foetida* oleo gum resin and glucantime for treating cutaneous leishmaniasis in BALB/c mice

Tayebeh Ghazaleh[1], Fariba Khoshzaban[2], Fatemeh Emadi[3,*], Fatemeh Ghaffarifar[4], Mohammad Kamalinejad[5], Maryam Iranzadasl[1], Elham Emaratkar[1]

[1] *Department of Traditional Medicine, School of Persian Medicine, Shahed University, Tehran, Iran*
[2] *Department of Parasitology, School of Medicine, Shahed University, Tehran, Iran*
[3] *Traditional Medicine Clinical Trial Research Center, Shahed University, Tehran, Iran*
[4] *Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
[5] *Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Article history:
Received: May 22, 2026
Received in revised form: Jul 29, 2026
Accepted: Jul 29, 2026
Epub ahead of print

**** Corresponding Author:***
Tel: +98-9122397934
Fax: +98-66464322
f.emadi@shahed.ac.ir

Keywords:
Ferula assa-foetida
Asafoetida
Cutaneous leishmaniasis
Lesion
Persian medicine

Abstract

Objective: To evaluate the antileishmanial activity of asafoetida topical ointment (*Ferula assa-foetida* oleo-gum-resin) against *Leishmania major***.**

Materials and Methods: Aqueous extract from asafoetida was achieved and two concentrations of 20 and 40% in eucerin base were prepared. BALB/c mice were divided into two set of animals with lesions ≤5 mm or lesions >5 mm. In one set four groups of asafoetida extract ointment (AEO) 40% (twice a day), eucerin, glucantime (20 mg/kg, sub cutaneous daily) and control were included for 8 weeks. In the other set, AEO 20% was used instead.

Results: In mice with lesions > 5 mm, a significant reduction in the lesions size was observed in the glucantime group compared to others ($p<0.05$). In terms of parasite load, a significant decrease was found between the AEO 40% and glucantime group compared to the eucerin and control groups ($p<0.001$). Parasite count in the spleen tissue revealed a significant difference in AEO 40% and glucantime group compared to the other groups ($p=0.002$). In the AEO 40% group, no amastigotes were observed in the spleen of any mice compared to the glucantime group (40% loss of amastigotes). In mice with lesions ≤5 mm, no reduction in the lesions size was observed in the AEO 20% group. However, a higher decrease in parasite load was observed in the lesions of all mice in the AEO 20% group and glucantime group ($p<0.001$). Amastigotes decreases in spleen in the glucantime and AEO 20% group compared to eucerin and control groups were significant ($p<0.002$).

Conclusion: Asafoetida may be recommended as an efficient topical treatment for improving the *L. major* -induced ulcers besides standard treatment.

Please cite this paper as:
Ghazaleh T, Emadi F, Khoshzaban F, Ghaffarifar F, Kamalinejad M, Iranzadasl M, Emaratkar E. Comparison of topical Ferula assa-foetida oleo gum resin with glucantime for treating cutaneous leishmaniasis in BALB/c mice. Avicenna J Phytomed, 2024. Epub ahead of print.

Introduction

Leishmaniasis encompasses a group of parasitic diseases caused by an intracellular protozoan from the genus *Leishmania*, affecting populations worldwide. Among the ten most significant protozoan diseases in tropical and subtropical regions, it is prevalent across the East Africa, the Americas, the Eastern Mediterranean, Europe, and Southeast Asia. The annual incidence of cutaneous leishmaniasis (CL) ranges between 700,000 and one million cases (WHO 2023; Moghaddamfar and Sharifpour 2016; Sundar et al. 2022).

Leishmaniasis manifests in a broad clinical spectrum, from self-healing cutaneous ulcers to life-threatening systemic conditions (WHO 2023; Habif 2021). In Iran, CL is still the most common form of leishmaniasis with over 20,000 cases reported annually, predominantly caused by *Leishmania major*. The estimated incidence is 31 per 100,000 people, with 99.5% of cases occurring in rural areas (Hajjaran et al. 2021; Shirzadi et al. 2016). Due to their high costs and operational challenges, traditional vector and reservoir control methods are ineffective in economically challenged regions. Timely diagnosis and treatment are crucial as untreated or inadequately treated patients serve as reservoirs, promoting disease transmission in areas where anthroponotic leishmaniasis is prevalent (Oryan and Akbari 2016).

Chemotherapy remains the cornerstone of leishmaniasis treatment as no effective vaccine exists. Therapeutic approaches for CL include physical and surgical treatments such as cryotherapy, local heat, curettage, argon laser, and medication (antimony compounds). Antimony compounds including sodium stibogluconate (pentostam) and meglumine antimonate (glucantime) are the pentavalent medication (Chakravarty and Sundar 2019; Hadighi et al. 2006). Glucantime is the first-line therapy for CL and visceral leishmaniasis (Torres-Guerrero et al. 2017). However, it has adverse side effects including gastrointestinal complications, renal failure, electrocardiogram (ECG) alterations, pancreatitis, and hematologic disturbances such as reduced red blood cells (RBCs), platelets, white blood cells (WBC), hemoglobin (Hb) and hematocrit (Hct) and increased mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), and eosinophils (Ayatollahi and Sharifi 2003). Other reported side effects include allergic reactions, headaches, chills, dizziness, nausea, vomiting, muscle pain, myocarditis, nephritis, and hepatitis (Alrajhi 2003). The toxicity and resistance to these synthetic drugs drive the search for fast-acting treatments that minimize scarring and adverse effects (Alvar and Arana 2018; Shirzadi 2013).

Access to treatment can be insufficient in rural villages with a high prevalence of this disease. Treatment-resistant lesions can result in secondary infections, and deforming skin lesions often persist for over a year. Even after standard treatment, residual scarring can cause persistent pain, leading to anxiety and depression (Bautista-Gomez et al. 2022; Erber et al. 2020; Okwor and Uzonna 2016; Torkashvand et al. 2016). The slow healing, recurrence, and treatment failure pose further challenges, along with the need for painful intralesional injections and high treatment costs (WHO 2023; Postigo 2010).

