

Full Length Research Paper

Antiviral activity of *Aloe vera* against herpes simplex virus type 2: An *in vitro* study

Keivan Zandi^{1*}, Moloud Abbas Zadeh¹, Kohzad Sartavi² and Zahra Rastian¹

¹Persian Gulf Health Research Centre, Bushehr University of Medical Sciences, Bushehr, Iran.

²Jahad Keshavarzi Research Centre, Bushehr, Iran.

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In this study we tested the antiviral activity of a crude hot glycerine extract of *Aloe vera* gel which was grown in Bushehr (Southwest of Iran) against HSV-2 replication in Vero cell line. The extract showed antiviral activity against HSV-2 not only before attachment and entry of virus to the Vero cells but also on post attachment stages of virus replication. The IC_{50} before attachment and entry of virus to the cells is 428 $\mu\text{g/ml}$ and the CC_{50} value which is the cytotoxicity of the extract for Vero cells is 3238 $\mu\text{g/ml}$, while the calculated selectivity index (SI) is 7.56. Also, IC_{50} of extract on post attachment stages of replication is 536 $\mu\text{g/ml}$ and the SI value for inhibition of the post attachment stages of HSV-2 replication is 6.04. Therefore, compounds of *Aloe vera* from Bushehr could be a good candidate as a natural source for antiviral drug development against HSV-2.

Key words: *Aloe vera*, antiviral, HSV-2, hot glycerine extract.

INTRODUCTION

People have been using herbal medicines to cure infectious disease from ancient times. In many studies for finding novel antiviral agents, some plants and algae extracts were tested on different viruses including the herpes viruses (Yoosook et al., 1999; Lopez et al., 2001; Sydiskis et al., 1991). Anthraquinone-containing extracts from different plant sources such as *Aloe vera* have shown a wide variety of pharmacological activities, such as antimicrobial, anti-inflammatory and antitumor activities (Bisset, 1994).

Aloe has been used medicinally for several thousands of years in many cultures; from ancient Egypt, Greece, and Rome to China and India. The plant has many common names and is often referred to as burn plant, first-aid plant, or medicine plant. Its name is most likely derived from the Arabic word *Alloeh*, meaning "shining bitter substance".

Herpes simplex virus type 2 (HSV-2) is an enveloped virus which causes genital herpes and some other important complications such as encephalitis, meningitis, eye infections and cold sore. This virus can produce latent

infection in the host for life and is reactivated by stimulus to cause recurrent infections and lesions (Fields, 2001). Considering the complications of this virus, some synthetic antiviral compounds such as acyclovir, penciclovir and vidarabine were developed for treatment of active herpetic infections, but they are not effective for the treatment of latent infections (Naesens and De Clercq, 2001). On the other hand the severe side effects and the development of some resistant mutants of this virus especially during long term medication with antiviral drugs were reported (Malvy et al., 2005; Pottage et al., 1995). Also, regarding the increasing prevalence of genital herpes, there is an urgent need to develop new anti-HSV-2 drugs. Because of the reasons mentioned above, finding new natural anti herpetic compound(s) is very interesting especially from some medicinal plants such as *A. vera*. One of the anthraquinones is emodin which has been reported to have antiviral activities to some kind of viruses, such as human cytomegalovirus, herpes simplex virus type 1 and poliovirus (Bernard et al., 1992; Cohen et al., 1996; Semple et al., 2001). It is obvious that the effect of the soil composition, climate and other environmental factors could affect the chemical and biological composition of *A. vera* in different area.

To the best of our knowledge, no research was done to evaluate the antiviral activity of *A. vera* which were grown

*Corresponding author. E-mail: keivanzandi@yahoo.com or zandi@dr.com. Fax: +98-771-2531933; Tel.: +98-917-371 2079.

in the south west of Iran. Here, we have attempted to evaluate the anti HSV-2 activity of this plant from Bushehr port in the south west of Iran.

MATERIALS AND METHODS

Preparation of *Aloe vera* extract

A. vera was collected from the pilot farm of the Jihad Keshavarzi Research Centre Bushehr (South west of Iran). The Aloe leaf was cut and the fresh gel within the leaf was extracted. The extracted gel corresponding to was dissolved in 10% glycerine solution. The mixture was boiled for 20 min in 105 °C. The hot glycerine extract was clarified by filtration using Whatman No.1 filter paper. It was, thereafter, sterilized by autoclaving.

