ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

Antileishmanial activity of *Ferula assa-foetida* oleo gum resin against *Leishmania major*: An *in vitro* study

Ali Fatahi Bafghi, Seyyed Majid Bagheri[1], Seyed Hassan Hejazian[1]

Departments of Parasitoloy and [1]Physiology, Shahid Sadoghi University of Medical Sciences, Yazd, Iran

ABSTRACT

Background: In Ayurveda, asafetida is introduced as a valuable remedy for flatulence, hysteria, nervous disorders, whooping cough, pneumonia and bronchitis in children and also considered as an aphrodisiac agent. Presently, Leishmaniasis is common in most countries of the world and is a serious health problem in the world. Some plant medicines and natural products have a new candidate for treatment of leishmaniasis. **Objective:** This study was designed to evaluate *Ferula assa-foetida* oleo gum resin (asafetida) on mortality and morbidity *Leishmania major in vitro*. **Materials and Methods:** Mostigotes were isolated from mice spleens and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C. A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of $RPMI_{1640}$ media to which different concentrations of 2.5, 5, 10 and 20 µg asafetida were added and each concentration was done in triplicates. Each run also included control. The mortality of parasitoids was measured by the slide and the enzyme-linked immunosorbent assay (ELISA) methods. **Results:** After 72 h, asafetida inhibited growth of parasites in all doses in stationary and logarithmic phases. The ELISA measurement suggested that the viability of parasites significantly decreased after 48h ($P < 0.05$). **Conclusion:** The results show that asafetida could prevent from growth and viability of parasites and this oleo gum resin can be useful for treatment of leishmaniasis.

Key words: Asafetida, antileishmanial effect, *Ferula assa-foetida*, *Leishmania major*

INTRODUCTION

Leishmaniasis is a parasitic disease, in which the sand fly is the common vector of transmission. This disease is regarded as a major problem of the World Health Organization (WHO) and is still considered as one of the most severe affections by this organization.[1] Around 1.0-1.5 million cases of cutaneous form are reported annually with 90% of the cases occurring in eight countries, namely, Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru.[2,3] In the absence of a vaccine, there is an urgent need for effective drugs to replace or supplement those in current use. The clinically used drugs, many of which are based on pentavalent antimony compounds, were developed and amphotericin B and pentamidine also are commonly used.[4] These synthetic drugs have severe toxic and side effects even after modification and duration of treatment. Plant products are frequently considered less toxic than synthetic ones. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.[5] Many natural products including naphthoquinones, lignans, neolignans, alkaloids, chalcones and triterpenoids showed antileishmanial activity.[6] The genus of *Ferula* belongs to the family of Apiaceae that 133 species distributed throughout the Mediterranean area and central Asia such as Iran and Afghanistan.[7] The part used is an oleo gum resin, obtained by incision from the stem and root, and called asafetida. This oleo-gum-resin of *Ferula assa-foetida* L. is considered as an important

Address for correspondence:
Mr. Seyyed Majid Bagheri-Msc, Department of Physiology, Shahid Sadoughi University of Medical Sciences, Professor Hesabi Bulvd. Shohadaye Gomnam Bulvd, Yazd, Iran.
E-mail: boss_bagheri@yahoo.com

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matter of pharmacological and industrial application. In Iranian folk medicine, asafetida have been used for anticonvulsant, antispasmodic, carminative, digestive, expectorant, sedative, antihysteric, laxative, aphrodisiac, antiseptic and analgesic activities.[8] In Iran, China and Nepal, it is traditionally used for infestation with intestinal parasites.[9,10] Asafetida contains about 40-64% resin, 25% endogeneous gum, 10-17% volatile oil and 1.5-10% ash. Its resin fraction consists of ferulic acid esters, free ferulic acid, umbelliferone and coumarin derivatives and its gum fraction are known to be glucose, galactose, L-arabinose, rhamnose and glucuronic acid.[8] Possible anti-leishmanial effect of *Ferula* species has been investigated using *Ferula szowitsiana*. Iranshahi *et al.,* showed that sesquiterpene coumarins were isolated from the roots of *Ferula szowitsiana* that have inhibiting activity against promastigotes of *Leishmania major*.[4] To our knowledge, there is no comprehensive study on the anti-leishmanial activity of asafetida. In the present study, we investigated the anti-leishmanial effect of asafetida on *Leishmania major* in stationary and logarithmic phases.

MATERIALS AND METHODS

Preparation of asafetida

Asafetida was collected from Tabas region (Yazd province, Iran) during the summer and the plant spices was botanically identified by the botanist in Yazd Agricultural Research Center and voucher number of the specimen was 2365. The whole dried *Ferula assa-foetida* oleo gum resin was powdered (10 g) and dissolved in distillated water (100 ml) for overnight at room temperature and the yielded suspension was used. Concentrations and dosages of asafetida were expressed as crude amount of the dried oleo gum resin used in preparing the stock solution.