Persian medicine (PM) has garnered attention in recent decades for its complementary and alternative approaches to disease treatment. Researchers have explored natural compounds and plant-derived substances for leishmaniasis treatment due to their affordability, availability and fewer side effects (Naseri et al. 2021; Baghizadeh et al. 2021; Seydi et al. 2023; Shirzadi 2013) but despite prodigious progress in diagnosis and treatment of human diseases, the herbal compounds are commonly used to treat some disorders such as cutaneous leishmaniasis without any experimental

evidence to explain their activity (Kazemi Oskuee et al. 2018)

Ferula assa-foetida L. one of the 60 species of Ferula genos. from the Umbelliferae family, distributed across Central Asia, Europe, and North Africa. The plant's oleo gum resin, asafoetida, is primarily sourced from Afghanistan and Iran (Amalraj and Gopi 2017; Sahebkar and Iranshahi 2010). In PM, asafoetida, known as "*Heltit*", is traditionally used to treat lesions and keloid scars due to its deep tissue penetration (Avicenna 2005). It is noted for its therapeutic effects in gastroenterology, neurology, dermatology, and urology. Pharmacological studies have revealed its antimicrobial, antiviral, antifungal, anthelmintic, antiprotozoal including antileishmanial activity, antioxidant, antidiabetic, antispasmodic, anti-inflammatory, antitumor, antiepileptic, antihypertensive, antinociceptive, and anti-bronchitis properties (Alijaniha et al. 2023; Iranshahy and Iranshahi 2011; Mohammadhosseini et al. 2019; Zargari 1996).

Previous studies have shown asafoetida's *in vitro* antileishmanial activity against *L. major* promastigotes, mediated by inhibiting parasite growth and viability (Bafghi et al. 2014; Barati et al. 2010; Khoshzaban et al. 2012; Salmani et al. 2015). This study aims to evaluate the antileishmanial effects of asafoetida extract ointment (AEO) on CL lesions in BALB/c mice.

Ethics statement

This interventional study was ethically approved by the Ethics Committee of Shahed University (IR.SHAHED.REC.1398.123) on February 10, 2020, in adherence to the "Ethical Guidelines for Animal Research." (Ahmadi-Noorbakhsh et al. 2021).

Materials and Methods

Materials

Novy-Mac Neal-Nicolle (NNN) (Biomedia, Iran)
Roswell Park Memorial Institute (RPMI) 1640 media (Biomedia, Iran)
fetal calf serum (FCS) (Biomedia, Iran).
Asafoetida was sourced from a reputable medicinal plant vendor.
Glucantime (Sanofi-Aventis, France) was commercially sourced.

Cultivation of *Leishmania major*

A standard strain of *L. major* (MRHO/IR/75/ER) was obtained from Pasteur Institute of Iran, Tehran. The parasite was maintained in BALB/c laboratory mice, serving as a reservoir, in the animal house of Faculty of Medicine, Tarbiat Modares University, Tehran, Iran. The parasite was cultured in Novy-Mac Neal-Nicolle (NNN) media supplemented with streptomycin, penicillin, and 20% heat-inactivated fetal calf serum (FCS) at 25±1°C. Promastigotes in the third passage in NNN medium were gradually adapted to Roswell Park Memorial Institute (RPMI) 1640 media, and FCS. Ultimately, the parasites were cultured in these biphasic (NNN) and monophasic (RPMI+FCS) media to induce lesions in the experimental mice.

Animals and preparation for treatment

Forty male BALB/c mice (8 weeks old, weighing approximately 20-25 g) were utilized as an *in vivo* model to evaluate the efficacy of AEO on leishmaniasis lesions. The mice were procured from Royan Embryo Cell Technology Company, Tehran, Iran. BALB/c mice were housed in standard laboratory conditions in a pathogen-free facility (21±1°C, 60±5% humidity, and 12-hour light/dark cycles) with free access to food and water. The mice were given a week to acclimate to their new environment.

Formulation preparation

Asafoetida was sourced from a reputable medicinal plant vendor and authenticated at the Herbarium of Faculty

of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (SBMU-8250). The plant's scientific name was verified using "The Plant List" (www.theplantlist.org) and "The World Flora Online" (www.worldfloraonline.org) databases. To prepare the asafoetida aqueous extract, 103.6 grams of asafoetida was stirred in 150 ml of water at 60°C. After 30 minutes, the mixture was cooled and filtered. After concentrating the achieved extract, the extract was dried and the extraction output was calculated (84%).

The essential oil of asafoetida was obtained using hydro-distillation method and analyzed using gas chromatography coupled with a mass spectrometer (GC/MS) according to our previous study (Alijaniha et al. 2023). The results demonstrated that cis and trans propenyl sec-butyl disulfide (23.3 and 43.9 %) are the most abundant components in the analyzed assafoetida essential oil. For preparing the formulation, asafoetida was mixed with eucerin to make the 20% and 40% AEO.

Glucantime (Sanofi-Aventis, France) was commercially sourced. Stock solutions of glucantime were prepared in distilled water (20 mg/kg/ bodyweight of Glu) (Molaie et al. 2019).

Infecting BALB/c mice

All of BALB/c mice was infected with *L. major* by subcutaneous inoculation at the tail base with 0.1 ml of promastigotes (2×10^6 cells/ml) in the stationary phase in the RPMI medium, using insulin syringes.

Treatment of the infected mice

Five weeks after inoculation, *Leishmania* lesions appeared and they were confirmed by microscopic examination of amastigotes. The mice were randomly categorized into two sets based on lesion size: a) lesions <5 mm after 45 days and b) lesions ≥5 mm after 53 days. Each set included four groups: AEO (20% or 40%), eucerin, glucantime (positive control), and control. Each group consisted of 5 mice.

The AEO groups (20% and 40%) and the eucerin group received topical treatment twice a day, while the glucantime group received intralesional injections of 20 mg/kg daily. Treatment was administered for 8 weeks. The control group remained untreated. Mice were monitored throughout the treatment for lesion progression, healing of the lesion and scar, mortality, and parasite presence in the lesions, spleen, and liver. Data was recorded and analyzed at the end of the 8-week treatment period.

Evaluation of lesion size and parasite load

At the beginning of the treatment, lesion healing was assessed based on reductions in lesion size and parasite count. A sterile steel instrument was used to scratch the lesion without drawing blood to quantify the parasites. Smears were prepared, fixed, stained, and examined under a light microscope to count parasites

Lesion size was measured weekly from baseline to week 8, using a scaled ruler in millimeters (interval between visits was 7±2 days). At the end of the treatment period, lesion samples were examined microscopically for parasite load again. Lesions were cleaned with ethanol and scraped, then smears were fixed with methanol, and stained with Giemsa. Parasite loads were graded under light microscopy using the following scale (Ezatpour et al. 2015): 0 parasites/10 fields (negative), 1 parasite/10 fields (1+), 1–10 parasites/10 fields (2+), 11–100 parasites/10 fields (3+), 101–1000 parasites/10 fields (4+), and >1000 parasites/10 fields (5+).

