

# Antiviral Effect of Ethanolic Extract of *Salvadora Persica* (Siwak) on Herpes Simplex Virus Infection.

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## ABSTRACT

**Aims:** To investigate the effect of ethanolic extract of *Salvadora Persica* extract on HSV-1 infection both in vitro and in vivo in the mouse model system. **Materials and methods:** Ethanolic extract of *Salvadora Persica* was used at different concentrations. BHK cells that grown in Eagles medium were used for virus isolation and titration using PFU/ml. The effects of different concentrations of *Salvadora Persica* on viral growth in BHK cells as well as cytolytic activity of HSV-1 were evaluated at different time post infection. The therapeutic efficacy of *Salvadora Persica* in vivo was studied in mice. Lesions were scored and viral isolation from infected skin and ganglia was titrated on BHK cells. **Results:** *Salvadora Persica* inhibited the replication of HSV-1 in BHK cells as well as the cytolytic activity of cell free virus. Topical application of *Salvadora Persica* on the skin of mice infected with HSV-1 reduced the development of cutaneous lesions and the viral titers in the skin and ganglia were also reduced. **Conclusion:** The results of this work may be beneficial for the treatment of recurrent oral herpes infections.

**Key Words:** Miswak; Antiviral, HSV-1

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## INTRODUCTION

Herpes simplex virus (HSV), a ubiquitous pathogen of humans, is responsible for a variety of conditions ranging in severity from mild cutaneous lesion to very rare fatal encephalitis<sup>(1)</sup>. In dentistry, HSV is responsible for many primary and recurrent oral diseases, such as primary gingivostomatitis and recurrent herpes labialis<sup>(2,3)</sup>.

*Salvadora Persica* (Miswak – siwak) is a medical plant whose roots, twigs or stems have been used for centuries as oral hygiene tool in many parts of the world<sup>(4)</sup>. It is available as chewing gum, toothpaste and tooth brushes<sup>(5)</sup>. Prophet Mohammed advised Muslims to use Miswak before every praying saying "The Miswak is an implement for cleaning mouth and for pleases Allah"<sup>(6)</sup>. Several studies have shown that extract of *Salvadora Persica* possess many biological properties including antibacterial<sup>(7)</sup>, antifungal<sup>(8)</sup>, anticaries<sup>(7,9)</sup>, anti-inflammatory<sup>(10)</sup>, antiplaque effect<sup>(11)</sup>, and reduces gingivitis and gingival bleeding<sup>(12,13)</sup>. Recently *Salvadora*

*Persica* extract was evaluated as root canal irrigant<sup>(14,15)</sup>.

As no laboratory study documented the effect of *Salvadora Persica* on herpes simplex virus; therefore, the present study was conducted to investigate the effect of ethanolic extract of *Salvadora Persica* extract on HSV infection both in vitro and in vivo in mouse model system.

## MATERIALS AND METHODS

### *Cells and Virus:*

Baby hamster kidney (BHK) cells (Flow Laboratory) were propagated in Eagle's medium containing 10% calf serum. Virus stock (HSV-1) was derived from patient isolate, grown and titrated in BHK cells using plaque assay method<sup>(16)</sup>. The highest titer obtained was  $1 \times 10^6$  plaque forming unit (PFU)/ml, frozen at  $-20\text{ }^{\circ}\text{C}$  until use.

### *Preparation of Salvadora Persica extract:*

*Salvadora Persica* chewing sticks (800 g) obtained from Saudi Arabia was cut into small pieces and grinded until bec-

oming powder. Then 120 ml of 60% ethanol was added to 40 g of powder in ed at 37C° for drying. The dried extract was stored in refrigerator in sterile screw capped vials<sup>(17,18)</sup>.

**Effect of *Salvadora Persica* on viral growth in vitro:**

50 mm petri-dishes containing confluent BHK monolayer cells were infected with HSV-1 at multiplicity of infection (m.o.i.) of 5 pfu. After absorption for one hour at 37 C°, monolayers were washed twice with Eagle's medium containing 5% calf serum and overlaid with Eagle's medium or *Salvadora Persica* extract diluted in Eagle's medium at concentrations (0.1%, 0.5%, 1% and 5%) and incubated at 37 C° for 24 h. Then cells were washed twice with phosphate buffered saline (PBS), harvested and titrated on BHK cells for virus yield<sup>(19)</sup>.