Cell line and virus

Vero cells (African green monkey kidney cell line) were used for HSV-2 replication and propagation. Briefly, the cells were grown in 50 ml cell culture flasks (NUNC) or 24 wells cell culture microplates (NUNC) by using Dulbeccos Minimum Essential Medium (Gibco) containing 10% foetal bovine serum (Gibco). Herpes simplex virus type 2 was isolated from clinical sample and confirmed by using anti HSV-2 type specific fluorescent monoclonal antibody (DAKO). The virus was propagated in Vero cells and the titre of propagated viral stock was determined as TCID₅₀/ml by using Karber method. The viral stock after titration was dispensed in some sterile tubes which were stored at -70°C.

Cytotoxicity test

The cytotoxicity of alga extract was determined by culturing of Vero cells for 72 h in the presence of increasing amounts of extract. Then viable cells were determined by the trypan blue exclusion test. The results were plotted at dose response curve, and by using STATA statistical software the 50% cell growth inhibitory concentration (CC₅₀) was obtained.

Antiviral activity assay

In this study we used the cytopathic effect inhibition assay for evaluation of antiviral activity of the aloe extract. To begin with, Vero cells were grown in 24-well plastic plates (7*10³ cells /well). Then the plates were incubated at 37°C in the presence of 5% CO₂ until the cells became confluent. Thereafter, the culture medium was removed from each well. 0.1 ml of virus suspension containing 10000 TCID₅₀ and 0.1 ml of DMEM containing 2% FBS were mixed in each well of 24-well plates and appropriate concentrations of the extract from minimal to maximal non-cytotoxic concentration were added to each well based on serial dilution preparation.

For the virus control 0.1 ml of virus suspension and 0.1 ml of culture medium without extract were used. For the cell control 0.1 ml of culture medium with maximal non cytotoxic concentration of extract were added. Also for evaluation of probable antiviral effect of 10% glycerine solution 0.1 ml of virus suspension and 0.1 ml of sterile 10% glycerine solution without extract were used. The plates were incubated at 37°C in a humidified CO₂ atmosphere (5%, CO₂) and were investigated everyday for CPE presentation until 5 day post infection. For testing the probable post attachment antiviral effect of the extract, same protocol which mentioned above was done but the adding of the extract was two hours post inoculation of cells with virus. The degree of inhibition was expressed as percent

yield of virus control (% virus control = CPE experimental group/ CPE virus control *100). The concentration of extract which reduced CPE 50% with respect to virus control was estimated from graphic plots defined as 50% inhibited concentration (IC₅₀) expressed in microgram per milliliter by using STATA modeling software. The selectivity index (SI) was measured from the ratio of CC₅₀/IC₅₀ (Kudi and Myrint, 1999; Kujumgier et al., 1999).

Statistical analysis

STATA statistical analysis package was used for the dose response curve drawing in order to IC₅₀ and CC₅₀ calculation.

RESULTS

The cytotoxicity of Aloe vera gel crude extract on Vero cells was determined by calculation of CC₅₀ which is 3238 µg/ml. Meanwhile, the 1000 µg/ml of hot glycerine extract of *A. vera* gel showed the cytotoxicity just for 10% of Vero cells, 5500 µg/ml of the extract was cytotoxic for 100% of treated cells.

Treatment of the Vero cells with different concentrations of crude extract at the same time of inoculation by HSV-2 was done based on the method mentioned in materials and methods section. Based on results, we understood that 100 µg/ml of the extract did not show any antiviral effect while the 700 µg/ml of that extract could inhibit the performing of cytopathic effect completely due to HSV-2 replication in Vero cells. Therefore the IC₅₀ of this extract by using STATA modelling software is 428 µg/ml (Figure 1). From the resulting IC₅₀ and CC₅₀ from extract, the SI value is 7.56 for *A. vera* gel hot glycerine extract.

The antiviral activity of the crude extracts was tested on post attachment stages of the virus replication cycle. It was observed that 100 µg/ml of the extract could not prevent the performing of cytopathic effect of HSV-2 in cell culture and 850 µg/ml of that extract inhibited the HSV-2 related CPE performing in Vero cells completely. Therefore the IC₅₀ value for filtered extract is 536 µg/ml, while the SI value for the antiviral activity is 6.04 (Figure 2).