Evaluation of the liver and spleen

After the 8 weeks, the mice were euthanized using a high dose of ether, and their liver and spleen samples were collected to investigate the parasite's ability to spread and involve other organs. The organs were removed and examined for the presence of parasites. A small tissue section was taken from each organ, and compression slides were prepared using glass slides. Slides were dried, fixed with

methanol, and Giemsa-stained for examination under a 100x microscope. Parasites in the organs were evaluated by counting the average number of parasites per 100 tissue cell nuclei. The presence of amastigotes was considered positive.

Statistical analysis

Data analysis was performed using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as mean±SEM. Multiple pairwise comparisons among the experimental groups were made using post-hoc Dunn's test following the Kruskal-Wallis test. A p-value of <0.05 was considered statistically significant.

Results

The set with lesions > 5 mm

In mice with lesions larger than 5 mm, a significant reduction in the lesion size was observed in the glucantime group compared to the AEO, eucerin, and control groups between the 3rd and 5th weeks ($p<0.05$). In the AEO 40% group, two mice survived until the end of the 8th week, while all the mice in the other groups succumbed from the 1st to the 8th week. Additionally, the surviving mice in this group were more active than those in other groups. However, none of the groups achieved complete healing by the end of the treatment period (Figure 1).

All groups had 2, 3, and 4+ parasite loads in the pre-treatment phase. Post-treatment, a statistically significant reduction in parasite load was observed in the glucantime and AEO 40% groups compared to the eucerin and control groups ($p<0.001$) (Figure 2).

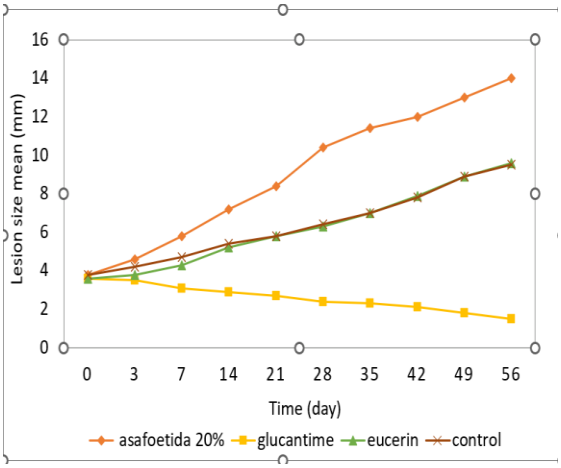

Figure 1. Comparison of lesion size in experimental groups asafoetida, glucantime, eucerin, and control in the set of mice with lesions>5 mm within 8 weeks.

Parasite load in the liver showed no significant difference among the groups ($p=0.368$) (Figures 3 and 4). However, spleen tissues showed a statistically significant difference in parasite load between the AEO 40% and glucantime groups compared to the others ($p=0.002$) (Figures 5 and 6). No amastigotes were found in any spleen tissue of the AEO 40% group (100% of mice group=5 mice) compared to the glucantime group; in glucantime group only 2 mice haven't any amastigote (40% of mice group=2 mice), (Figures 5 and 6).

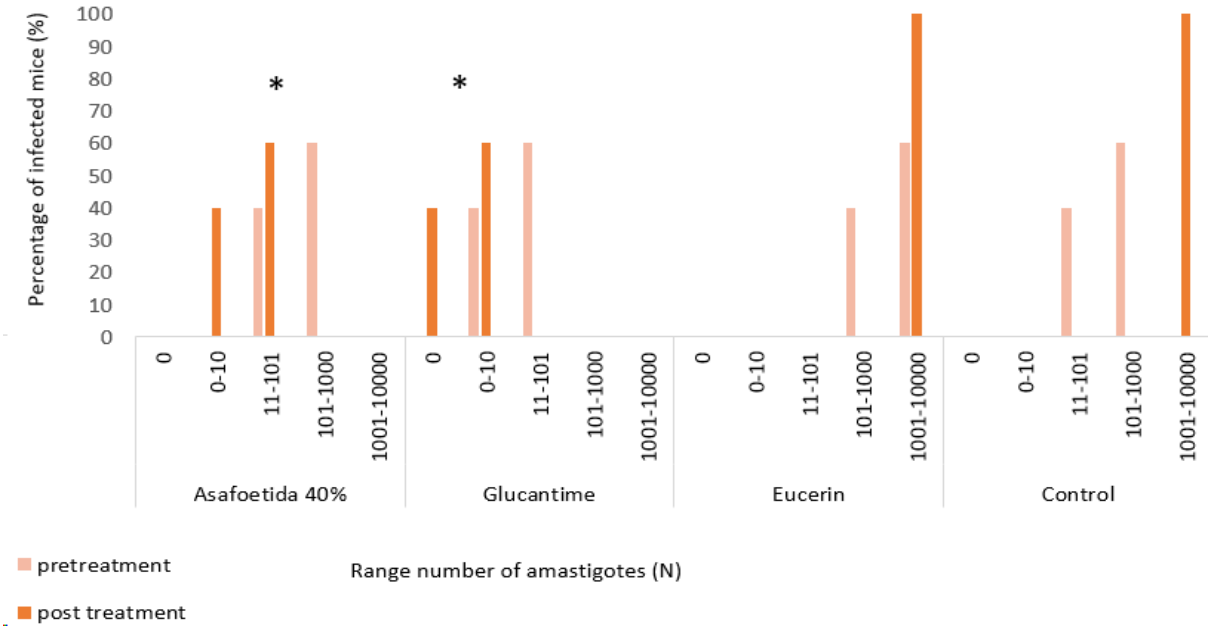

Figure 2. The count of amastigotes in lesion and percentage of infected mice in experimental groups: Asafoetida, Glucantime, Eucerin, Control in the set of mice with lesions > 5 mm within 8 weeks (*p<0.001: statistically significant in the glucantime and asafoetida groups compared with eucerin and control. Percentage of infected mice (%): The number of mice per percentage that has one level of count of parasite (amastigote) 0 amastigote, 1- 10 amastigote, 11-100 amastigote, 101-1000 amastigote or 1001-10000 amastigote. Range number of amastigotes (N): The number of amastigotes in each vision square with microscop. Pretreatment: Parasitic load count before treatment. Postreatment: Parasitic load count after treatment

