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ORIGINAL ARTICLE

Antiviral activity of *Ferula assa-feotida* on HSV-1, 2 *in vitro*

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ABSTRACT

Background and Objectives: Medicinal plants are the primary treatment for many infectious and non-infectious diseases. In this study, we evaluated the antiviral activity of *Ferula assa-feotida* against herpes simplex viruses 1 and 2, and compared it with the antiviral drug acyclovir.
Materials and Methods: In our experimental study, *Ferula assa-feotida* was dissolved in DMSO, then diluted in DMEM medium. Acyclovir was used at a concentration of 100 µM in all procedures. The antiherpetic activity and Antiviral activity of *Ferula* were evaluated in Vero cells (African green monkey kidney cells) by using the plaque reduction assay.
Results: Inhibitory concentrations of 50% (IC_{50}) of *Ferula assa-feotida* for HSV-1 and HSV-2 were determined at 0.00025% and 0.00015%, respectively. *Ferula* was introduced at various stages of viral infection and significantly inhibited HSV-1 and HSV-2 infectivity by > 95.5% and 89%, respectively, when virus was pre-treated before addition to the cells. No HSV-1 or HSV-2 activity was detected in cells treated prior to and following viral infection.
Conclusion: These results indicate that *Ferula assa-feotida* demonstrates antiherpetic activity in the early phase of viral infection and could be used as potential antiviral agent.

Keywords: *Ferula assa-foetida*; Antiviral; Herpes simplex virus type 1; Herpes simplex virus type 2

INTRODUCTION

Herpes simplex viruses (HSVs) are composed of two major antigenic types, HSV-1 and HSV-2. These viruses are important human pathogens which replicate in mucosal surface cells at the site of entry (1). The most important characteristic of these viruses is their ability to establish persistent lifelong infections in their hosts. During the latent phase, viruses may reactivate and cause recurrent infections in some individuals. While others experience limited recurrent HSV-1, infection is very common in adults and primarily affects this population (2). Antiviral agents such as acyclovir, famciclovir and valacyclovir can be used to shorten the duration and severity of clinical symptoms (3, 4). However, these medications may have a variety of side effects. Furthermore, the prevalence of resistance to standard anti-herpetic agents

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is increasing, particularly in immunocompromised patients (5, 6).

Medicinal plants have been extensively used to treat a variety infectious and non-infectious disorders and are an abundant source of new bioactive secondary metabolites. In addition to synthetic therapeutic agents, phytomedicine is still an important basis of advanced drugs for different diseases including infections (7). *Assafoetida* is an oleo-gum-resin obtained from *Ferula* species such as *Ferula foetida, Ferula rubricaulis, Ferula rigidula, Ferula alliacea* by cutting of the roots or removal of the stems and is widely used in traditional medicine (8). *Ferula assafoetida* Linnaeus. grows naturally in central Asia particularly, in Iran and Afghanistan (9). It has antispasmodic, anti-hypotensive, anticancer, antidiabetic properties, antibacterial and antiviral activity (10). Oleo-gum-resin from the plant *F. foetida* commonly known as *assa-foetida* is composed of volatile oil (4% to 20%), resin (40% to 60%), and gum (25%). Furthermore, chemical compounds such as sugars, sesquiterpene coumarins and polysulfides were isolated from *F. foetida* (11, 12).

In Iran, *Ferula assa-foetida* has been used as an aromatic, carminative, antispasmodic, digestive, expectorant, laxative, sedative, nervine, anthelmintic, analgesic, aphrodisiac, and antiseptic agent (13). In the ancient Indian ayurvedic system and other traditional medicines such as America and Brazil, *Assa-foetida* is considered as an aphrodisiac agent (14). New pharmacological studies have demonstrated that *assa-foetida* and its constituents have antiviral, antispasmodic properties (15). Previous studies have evaluated the effects of certain *Ferula* species on reproductive activities. In recent years, most studies on medicinal plants have focused on determining the mechanism of action and clarifying the hidden aspects of their pharmacological properties. Therefore, due to its antioxidant, antiviral, antifungal, anti-diabetic, and anti-muscle contraction activities (16) and considering that this plant is a native plant of Iran, the purpose of this study was to assess the therapeutic effect of *Ferula* on HSV-1, 2 infection *in vitro.*