Animal host

Laboratory bred male mice weighing (20-30g) were used as the experimental host. These were housed in plastic cages in climatically controlled rooms and fed with standard rodent food pellet and water *ad libitum*. Animal experiments were performed as per ethical guidelines laid down by Shahid Sadoughi University of medical sciences animal ethics committee.

Source of parasites

Leishmania (L) *major* strain [MRHO/IR/75/ER] promastigotes were obtained from the medical parasitology department/school of medical sciences/Tarbiat Modares University. *Leishmania major* strain (MRHO/IR/75/ER) was maintained in BALB/c mice. A mostigotes were isolated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN). Subsequently, the third passage promastigotes from NNN medium were progressively adopted to RPMI 1640 media (gibco) supplemented with antibiotics, glutamine and FCS supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C (2).

The slide method

A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of RPMI 1640 media to which different concentration of 2.5, 5, 10 and 20 μg of asafetida were added and each concentration was done in triplicates. Each run also included control. The vials were then incubated at 26°C for promastigotes respectively. On the next four days the culture were counted. A 1:10 dilution in saline together with the appropriate dye was prepared. The dye for promastigotes was 0.4% trypan blue. The promastigotes permeable to the blue dye were dead while viable ones exclude the dye (Jaffe *et al*., 1987). The chamber of a Neubar slide is charged and the numbers of organisms in 16 small corners square are counted. The total number per ml = N (counted) ×10 (number in 1 mm^3 × 103 number 1 ml) ×10 (dilution factor). Trypan blue 0.4% was used for promastigotes. One drop containing the parasites was put on a slide together with a drop of a drug solution and covered by a cover slip. The slides were examined under the microscope and the percentages of stained parasites were noted. Normal saline was used as a control.

The cell proliferation ELISA, nrdu (chemiluminescent) method

The cell proliferation of enzyme-linked immunosorbent assay (ELISA), Nrdu (Chemiluminescent) was performed as described by Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim Germany (Version march 2005, Cat. No. 11 669 915 001) that in brief is:

1. A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of liquid medium to which different concentrations of 2.5, 5, 10 and 20 μg of asafetida were added. Each concentration was done in triplicates and each run included control
2. It was stimulated with acetone in the period
3. Dioxy bromoorydin was added and it was incubated at 37°C for 8 hours
4. Supernatant was removed
5. Fixator was added to the permeable membrane
6. Anti-oxibromoouridin conjugated with POD was added and incubated for 3 hours
7. Chromogen was added and incubated
8. And finally, it was terminated and read at 450 nm.

Statistical analysis

The results were expressed as mean ± SEM. Comparisons among the experimental groups were done by one-way ANOVA test using graph pad prism5 software program. The upper level of significance was chosen as $P < 0.001$.

RESULTS

The percentage inhibition of asafetida on stationary and logarithmic phases of *Leishmania major* is presented in Figures 1 and 2. The results showed that the percentage inhibition is time dependent in two phases. After 72 h, the percentage inhibition was upper 90% in all doses in stationary and logarithmic phases. The result of ELISA measurement is showed in Figures 3 and 4. As shown in these figures, after 48 h, viability of parasites in stationary and logarithmic phases significantly decreased in asafetida 5, 10 and 20 μg/kg treatment compared to control groups.

DISCUSSION

In this study, we investigated the anti-leishmania activity of asafetida on *Leishmania major* parasites *in vitro*. Our result indicated that asafetida could increase mortality of *L. major* and this effect was dependent to time. The time dependent effect of these plant products may be due to the uptake of the active moiety, which progressively increases the amount of active component in leishmania body with increase in exposure period or the possibility that the active component (s) could change into more toxic forms in the leishmania body, by the action of different enzymes. Cytotoxic activity of asafetida was researched in several different studies. Recently, antiviral activity of asafetida was assessed against some human rhinovirus serotypes.[11] Molluscicidal activity of different root extracts of *Ferula assa-foetida* was reported against the snail *Lymnaea acuminata* by Kumar *et al.* They concluded that this effect might be due to the ferulic acid and umbelliferone.[12] There are a few anti-parasitic reports of asafetida, including activity against *Trichomonas vaginalis*[13] and *Schistosoma mansoni*.[14] Antifungal activity of asafetida was studied and researchers found that asafetida possesses moderate antifungal properties against *Aspergillus parasiticus*.[15] Rani *et al.*, also showed that different concentrations of *Ferula assa-foetida* with some unsaturated carbonyl compounds have synergistic fungicidal activity against *Sclerotium rolfsii* and *Macrophomina phaseolina in vitro*.[16] A previous report states about the antibacterial activity of asafetida against *Clostridium perfringens* and *Clostridium sporogenes*.[17] From the above studies, it can be concluded