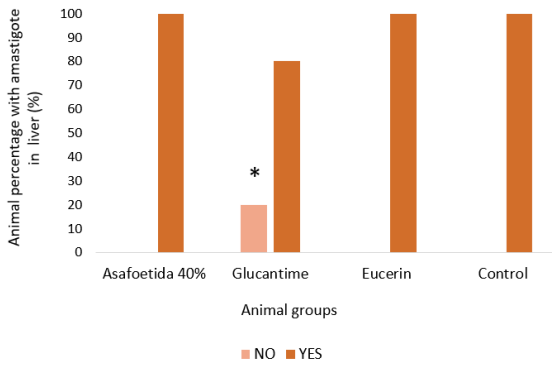

Figure 3. The percentage of infected liver of animals with amastigote in each of the experimental groups: Asafoetida 40%, Glucantime, Eucerin, and Control in the set of mice with lesions > 5 mm within 8 weeks (p=0.365: The glucantime group with other group). Animal percentage with amastigote in liver: The number of mice based on percentage that has amastigote in its liver. Yes: The mouse has amastigotes in its liver. No: The mouse dosn't have any amastigotes in its liver

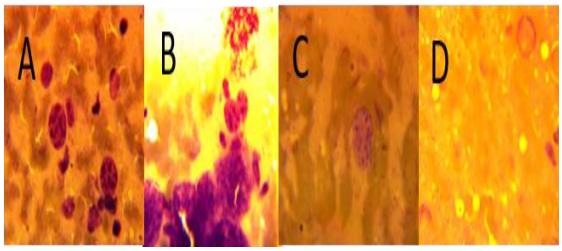

Figure 4. Presence of *Leishmania* parasite in liver cells of mice in the set of mice with lesions ≤ 5 mm: A- Asafoetida 20%, B- Eucerin, C- Control, and D- Glucantime

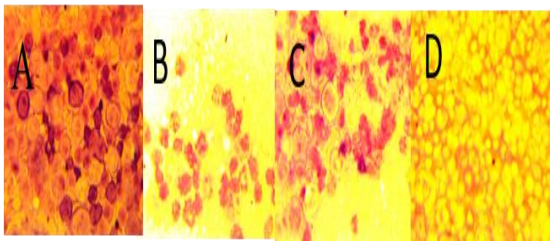

Figure 5. Presence of *Leishmania* parasite in spleen cells of mice in the set of mice with lesions ≤ 5 mm: A- Asafoetida 20%, B- Eucerin, C- Control, and D- Glucantime

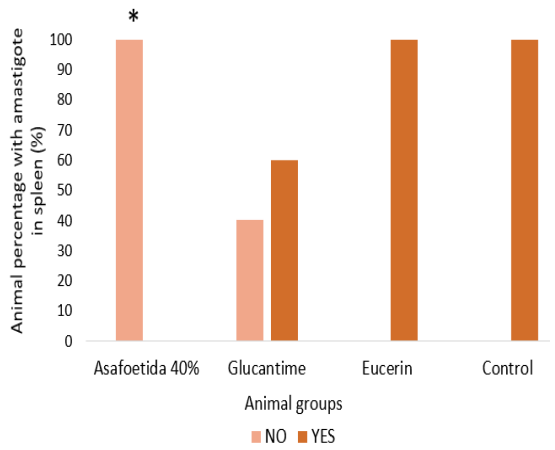

Figure 6. The percentage of infected spleen of animals with amastigote: in each of the experimental groups Asafoetida 40%, Glucantime, Eucerin, and Control in the set of mice with lesions > 5 mm within 8 weeks (*p=0.002). Animal percentage with amastigote in spleen: The number of mice based on percentage that has amastigote in its spleen. Yes: The mouse has amastigotes in its spleen. No: The mouse dosn't have any amastigotes in its spleen

The set with lesions ≤ 5 mm

In the AEO 40% group, topical hair loss, restlessness, and excessive movement were observed, likely due to a burning sensation. Therefore, a lower concentration (20%) was used for lesions ≤ 5 mm.

In mice with lesions ≤ 5 mm, the glucantime group exhibited an apparent reduction in lesion size, whereas no healing was observed in any other groups, and lesion size increased. From the 1st to the 8th week, there was a significant difference in lesion size between the glucantime group and all other groups ($p<0.05$) (Figure 7).

The parasite burden in the glucantime group was zero in 100% of mice. The AEO 20% group showed a reduction in parasite load +1 to +2, while the eucerin and control groups exhibited an increase ($p<0.001$) (Figure 8).

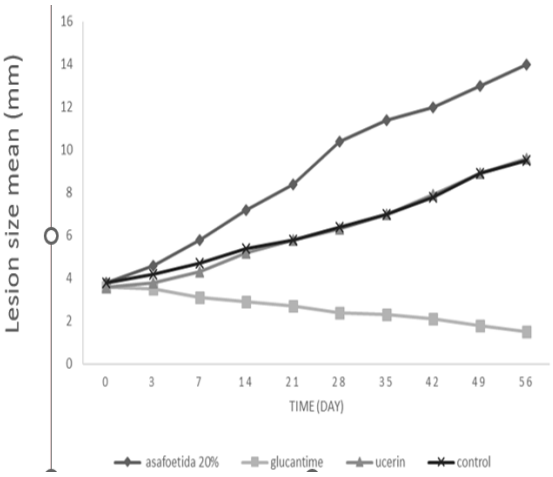

Figure 7. Comparison of lesion size in experimental groups: Asafoetida 20%, glucantime, eucerin, and control in the set of mice with lesions ≤5 mm within 8 weeks.

No amastigotes were observed in the livers of 80% (4 mice) and spleens of 100% (5 mice) of the glucantime group. Amastigotes were found in the liver of all 5 mice in the AEO 20% group, but none was observed in their spleen. The livers and spleens of the eucerin and control groups were full of amastigotes. The difference betweenthe range of the amastigotes in AEO 20% and glucantime groups compared to the eucerin and control groups was statistically significant (liver: p=0.002; spleen: p=0.001) (Figures 4, 5, 9, and 10). Glucantime effectively inhibited the entry of infected macrophages into the liver and spleen, while AEO 20% successfully prevented macrophages entry into the spleen only.

