Full Length Research Paper

Evaluation of in vitro antiviral activity of Chelidonium majus L. against herpes simplex virus type-1

Seyed Hamidreza Monavari¹*, Mahmoud Shamsi Shahrabadi², Hossein Keyvani² and Farah Bokharaei-Salim²

¹Department of Virology and Antimicrobial Resistance Research Center, Tehran University of Medical Sciences, Tehran, Iran.
²Department of Virology, Tehran University of Medical Sciences, Tehran, Iran.

Accepted 12 December, 2011

Chelidonium majus Linnaeus (L) has been used in a variety of practical applications in medical sciences. Its antiviral activity against herpes simplex virus type-1 (HSV-1) was investigated in cell culture. C. majus L. extract was found to be non-toxic to Vero cells (African green monkey kidney cells) up to concentration 1000 µg/ml and inhibited the growth and development of HSV-1 in dose-dependent manner in Vero cells. In order to study the possible mechanisms of the antiviral activity of C. majus L. extract, cells were treated with extract before, during and after infection, and the viral titers were tested by TCID₅₀ assay. The antiviral effects in treatment of post and during virus infections were more remarkable than the treatment of pre-infection. For further investigation indirect immunofluorescence technique was used to elucidate the antiviral mechanism of the extract by infecting Vero cells at different times and monitoring the synthesis of viral proteins. Although the precise mechanism has not yet to be defined, our work indicated that C. majus L. extract could inhibit growth and development of HSV-1 in cells in vitro.

Key words: Chelidonium majus L, herpes simplex virus type-1 (HSV-1), antiviral activity, Vero cell, TCID50.

INTRODUCTION

Herpes simplex virus-1 (HSV-1) is capable of causing a widespread spectrum of mild to severe disorders. These include acute primary and recurrent mucocutaneous diseases. Herpes simplex virus-1 causes several neuronal diseases; it spreads in sensory axons and infects sensory neurons in the ganglia of the peripheral nervous system establishing latent (Townsend and Collins, 1986; Cook and Stevens, 1973).

Today, in the treatment of herpes virus infections, antiviral drugs like acyclovir (ACV), vidarabine (ara-A) and ganciclovir (DHPG) are used (Crumpacker et al., 1982; Corey and Spear, 1986). The mechanism of action of these drugs is basically dependent on their abilities to inhibit the virus-specific enzymes, thymidine kinase and the DNA polymerase (Konson et al., 2004; Malvy et al., 2005). Because of their cytotoxic effects, these drugs except of acyclovir are not widely used (Erlich et al., 1989). In recent years, however, acyclovir and other drugs have been reported to be inefficient in treating genital herpes infections. HSV-1 has also been reported to acquire resistance to these drugs (Crumpacker et al., 1982; Konson et al., 2004).

Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases (Vijayan et al., 2004). There is an increasing need for substances with antiviral activity since the treatment of viral infections with the available antiviral drugs often leads to the problem of viral resistance (Vijayan et al., 2004). There is a need to search for new and more effective antiviral agents (Gilca et al., 2010). One of the plant derivatives that has been occupied an important place in the field of chemotherapeutic research in recent years is C. majus Linnaeus (L) (Biswas et al., 2008; Gilca et al., 2010). The greater celandine (C. majus L.) is a species of the Papaveraceae family, which is widely
distributed in Europe and Asia, is a plant of great interest for its wide use in various diseases in European countries and in Chinese herbal medicines. Drug contains various isoquinoline alkaloids, flavonoids and phenolic acids that exhibit a broad spectrum of biological activities (anti-inflammatory, anti-microbial, anti-tumoral, analgesic, hepatoprotective) that support some of the traditional uses of C. majus L. However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied (Colombo and Bosisio, 1996; Jang et al., 2004; Pieroni et al., 2005; Gilca et al., 2010).

Extracts of C. majus L are traditionally used in various complementary and alternative medicine (CAM) systems including homeopathy in combating diseases of the liver and various skin disorders (Taborska et al., 1995; Kim et al., 1997).

MATERIALS AND METHODS

Preparation of the crude extract

Dried plant material (20 g) was macerated with 90% ethanol (450 ml) for 5 days, filtered and the marc was exhaustively percolated with the same solvent. Filtrate was evaporated to a thick residue at 50°C. The yield of the extract was 40%. The extract was re-suspended in 90% ethanol (180 ml) and used for in vitro experiments.

Cell culture and virus

Vero cells were cultured with Dulbecco’s Modified Eagele’s Medium (DMEM) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2 and were subcultured two or three times a week.

HSV-1 was isolated from patients and identified by specific monoclonal antibodies. Viruses were quantified in terms of the 50% tissue culture infective dose (TCID50) by endpoint dilution, with the infectious titer determined by the method of Reed and Munch (Flint et al., 2000), and stored in small aliquots at -70°C until use.