**Effect of *Salvadora Persica* on cytolytic activity of HSV-1 in vitro:**

One ml of HSV-1 containing 1x10<sup>6</sup> pfu/ ml was mixed with equal volume of either Eagle's medium or fresh-ly prepared *Salvadora Persica* at different concentrations (0.1%, 0.5%, 1% and 5%). The mixture was incubated at 37 C° for 10, 20 and 60 min. At the end of incubation, mixtures were centrifuged at 50000 rpm for 1 hr. at 4 C° and viral pallet was resuspended in PBS and titrated for virus yield<sup>(20)</sup>.

**Animal inoculation:**

Three weeks old Albino mice were used. Mice were anaesthetized and the forehead skin was scarified and 50µ (1x10<sup>5</sup> pfu/ml) of virus was applied.

sterile flask and left for 3 days at room temperature and then filtered and incubat-  
**Effect of *Salvadora Persica* on HSV-1 in vivo:**

In order to study the therapeutic efficacy of *Salvadora Persica*, infected mice were divided into 3 groups (10 mice in each group). Group 1: control (no treatment); group 2: topical application of 5% *Salvadora Persica* 2 hr. after viral application and group 3: topical application of *Salvadora Persica* 24 hr. after viral application. *Salvadora Persica* was applied 3 times for 10 days<sup>(20, 21)</sup>. Viral lesions were examined daily and recorded changes giving the score of 1. erythema 2. erythema with edema 3. erythema with one or few vesicles 4. erythema with numerous vesicles 5. numerous large vesicles.

To study the correlation between clinical course of HSV-1 infection and amount of virus in the skin and corresponding ganglia, Infected skin and trigeminal ganglia were excised from three mice in each group on 6 and 8 days post infection. Tissues were homogenized and titrated for virus yield on BHK cells<sup>(21)</sup>.

**Statistical Analysis:**

Data were analysed statistically using T-test and Duncan's Multiple Range Test.

**RESULTS**

The effect of *Salvadora Persica* on viral growth in vitro is shown in Figure (1). It is clear that *Salvadora Persica* inhibited the growth of HSV-1 at all concentrations ( $p < 0.05$ ) and the greater the concentration (5%), the higher inhibition although inhibition of virus growth at 0.1% was not significant.

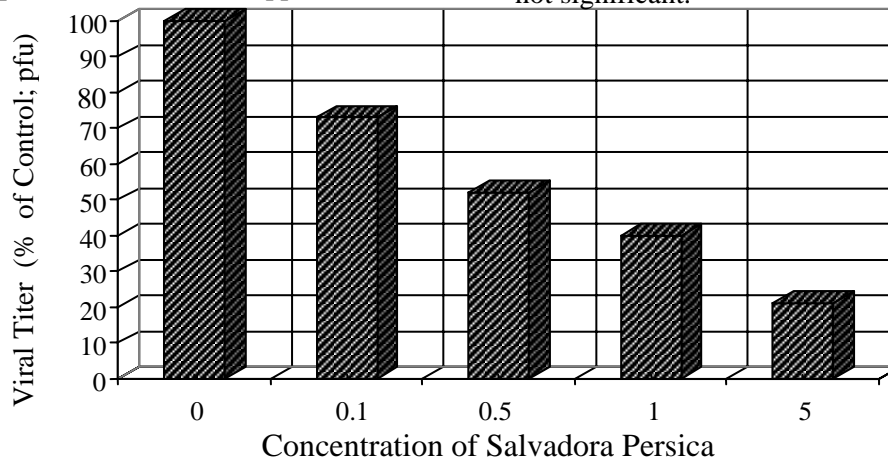
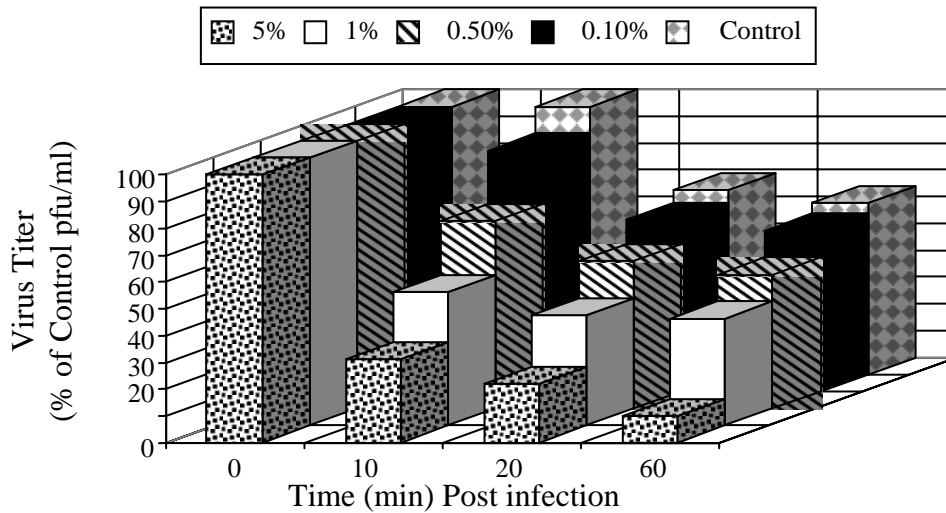