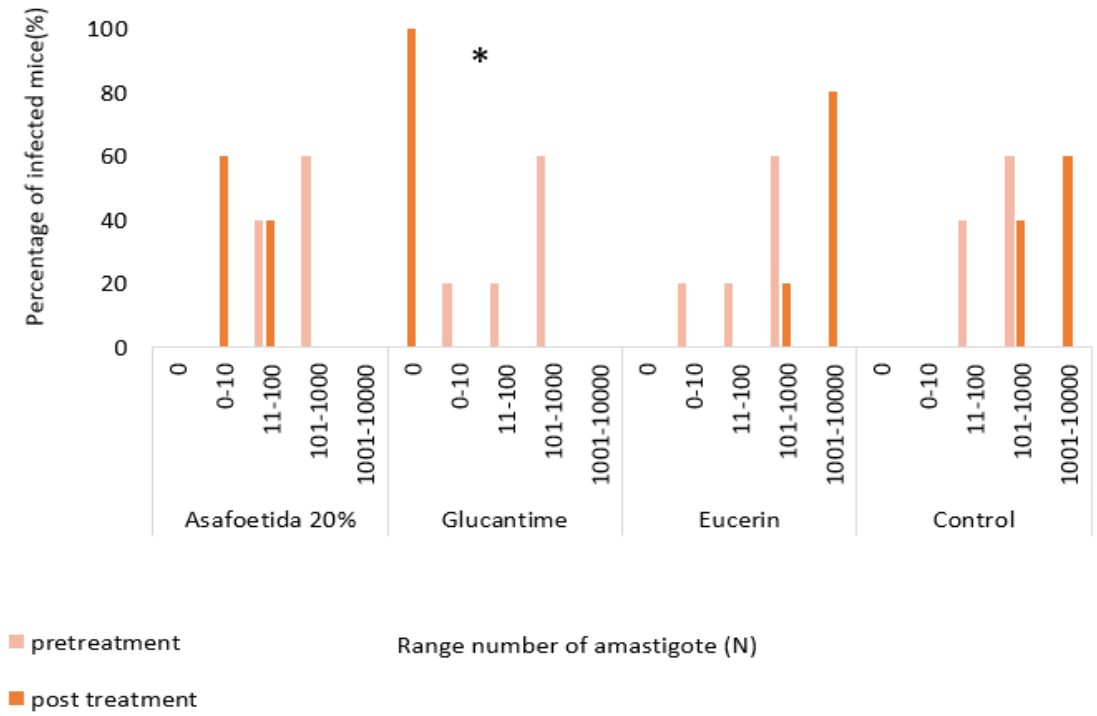

Figure 8. The count of amastigotes in lesions and percentage of infected mice in experimental groups: Asafoetida 20%, Glucantime, Eucerin, and Control in the set of mice with lesions ≤ 5 mm within 8 weeks (*p<0.001: statistically significant in the glucantime group compared with Asafoetida, eucerin and control groups).

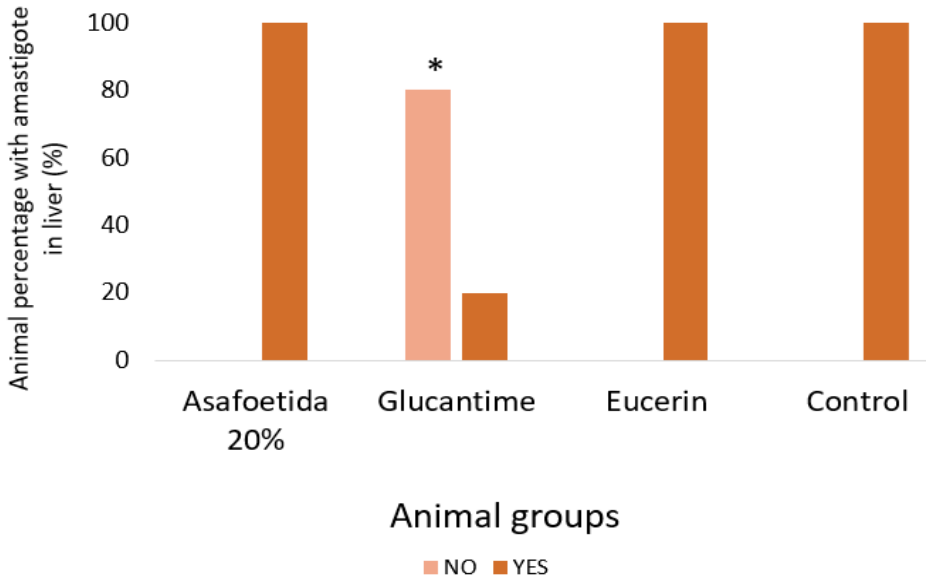

Figure 9. The percentage of infected liver of animals with amastigote in each of the experimental groups: Asafoetida 20%, Glucantime, Eucerin, and Control in the set of mice with lesions ≤ 5 mm within 8 weeks (*p=0.002: statistically significant in the glucantime group compared with Asafoetida, eucerin and control groups).