MATERIALS AND METHODS

Plant and drug preparations. *Ferula assa-foetida*, a plant Oleo gum resin was Collected from Tabas region (Yazd province, Iran) during the summer. The dried powder was dissolved in distilled water overnight at room temperature. The concentration of *F. assa-foetida* used in all experiments was 1 mg/ml. *Ferula* was dissolved in DMSO (Dimethylsulfoxide) from 0.001 to 10% and added to cell culture medium at a maximum final concentration of 1% DMSO without affecting on virus and cells. Acyclovir was manufactured by Amin Pharmaceutical Company (Iran) and was dissolved in distilled water to prepare a stock solution with a concentration of 100 μM. This study has been approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences in Yazd, Iran.

Cells, viruses, and cytotoxicity assay. Vero cells (African green monkey kidney cells) were grown in monolayer culture in Dulbecco´s modified Eagle´s medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin (17). Herpes simplex virus type 1 (HSV-1) strain KOS and HSV-2 strain HG52 were used in all experiments. In cytotoxicity assay. The monolayer cells were seeded in 96-well culture plates for cytotoxicity and propagated at 37ºC in an atmosphere of 5% CO_2. The cytotoxicity of *Ferula* was evaluated against Vero cell using MTS (4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay kit (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, a monolayer of cells was prepared in a 96-well cell culture plates. The cells were then treated with increasing dilution from 10-0.001% of the *Ferula* in triplicate, followed by incubation at 37°C with 5% CO_2 for 48 hours. The MTS solution was then added, and the cells were incubated for 4 h at 37°C with 5% CO_2. The absorbance of each well was measured at a 495 nm wavelength using Infinite 200 Pro multiplate reader (Tecan, Männedorf, Switzerland). The half toxicity concentration (TC_{50}) and the maximum non-toxic dose (MNTD) were determined using dose-response curves.

Plaque inhibition assay. Inhibition of HSV infection was measured with a plaque reduction assay. Usually, 2×10^3 plaque-forming units (pfu) were serially diluted from 0.005-0.00001% of *Ferula assa-feotida* for 1 h at room temperature, then the viruses were allowed to get adsorbed to the cells at 37°C for 1 h. The residual inoculum was discarded and infected cells were overlaid with a medium con-

taining 0.5% methylcellulose. Each concentration was performed in three replicates. After incubation for 3 days at 37ºC, monolayers were fixed with 10% formalin. The cultures were stained with 1% crystal violet and subsequently plaques were counted. Inhibitory concentration (IC_{50}) was expressed as antiviral activity, which inhibited plaque numbers by 50% compared with untreated control and was determined from dose–response curves.

Time of addition studies. Approximately 2×10^3 pfu of HSVs were incubated in a medium containing the maximum non-toxic concentration (0.005%) of the *Ferula* for 1 h at 25ºC before inoculation of Vero cells (pretreatment of viruses). Cells were infected with viruses prior to incubating with the *Ferula* (during replication). The drug was added to the cells before the HSVs were added (Pretreatment of cells with dissolved *Ferula*). Each experiment was performed in three duplicates. Plaque reduction experiments were performed as described above and the count of plaques in cells treated with plant and viruses were evaluated with untreated controls.

Statistical analysis. Half toxicity concentration (TC_{50}) to half maximal inhibitory concentration (IC_{50}) ratio was used to determine the selectivity index (SI). Three independent experiments were conducted, and all experiments were done in triplicate. The data was presented in mean ± SD. $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using SPSS software (SPSS for Windows Inc. Version 22. Chicago, Illinois).

RESULTS

Ferula assa-foetida toxicity on Vero cells was determined using MTT assay. *Ferula* was dissolved in DMSO at a concentration range of 0.001-10% of 1 mg/ml through serial dilution. The cytotoxic concentration of the resin which reduced viable cell number by 50% (TC_{50}) was determined from dose-response curves which was determined at 0.075% (Fig. 1). To determine the appropriate concentration of the HSV-1, 2 which leads to pathological changes in 50% of the cells, plaque reduction assay method was used. In all experiments, the controls consisted of untreated virus-infected cells and treated cells with acyclovir. In antiviral activity tests, *Ferula* showed concentration-dependent antiherpes activity and was able to significantly inhibit viral infection (Fig. 2). The IC_{50} values for HSV-1 and 2 were determined to be 0.00025% and 0.00015% for *F. assa-foetida*, respectively. A selectivity index for the Ferula was calculated as the TC_{50}/IC_{50} ratio (Table 1).