Figure (1): Effect of *Salvadora persica* on viral growth of HSV-1 in BHK cells.

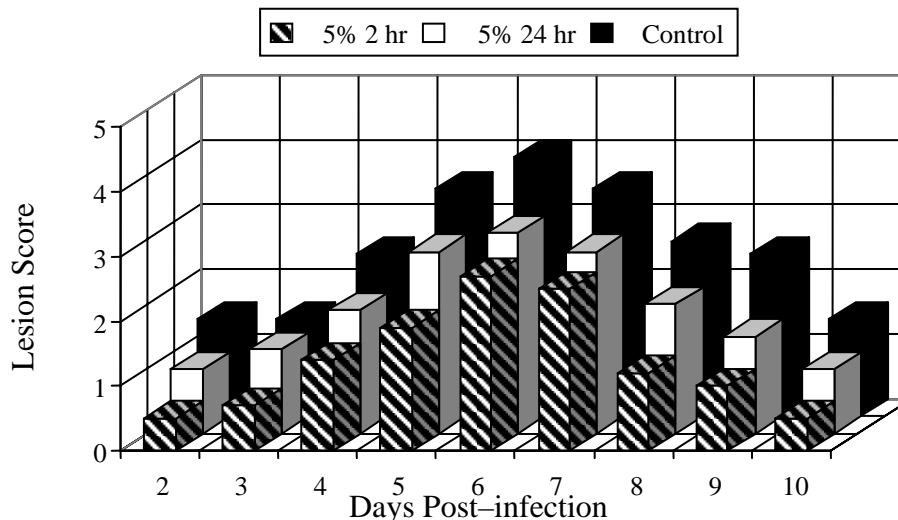
It is obvious that the cytolytic activity of HSV-1 was reduced in the presence of 0.5%, 1% and 5% of *Salvadora Persica* extract when measured after 10 min incubation which is statistically significant ( $p < 0.05$ ). This activity was further prohibited after 60 min, Figure (2). The greater reduction of cytolytic activity (50%) was achieved using 5% *Salvadora Persica* when examined after 60 min ( $p < 0.01$ ).

In vivo effect of *Salvadora Persica* on the development of cutaneous lesions induced by HSV-1 at different times is shown in Figure (3). The application of *Salvadora Persica* at 2 hr. post infection yielded a significant improvement ( $p < 0.01$ ) in the pathological changes (skin lesions) on days (2, 3, 4 and 5). Such effect was not seen when *Salvadora Persica* was applied 24 hr. post infection.



Duncan's Grouping: Control: A; 0.1%: A; 0.5%: B; 1%: B; 5%: C. Means with different letters were statistically significant ( $p < 0.05$ ).