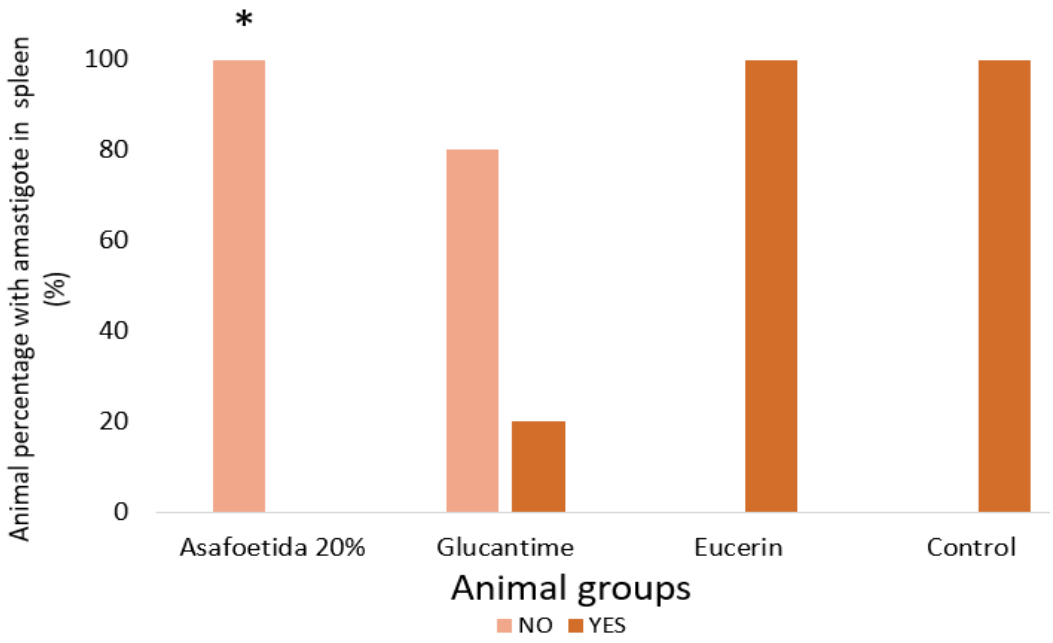

Figure 10. The percentage of infected spleen of animals with amastigote in each of the experimental groups: Asafoetida 20%, Glucantime, Eucerin, and Control in the set of mice with lesions ≤5 mm within 8 weeks (*p= 0.001: statistically significant in the Asafoetida group compared with glucantime, eucerin, and control groups).

Discussion

CL as the most prevalent form of leishmaniasis, is endemic in over 80 countries worldwide and poses a significant health threat in the Middle East, including Iran (Shirzadi et al. 2016). Due to their high costs and logistical challenges, traditional vector and reservoir control methods are often ineffective in low-income regions (Oryan and Akbari 2016). Effective control of the *Leishmania* parasites is dependent on CD4+ T helper cells (Th1) that are recruited in the presence of interleukin-2 (IL-2) and activate the macrophages to kill the amastigote of *Leishmania* by production of interferon gamma (IFN-γ) to start the healing process. Specific parasite and host factors play an important role in CL. *Leishmania* parasites reduce the expression of Th1-type cytokines, including IL-2, IFN-γ, and interleukin 12 so can cause more stability of itself inside the macrophages. If these pro-inflammatory cytokines increase the immune response, they are able to activate macrophages and kill parasites leading to disease improvement; thereby boosting the immune system, especially the cellular immunity and the increase of Th1 immune responses are necessary for the successful treatment of leishmaniasis (Campos-Neto 2005; Zuhair et al. 2003).

Hence methods or drugs that enhance the Th1 immune response in the host can potentially control the disease by eliminating the parasites. Recent studies have evaluated various herbal and natural products as immune system boosters (Azizi et al. 2019). *F. assa-foetida* is one of the few plants with confirmed growth-reducing and strong apoptotic effects against *Leishmania sp.* in the culture medium (Bafghi et al. 2014; Barati et al. 2010; Khoshzaban et al. 2012; Salmani et al. 2015). Its bioactive compounds, including sesquiterpene coumarins like umbeliprenin and galbanic acid, can modulate nuclear factor kappa B (NF-KB), transforming growth factor-β (TGF-β), caspase-3, and tumor necrosis factor alpha (TNF-α) expression to induce apoptosis (Appendino et al. 2006; Iranshahy and Iranshahi 2011; verma et al. 2019) Additionally, the *Ferula sp.* essential oil and some of constituent such . as caryophyllene have shown potent antileishmanial activity against *L. major*

and *L. infantum* (Sonigra and Meena 2021). 7-prenyloxycoumarins isolated from the roots of *F. szowitsiana* have also shown antileishmanial activity against *L. major* promastigotes (Iranshahi et al. 2007).

This study assessed the efficacy of AEO on CL lesions in an experimental BALB/c mice model. The results showed that in the lesions exceeding 5 mm, the AEO 40% group exhibited reduced lesion size by the fourth week, but none of the lesions achieved complete healing, with all mice dying by the eighth week except for two in the AEO group. The lack of lesion reduction and recovery in the asafoetida 40% group may have occurred due to secondary microbial infections. Only the glucantime group showed a reduction in lesion size, though complete recovery was not achieved.

In this study, AEO and glucantime were administered without antibiotics which are commonly used alongside anti-CL drugs to prevent secondary infections (Abdellahi et al. 2020). The observed reduction in lesion size and survival of two mice in the AEO 40% group could be attributed to the limited spread of the parasite to other organs, a key indicator of drug efficacy in BALB/c mice (Takeoka et al. 2001).

Analyzing lesion spread revealed significant differences in parasite load between groups at the end of treatment ($P<0.001$). A reduction in parasite load (+2) was noted in the lesions of all mice in the AEO 40% and glucantime groups, while an increase (+1 to +2) was observed in the eucerin and control groups.

Significant differences were found in spleen tissue among the groups (p=0.002). In the AEO 40% group, no amastigotes were detected in any spleen tissue of the mice (5 mice), while in the glucantime group the spleen of 60% of the mice (3 mice) did not show any amastigotes. However, the spleens of the eucerin and control groups were full of amastigotes. Glucantime and AEO 40% effectively reduced the parasite load, with AEO 40% effectively preventing infected macrophages from infiltrating the spleen.

There were no significant differences among the groups in their ability to inhibit the entry of infected macrophages into the liver (p=0.368), and all groups had amastigotes in their livers.

For mice with lesions ≤5 mm, none of the groups showed lesion reduction or healing, except for the glucantime group which demonstrated a reduction in lesion size from the 1st to 8th week ($p<0.05$) with a significant difference with AEO 20% group ($p<0.05$).

There was a significant difference between the AEO 20% and glucantime groups compared to the control and eucerin groups regarding parasite load in the lesions ($p<0.001$). The glucantime group exhibited zero parasite load in all lesions, while the AEO 20% group showed a reduction to +1 or +2. The eucerin and control groups had a +1 to +2 increase in parasite load.

The liver and spleen showed significant differences in their ability to inhibit infected macrophages. In the AEO 20% group, no amastigotes were observed in the spleens of any of mice (100% of group=5 mice) compared to the glucantime group (80% of group=4 mice), (p=0.002 and p=0.001). Amastigotes were not observed in the livers of 80% (4 mice) of the glucantime group. In contrast, all other groups had amastigotes in their livers.

The potential antileishmanial activity of asafoetida extracts on *L. major* promastigotes was demonstrated *in vitro* by Barati et al. (2010). Asafoetida oleo gum resin exhibited time-dependent suppression of *L. major* growth and viability at concentrations of 2.5, 5, 10, and 20 µg, which aligns with our findings. The effect of Ferula sp. oleo gum resin likely depends on the uptake of their active components, which gradually accumulate within *Leishmania* over time. Additionally, activated enzymes within *Leishmania sp.* may convert the active components into more toxic forms (Bafghi et al. 2014).