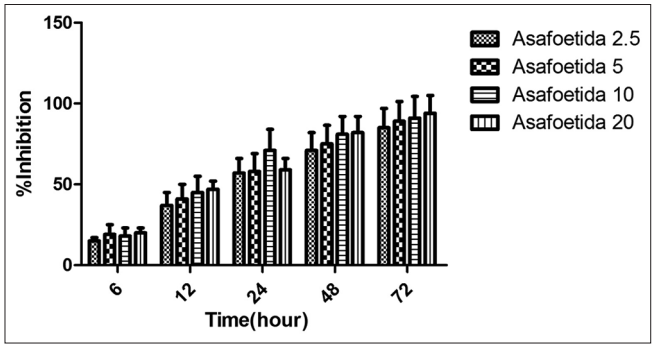

Figure 1: % inhibition asafetida against *Logaritmic* Phase *Leishmania major* in concentrations of 2.5, 5, 10 and 20 μg. Results are compared to the control on 6-72 hours

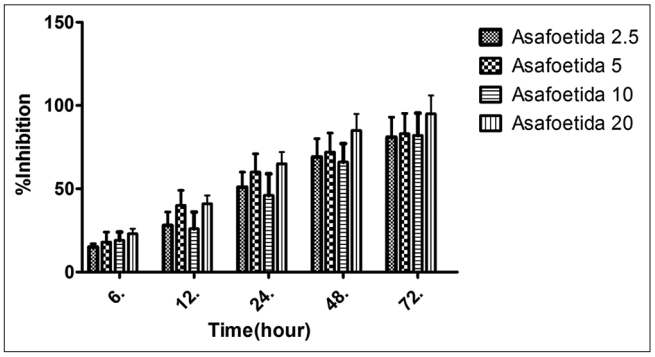

Figure 2: % inhibition asafetida against stationary Phase *Leishmania major* in Concentrations of 2.5, 5, 10 and 20 μg. Results compared to the control on 6-72 hours

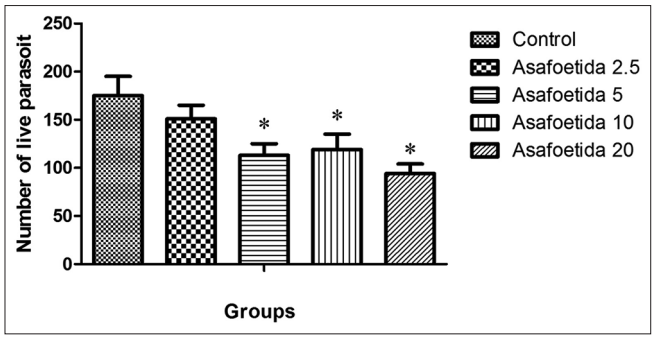

Figure 3: Effect of asafetida on viability *leishmania major* in Stationary of Phase. Each bar represents means ± SD. promastigotes were cultivated in the presence of different concentrations of the 2.5, 5, 10 and 20 μg and counted after 48 h

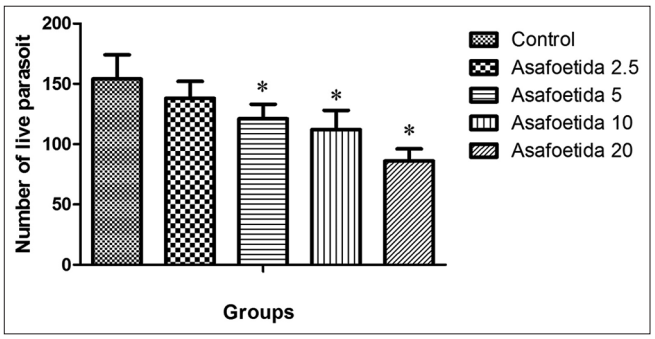

Figure 4: Effect of asafetida on viability *leishmania major* in logarithmic of Phase. Each bar represents means ±SEM. promastigotes were cultivated in the presence of different concentrations of the 2.5, 5, 10 and 20 μg and counted after 48 h

that asafetida, may be used as potent anti-leishmania. In a previous study, possible anti-leishmanial effect of asafetida has been investigated by Barati *et al*[18] This study showed that asafetida methanolic extract can prevent the growth of *Leishmania major* and the IC_{50} was 5.9 μg/ml. Iranshahi *et al.,* also showed that two sesquiterpene coumarins, named szowitsiacoumarin A and szowitsiacoumarin B, isolated from the *Ferula szowitsiana* roots, Umbelliprenin and auraptene have significant activity against promastigotes of *Leishmania major* after 48 h.[4] *Ferula* is a genus rich in coumarins, particularly sesquiterpene coumarins and many sesquiterpene coumarins have been identified from this genus recently. A large number of different sesquiterpene coumarins also have been isolated from asafetida that is similar to *F. szowitsiana* coumarins [8]. It also contains some other compounds belonging to different classes of natural products, such as diterpenes, phenolics and sulfur wherein different investigations confirmed that some of these constituents have anti-leishmania effect[6]

CONCLUSION

This research indicated that asafetida has a power anti-leishmania potent and for proper utilization of this oleo gum resin as antileishmania further studies are, however, necessary to elucidate the mechanism of action in parasite body.

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