Figure (2): Effect of *Salvadora Persica* on the cytolytic activity of HSV-1 in vitro.



Duncan's Grouping: Control: A; 5% 24 hrs: A; 5% 2 hrs: B. Means with different letters were statistically significant ( $p < 0.01$ ).

Figure (3): Effect of topical application of *Salvadora Persica* on the development skin lesions of mice infected with HSV-1.

Virus was isolated from skin treated 2 and 24 hr. post infection with *Salvadora Persica*. The titer of virus isolated from skin treated with *Salvadora Persica* 2 hr. post infection on day 6 was moderately reduced compared to control and 24 hr. treatment but not significant and significantly ( $p < 0.05$ ) reduced the viral titer on day 8. While application of *Salvadora Persica* 24 hr post infection slightly alter the

amount of the virus in the skin but not significant, as in Figure (4).

Viral titers in trigeminal ganglia were sharply reduced with topical application of *Salvadora Persica* initiated 2 hr. post infection on days 6 and 8 ( $p < 0.05$ ), while topical application of *Salvadora Persica* 24 hr. post infection did not alter the viral content in ganglia compared to control, Figure (5).

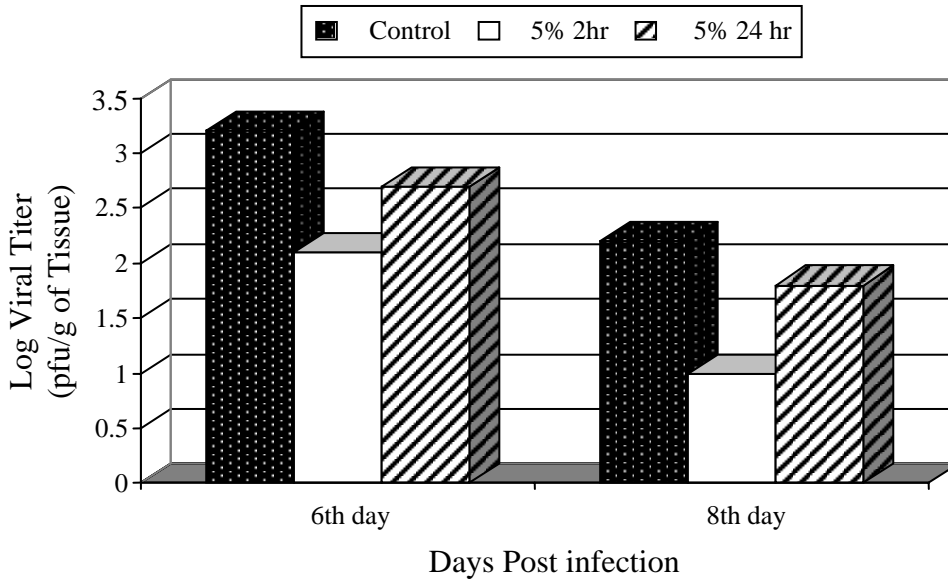


Figure (4): Effect of topical application of 5% *Salvadora Persica* on the titer of HSV-1 in the skin lesion of infected mice on days 6 and 8 post infection.