In the present study, no reduction in lesion size was observed in the AEO 20% group in mice with lesions <5 mm despite their smaller size. Regarding the parasite load, the AEO 40% group exhibited a more significant reduction than the AEO 20% group, indicating a dose-dependent effect of asafoetida (Barati et al. 2010; Bafghi et al. 2014; Khoshzaban et al. 2012; Salmani et al. 2015). Salmani et al. (2015) also demonstrated that *F. assafoetida* extract could inhibit *L. major* promastigote growth in a concentration and time-dependent manner.

Our findings showed that asafoetida inhibits *L. major* growth in a dose-dependent manner. Asafoetida may be considered a promising herb for improving *L. major*-induced ulcers. Further studies are needed to elucidate the full spectrum of its toxicological effects and mechanisms of action. Additionally, it is recommended to explore the synergistic effects of asafoetida extracts and glucantime in combination with other drugs to evaluate the synergistic effects.

Acknowledgment

This manuscript was derived from a Ph.D. dissertation.

Conflicts of interest

The authors have declared that there is no conflict of interest.

Funding

The authors express gratitude for the financial support of Shahed University.

Ethical Considerations

This interventional study was ethically approved by the Ethics Committee of Shahed University (IR.SHAHED.REC.1398.123) on February 10, 2020.

Code of Ethics

The study was done in adherence to the "Ethical Guidelines for Animal Research." (Ahmadi-Noorbakhsh et al., 2021).

Authors' Contributions

Conception and Design: Fariba Khoshzaban, Fatemeh Emadi, Tayebeh Ghazaleh; Ideation and supervision of drug preparation and use: Fatemeh Emadi, Fariba Khoshzaban, Mohammad Kamalinejad; Cooperation and supervision in animal executive stages: Fatemeh Ghaffarifar, Fariba Khoshzaban; Data acquisition: Tayebeh Ghazaleh; Data analysis: Fatemeh Emadi Data interpretation: Tayebeh Ghazaleh, Fatemeh Emadi Drafting: Tayebeh Ghazaleh, Critical revision: Fatemeh Emadi Final approval: all authors.

References

Abdellahi L, Hejazi SH, Amirmozafari N, Sotoodehnejadnematalahi F (2020) Comparison of three different therapies for cutaneous leishmaniasis and identification of the etiologic isolates in Isfahan, Iran. Arch Iran Med 23: 740-748

Ahmadi-Noorbakhsh S, Mirabzadeh Ardakani E, Sadighi J, Aldavood SJ, Farajli Abbasi M, Farzad-Mohajeri S, Ghasemi A, Sharif-Paghaleh E, Hatami Z, Nikravanfard N (2021) Guideline for the care and use of laboratory animals in Iran. Lab Anim 50: 303-305

Akhzari S, Rezvan H, Nourian A, Zolhavarieh M, Shamsi M (2018) Evaluation of Pro-Inflammatory Genes Expression in the Spleen and Lesions of BALB/c Mice Infected with *Leishmania Major*. J Ilam Uni Med Sci 26: 77-86

Alijaniha F, Emadi F, Naseri M, Bahaeddin Z, Dehparvar N (2023) Some physicochemical and phytochemical characteristics of Iranian *Ferula assa-foetida* L. oleo-gum resin. J Med Plants 22: 89-97

Alrajhi AA (2003) Cutaneous leishmaniasis of the old world. Skin ther lett 8:1-4

Alvar J, Arana B (2018) Leishmaniasis, impact and therapeutic needs. In: Drug discovery for leishmaniasis, PP 3-23

Amalraj A, Gopi S (2017) Biological activities and medicinal properties of Asafoetida: A review. J Tradit Compl Med 7: 347-359

Appendino G, Maxia L, Bascope M, Houghton PJ, Sanchez-Duffhues G, Muñoz E, Sterner

O (2006) A meroterpenoid NF-κB inhibitor and drimane sesquiterpenoids from asafoetida. J Nat Prod 69: 1101-1104

Ayatollahi J, Sharifi MR (2003) Effect of glucantime on globular blood factors in cutaneous leishmaniasis Patients. Journal of yazd shahid sadooghi university of medical sciences and health services 10: 51-5

Azizi M, Yousefi R, Yeganeh F, Hoseini MH M (2019) Study of the immune adjuvant effect of chitin microparticles on plasma levels of IgG1 and IgG2a during immunization against Leishmania infection in BALB/c mice. *Res Med 43:* 64-70

Bafghi AF, Bagheri SM, Hejazian SH (2014) Antileishmanial activity of *Ferula assa-foetida* oleo gum resin against *Leishmania major*: An in vitro study. J Ayurveda Integr Med 5: 223-226

Baghizadeh A, Davati A, Heidarloo AJ, Emadi F, Aliasl J (2021) Efficacy of *Plantago major* seed in management of ulcerative colitis symptoms: A randomized, placebo controlled, clinical trial. Complement Ther Clin Pract 44: 101444

Barati M, Sharifi I, Sharififar F (2010) Antileishmanial activity of Artemisia aucheri, Ferula asa-foetida and Gossypium hirsutum extracts on *Leishmania major* promastigotes in vitro. Ann Mil Health Sci Res 8: 166-172

Bautista-Gomez MM, Doerfler J, del Mar Castro M (2022) Barriers to cutaneous leishmaniasis care faced by indigenous communities of rural areas in Colombia: a qualitative study. BMC Infect Dis 22: 1-11

Campos-Neto A (2005) What about Th1/Th2 in cutaneous leishmaniasis vaccine discovery? Braz Med Biol Res 38: 979-984

Chakravarty J, Sundar S (2019) Current and emerging medications for the treatment of leishmaniasis. Expert Opin Pharmacother 20: 1251-1265

Erber AC, Arana B, Ben Salah A, Bennis I, Boukthir A, Castro Noriega MdM, et al (2020) Patients' preferences of cutaneous leishmaniasis treatment outcomes: Findings from an international qualitative study. PLoS Negl trop dis 14: e0007996

Ezatpour B, Saedi Dezaki E, Mahmoudvand H, Azadpour M, Ezzatkhah F (2015) In vitro and in vivo antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. Evid Based Complement Alternat Med 2015: 1-6

Habif P (2021) Clinical dermatology: A color guide to diagnosis and therapy. Mosby, USA, P 1040