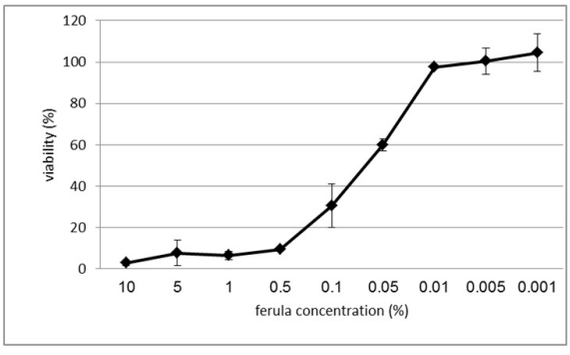

Fig. 1. Determination of TC_{50} of *Ferula assa-feotida* on Vero cells. The cytotoxic concentration that reduced viable cell number by 50% (TC_{50}%) was determined from dose-response curves. Experiments are the mean of three experiments and repeated independently.

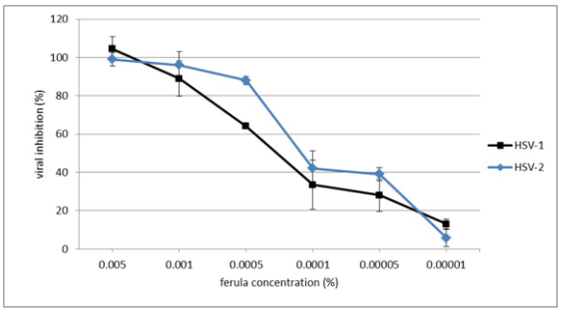

Fig. 2. Determination of the IC_{50} of *Ferula assa-feotida* on HSV-1 and HSV-2. Viruses were incubated for 1 hour at room temperature with different of concentration of *Ferula-asa feotida* and then, were tested for plaque reduction assay.

Table 1. Selectivity index of *Ferula assa-feotida* against HSV-1 and HSV-2

***Ferula* extract**	**max. noncytotoxic (%)**	**TC_{50} (%)**	**IC_{50} (%)**	**Selectivity index TC_{50}/IC_{50}**
HSV-1	0.03	0.075	0.00025	300
HSV-2	0.03	0.075	0.00015	500

Experiments were repeated independently, and data presented were the mean of three experiments.

To evaluate the mode of antiviral activity of *Ferula assa-foetida*, it was added at various phases during viral infection. Acyclivir as a control was used in all antiviral activity methods. When host cells were pretreated with *F. assa-foetida*, the tested showed little effect on the viral infection (Fig. 3a). However, pretreatment of HSV-1, 2 with this resin for 1 hour prior to infection showed a significant decrease in plaque formation by >95.5% and 89% for HSV-1 and HSV-2, respectively (Fig. 3b). When cells were infected with HSV-1 and HSV-2 before adding the Ferula, zero and a few reductions were observed, respectively. On the other hand, Acyclovir demonstrated the most antiviral effect during the replication stage, with a suppression of viral replication of 97% for HSV-1 and 90% for HSV-2 (Fig. 3c).

DISCUSSION

Currently approved antiviral agents for the treatment of herpesvirus infections include acyclovir and derivatives, nucleoside analogues that act as DNA chain terminators, ultimately preventing the elongation of viral DNA (18). The duration of herpes recurrences can be reduced by 10-15% with the most commonly used topical treatments, but not the functional symptoms (19). Usual treatment with acyclovir and penciclovir is inadequate due to some factors, consisting of low efficiency on symptoms and pruritus during healing (20). Several essential oils and plant extracts have shown antiherpetic effects, acting before virus penetration and by a mechanism that differs from the commonly used synthetic antiviral drugs (21-23). Although natural products are promising antiviral agents for topical therapeutic application (24-26), the demand for new antiviral agents with different or combined modes of antiviral action remains.