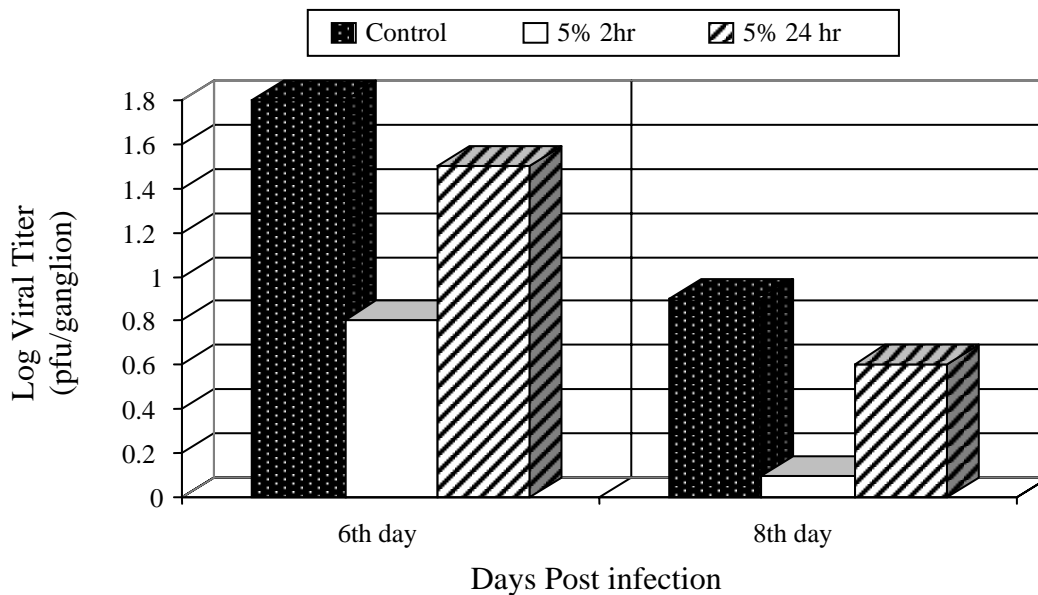


Figure (5): Effect of topical application of 5% *Salvadora persica* on the titer of HSV-1 in the trigeminal ganglia of infected mice on days 6 and 8 post infection.

## DISCUSSION

Salvadora Persica has been shown to have many biological properties<sup>(7-13)</sup>. In the present study, the antiviral activity of Salvadora Persica on HSV-1 was investigated both in vitro and in vivo in mouse model system. Salvadora Persica inhibited both replicating and cell free virus *in vitro*. The mechanism of such inhibition is not yet known. Inhibition of cell free virus is probably due to virucidal activity of some components of Salvadora Persica. It has been reported that benzylisothiocyanate isolated from Salvadora Persica affected HSV-1 replication<sup>(21)</sup>. The best concentration of Salvadora Persica which showed a significant effect was 5%. Higher concentrations have toxic effect on cells in vitro (data not shown), although other reports demonstrated the safe use of Salvadora Persica in concentration 10% in experimental injection of laboratory animals<sup>(17,18)</sup>.

The reduction of virus titer of cell free virus in control group was probably due to temperature inactivation of the virus. But beyond this temperature inactivation of the virus, the effect of Salvadora Persica on cytolytic activity of HSV-1 was significant ( $p < 0.05$ ).

The in vivo results showed that the application of 5% Salvadora Persica 2 hr. post infection was significantly ( $p < 0.01$ ) inhibited the development of cutaneous HSV-1 in early stage of disease. However, when topical application of 5% Salvadora Persica 24 hr. post infection was used, therapeutic effect was not observed. The skin content of virus was moderately reduced but, not significant.

A close correlation was observed between viral lesions and viral contents in the skin on days 6 and 8 post infection. Several studies demonstrated the close relationship between skin lesions and viral titer in different animal experiments using different antiviral agents<sup>(20, 23)</sup>.

Following primary infection of HSV-1 at peripheral site, virus attaches to the sensory nerve terminals and travels centripetally via neural route to sensory ganglia where it establishes latent infection which takes approximately 24 hr after primary infection<sup>(24, 25)</sup>. Topical application initiated as early as 3 hr. after viral infection with effective antiviral agents, has been

shown to prevent the appearance of HSV in corresponding sensory ganglia<sup>(26)</sup>. Therefore, the viral titer in trigeminal ganglia of control and Salvadora Persica treated mice was examined in this study. Salvadora Persica application initiated 2hr. or 24 hr. after viral infection lowered viral titers in ganglia.

The data presented in this work clearly demonstrated a significant antiviral activity of Salvadora Persica in vitro and a moderate antiviral effect in vivo. It has been shown that some antiviral agents lack activities following topical application in laboratory animals. This may be in part due to poor lipid solubility that retarded their penetration through the skin<sup>(27)</sup>.

Salvadora Persica extract has been shown to be an effective when used as root canal irrigant<sup>(14,15)</sup>. The results of this work may be beneficial for the treatment of recurrent oral herpes infections. Trials to treat aphthous stomatitis, angular cheilitis with Salvadora Persica extract are in progress.

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