Hadighi R, Mohebali M, Boucher P, Hajjaran H, Khamesipour A, Ouellette M (2006) Unresponsiveness to Glucantime treatment in Iranian cutaneous leishmaniasis due to drug-resistant *Leishmania tropica* parasites. PLoS Med 3: e162

Hajjaran H, Saberi R, Borjian A, Fakhar M, Hosseini SA, Ghodrati S, Mohebali M (2021) The geographical distribution of human cutaneous and visceral leishmania species identified by molecular methods in Iran: a systematic review with meta-analysis. Front Public Health 9: 661674

Ibn-Sina H (2005) Al-Qanun fi al-Tibb (The Canon of Medicine). Darolehya-e-Toras- e-Alarabiye, Beirut

Iranshahi M, Arfa P, Ramezani M, Jaafari MR, Sadeghian H, Bassarello C, Piacente S and C Pizza (2007) Sesquiterpene coumarins from Ferula szowitsiana and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes. Phytochemistry 68: 554-561

Iranshahy M, Iranshahi M (2011) Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. J Ethnopharmacol 134: 1-10

Kazemi Oskuee R, Jaafari MR, Moghaddasi M, Rivandi M, Afzaljavan F, Mohajeri M, Ramezani M (2018) Evaluation of Leishmanicidal effect of Euphorbia petiolata extract by in vivo anti-leishmanial assay using promastigotes of Leishmania major. Avicenna J Phytomed 8(6): 524-532

Kavoosi G, Rowshan V (2013) Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from Ferula assa-foetida oleo-gum-resin: effect of collection time. Food Chem 138: 2180-2187

Khoshzaban F, Naiini A, Saboori M, Vahdanii M, In vitro effects of aqueous *Frula assa-foetida* extacts on *leishmania major* promastigtes, 16- 17 May (2012) Proceedings of the National congress on medicinal plants, Kish Island, Iran

Moghaddamfar M, Sharifpour MA (2016) Cutaneous leishmaniasis. Paramedical Sciences and Military Health 11: 61-67

Mohammadhosseini M, Venditti A, Sarker SD, Nahar L, Akbarzadeh A (2019) The genus Ferula: Ethnobotany, phytochemistry and bioactivities–A review. Indl Crops Prod 129: 350-394

Molaie S, Ghaffarifar F, Hasan ZM, Dalimi A (2019) Enhancement effect of shark cartilage extract on treatment of *Leishmania infantum* with artemisinin and glucantime and evaluation of killing factors and apoptosis in-vitro condition. IJPR 18: 887-902

Naseri V, Chavoshzadeh Z, Mizani A, Daneshfard B, Ghaffari F, Abbas-Mohammadi M, Gachkar L, Kamalinejad M, Jafari Hajati R, Bahaeddin Z, Faghihzadeh S, Naseri M (2021) Effect of topical marshmallow (*Althaea officinalis*) on atopic dermatitis in children: A pilot double-blind active-controlled clinical trial of an in-silico-analyzed phytomedicine. Phytother Res 35: 1389-1398

Okwor I, Uzonna J (2016) Social and economic burden of human leishmaniasis. Am J Trop Med Hyg 94: 489-493

Oryan A, Akbari M (2016) Worldwide risk factors in leishmaniasis. Asian Pac J Trop Med 9: 925-932

Patil S, Shinde S, Kandpile P, Jain A (2015) Evaluation of antimicrobial activity of asafoetida. Int J Pharm Sci Res 6: 722-727

Postigo JAR (2010) Leishmaniasis in the world health organization eastern mediterranean region. Int J antimicrob Agents 36: S62-S65

Sahebkar A, Iranshahi M (2010) Biological activities of essential oils from the genus Ferula (Apiaceae). Asian Biomed (Res Rev News) 4: 835-847

Seydi F, Salehi M, Hashem-Dabaghian F, Emadi F, Gholami-Fesharaki M, Iranzadasl M (2023) Efficacy of topical galbanum oil with dry cupping in hospitalized COVID-19 patients: A randomized open-label clinical trial. Avicenna J Phytomed 13: 388-399

Salmani A, Taghizadeh M, Takhtfiroozeh S, Delavari M, Fallah F, Evaluation of the effect of *Ferula assa–foetida* extract on *Leishmania major* in vitro (2015) Proceedings of the 1st International Congress of physiology and pharmacology, Kashan, Iran

Shirzadi MR (2013) Guide care cutaneous leishmaniasis (seeker) in Iran. Ministry of Health and Medical Education. Department of Health. Center of management of Contagious Disease. Management of human-animal communicable diseases 1391, PP. 21, 23, 67

Shirzadi M, Esfahania S, Mohebalia M, Ershadia M, Gharachorlo F, Razavia M, Postigo J (2016) Epidemiological status of leishmaniasis in the Islamic Republic of Iran 1983-2012. East Mediterr Health J 73: 1-8

Sonigra P, Meena M (2021) Metabolic profile, bioactivities, and variations in the chemical constituents of essential oils of the Ferula genus (Apiaceae). Front Pharmacol 11:608649

Sundar S, Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson J, Loscalzo J (2022) Leishmaniasis. In: Harrison's Principles of Internal Medicine: McGraw-Hill Medical, New York

Takeoka GR, Güntert M, Engel KH (2001) Aroma active compounds in foods (chemistry and sensory properties). A. C. S. symposium series, American Chemical Society

Torkashvand F, Rezaeian M, Sheikh Fathollahi M, Mohammadreza Khaninezhad S, Hatami P, Bidaki R (2016) A survey on psychiatric disorders in patients improved cutaneous leishmaniasis in the city of rafsanjan in 2014. J Rafsanjan Univ Med Sci 14: 879-894

Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R (2017) Leishmaniasis: a review. F1000Research 6: 1-15

Verma S, Khambhala P, Joshi S, Kothari V, Patel T, Seshadri S (2019) Evaluating the role of dithiolane rich fraction of Ferula asafoetida (apiaceae) for its antiproliferative and apoptotic properties: in vitro studies. Exp Oncol 4141: 90–94

WHO.Leishmaniasis[Available from:https://www.who.int/newsroom/fact-sheets/detail/leishmaniasis]

Zargari A (1996) Medicinal Plants. Tehran University Publications, Tehran

Zuhair MH, Yaraee R, Zare N, Ghazanfari T, SarrafNejad AH, Nozar B (2003) Immunomodulatory effect of R10 fraction of garlic extract on natural killer activity. Int Immunopharmacol 3:1483-1489