In this study, we assessed the antiviral activity and mode of antiviral activity of *Ferula assa-foetida* for HSV-1 and 2. The *Ferula assa-feotida* oligo gum resins include five major types of the sesquiterpene coumarins named, badrakemin, conferone, isosamarcandin, feslol and samarcandin (27). Antiherpetic effects have been revealed by numerous monoterpenes and coumarins in the past, and coumarins with sesquiterpene have been evaluated for HSV-1 management. Lee et al. (2009) showed that sesquiterpene coumarin compounds from *Ferula assa-feotida* have

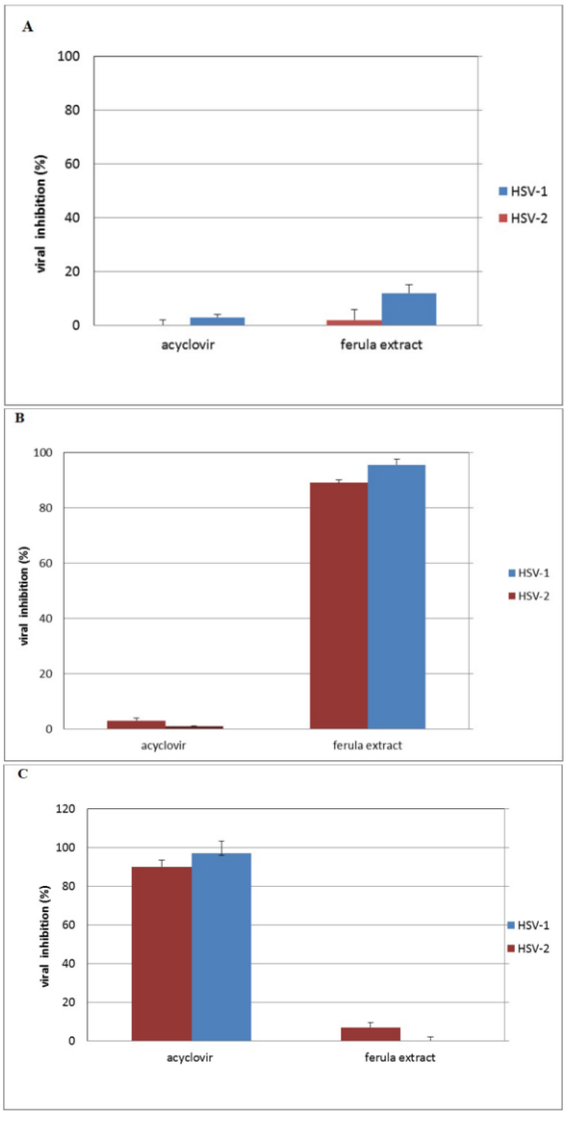

Fig. 3. Mechanism of action of maximum nontoxic concentration of *Ferula assa-feotida* and acyclovir against HSV-1 and HSV-2

(A) Pretreatment of cells with drugs

(B) Pretreatment of virus with drugs

(C) During intracellular replication

antiviral activity against influenza virus A H1N1. These compounds were more powerful against the flu A virus than amantadine (28). Anti-HIV activity of constituents of other species of *Ferula*, *Ferula* Sumbul, was confirmed by Ping et al. (29). Sesquiterpene coumarins from *Ferula* inhibit the cytopathic effect of human rhinoviruses for serotypes 1A, 2, 14, and 16 (30). Different species of *Ferula* coumarins were examined for their potential inhibitory effects on EBV early antigen activation (31). Some studies

cover the antiviral activities of *Ferula* species and their components. Recently, the antiviral activity of sesquiterpene coumarins from *Ferula assa-foetida* was evaluated against HSV-1 (32). Similar to the results of previous studies, this study also revealed that using *Ferula assa-feotida* before infection can prevent the HSVs to infect host cells. In addition, we showed a desired outcome with its low toxicity and anti-herpetic activity under *in vitro* condition and *in vivo* experiments could be the next step in assessing the efficacy in animal models. In summary, *Ferula assa-feotida* is a natural agent and has low toxicity and anti-herpetic activity, it has the potential to be a promising topical therapeutic agent for the treatment of recurring herpetic infection.

CONCLUSION

In conclusion, *Ferula assa-feotida* demonstrated low toxicity and siginificant antiherpetic activity *in vitro*. The mode of antiviral activity is different to the widely used drug acyclovir. *Ferula assa-feotida* affects HSV-1 and HSV-2 at early stages during viral replication and inhibits infection of host cells, whereas acyclovir targets viral DNA synthesis later during viral replication. Considering the lipophilic nature of piroxicam, which enables it to penetratethe skin, it might be suitable for topical treatment of herpetic infections, especially for those patients who experience frequent recurrences.

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