DISCUSSION

A. vera is a member of the Liliaceae. Topical aloe has been used for wounds such as cuts and burns owing to its perceived effectiveness in improving healing (Perfect et al., 2005). The aloe plant is the source of two herbal preparations; aloe gel and aloe latex. Aloe gel is often refers to the clear gel or mucilaginous substance produced by parenchymal cells located in the central region of the leaf. Diluted aloe gel is commonly referred to as "A. vera extract". The gel is composed mainly of water (99%) and mono- and polysaccharides (25% of the dry weight of the gel). The most prominent monosaccharide in AG is mannose-6-phosphate, and the most common polysac-

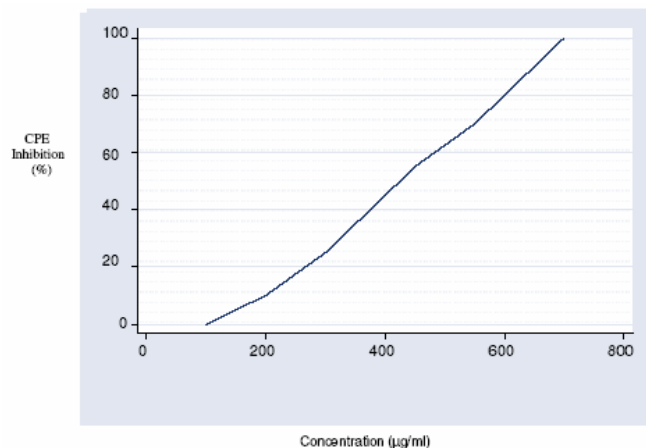


Figure 1. Dose response curve of *Aloe vera* extract against HSV-2 (at the same time of virus inoculation).

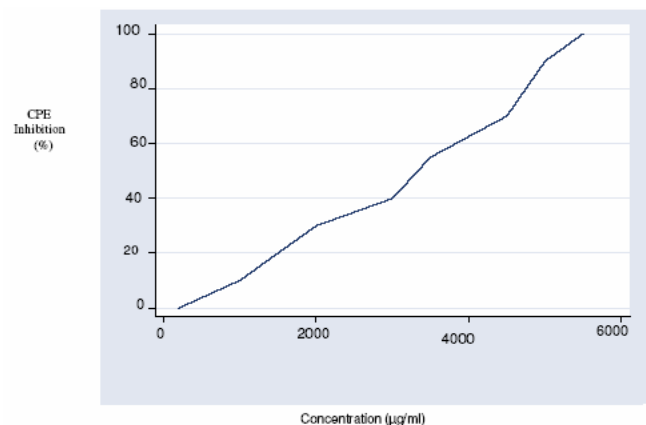


Figure 2. Dose response curve of *Aloe vera* extract against HSV-2 (after virus attachment).

charides are called gluco-mannans (Shelton, 1991).

Also, there are some publications about antiviral properties of different kinds of plant extracts such as *A. vera* especially investigation about the antiviral activity of its anthraquinones (Sydiskis, 1991; Andersen et al., 1991). Interest in employing antiviral compounds from natural sources like plants or algae has been enhanced by researchers and also consumers' preference for natural medicines and concerns about the toxic effects of synthetic antiviral materials. In the present study, we chose HSV-2 for our research because of its ability for performing the different clinical complications and its increasing prevalence in communities (Rosen, 2006). This is the first study about the anti HSV-2 activity of hot glycerine extract of *A. vera* which were grown in Bushehr (South west of Iran). We have prepared hot glycerine extract of *A. vera*, because the glycerine extract is enriched for anthraquinones present in plants (Sydiskis, 1991). It is obvious that the resulting IC_{50} and CC_{50} values are not

comparable with their counterpart studies in which the purified effective components such as emodin or another anthraquinones were tested.

For the sterilization of the extract, autoclaving was used, and we found that the autoclaved extract showed the acceptable IC_{50} . Based on SI values of this extract, it could be a good choice for anti- HSV-2 natural compound, although in most studies the filtering method was used for extract sterilization. Therefore the *A. vera* hot glycerine extract could be a good choice for preventing of virus adsorption, attachment or entry to the host cell. A previous study has established that aloe emodin could disrupt the envelope of viruses (Sydiskis, 1991). Results of the cytotoxicity test indicate that 5500 µg/ml of the extract was cytotoxic for 100% of Vero cells. Meanwhile other workers reported that aloe emodin of *Aloe barbadensis* did not show remarkable cytotoxic effect (Sydiskis, 1991). This difference could be due to the type of extract and/or the species of plant or some unknown factors. In other studies, stimulation of immune system against some viral complications due to Aloe extract were established (Gauntt et al., 2000; Ilijazovic, 2006). Therefore, an *in vivo* study on the antiviral activity or another aspect of medical applications of the *A. vera* of Bushehr is recommended. Also, further investigation on the antiviral activity of this plant on naked viruses could be interesting. It is obvious that for future works, identification of the effective compounds of the extract and the quantitation of these elements are necessary.

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