Cellular cytotoxicity assay

In order to test the effect of the C. majus L. extract on Vero cells, Cytotoxicity was evaluated by the neutral red staining (NRS) method. Briefly, Vero cells were grown in 24-well plates. After formation of complete monolayer (Figure 3a), the culture medium was replaced by similar medium containing various concentration of extract (10, 100, 200, 400, 600, 800, 1000 and 2000 µg/ml). After 4 days of incubation at 4°C, 100 µl of neutral red (120 µg/ml) was added to each cell monolayer and incubated for 3 h at 37°C. After that the cells were washed with 0.5% formaldehyde containing 1% CaCl2. The cells were stained indirectly with fluorescein conjugated anti-rabbit IgG using specific viral antiserum. Cover slips were mounted in glycerol buffer and examined in a UV equipped microscope.

RESULTS

Cytotoxicity

Neutral red staining method showed that the C. majus L. extract had no serious effect on the proliferation of cells, up to concentration of 1000 µg/ml. Therefore, we could draw a conclusion that the CC50 (the concentration which causes 50% cytotoxic effect) was more than 1000 µg/ml (Figure 1).

Antiviral activity of the extract by TCID50 assay

The inhibition of virus yield by the extract was evaluated by TCID50 assay in Vero cells. When the cells were incubated with the extract prior to viral infection, the virus titer of the supernatant did not show any significant change. To examine the direct effect of the extract to virus, HSV was mixed with nontoxic concentration of the extract and the mixture was used for infecting cells; when the result of virus yield inhibition showed no distinct drop

Virus yield inhibition assay

Nontoxic concentration of test drug was checked for antiviral property by virus yield reduction assay against virus challenge dose of 100 TCID50/ml. In virus yield assay, reduction in the yield of virus when cells were treated with C. majus L. extract before, during and after virus infection was determined, as described in the following section.

Incubation of cells with the extract before, during and after virus infection

C. majus L. extract was dissolved in serum free DMEM and incubated with semi-confluent cell in 24 well tissue culture plates in concentration of 1000 µg/ml for 1 and 4 h at 37°C. After removal of the extract, the cells were washed with phosphate buffered saline (PBS) and then infected with HSV-1 at multiplicity of infection (MOI) of 0.1. After 1 h incubation, the unabsorbed virus was removed; the cell character was washed with PBS and further incubated in DMEM with 2% PBS. Controls consisted of Vero cells untreated and Vero cells infected with HSV-1. For determination of antiviral activity of the extract during and post virus infection the assay was performed as described above, with the exception that the extract was added together with the virus and after 1, 4, 8, and 12 h post infections, correspondingly. After 48 h incubation at 37°C, virus titer was determined by the endpoint dilution method and expressed as TCID50/ml by comparison with virus control.

Immunofluorescence

Confluent Vero cells grown on 13 mm cover slips were infected with 100-fold TCID50/ml HSV-1 for 16 h in the presence of 1000 µg/ml of C. majus L. extract and compared with untreated infected cells. Cover slips were removed, washed in PBS and fixed in acetone at 4°C then were stained indirectly with fluorescein conjugated anti-rabbit IgG using specific viral antiserum. Cover slips were mounted in glycerol buffer and examined in a UV equipped microscope.
Figure 1. Effect of *Chelidonium majus* L. on viability of Vero cells. The high absorbency represents more uptake of neutral red and more cell variability.

Figure 2. Effect of the extract (pre, during and post-infection) on virus yield in Vero cells.

Incubation of infected cell monolayer with the extract for 1, 4, 8 and 12 h after infection showed antiviral activity. Results are shown in Figure 2. The inhibitory effect of the extract was shown 1 h after infection, when the virus titer of the supernatant dropped from 106.7 TCID$_{50}$/ml to 103.2 TCID$_{50}$/ml. Inhibitory effect of the extract was not significant in other cases.

Immunofluorescence

Semi-confluent monolayer's of Vero cells infected with HSV-1 (Figure 3b) in the presence and absence of *C. majus* L. extract were fixed and processed for indirect immunofluorescence. The expression of HSV-1 proteins was evident after 18 hr of infection correlating with the appearance of its characteristic cytopathic effect (Figure 3c, d). When the same experiment was repeated in the presence of the extract, expression of the HSV-1 antigens were very weak and did not show fluorescence apparently (Figure 3e).

DISCUSSION

The herpes simplex virus type-1 (HSV-1) is the primary cause of oral-facial and pharyngeal infections and may cause herpetic whitlow, as well as severe and sometimes dangerous infections of the eyes and brain. HSV-1 also accounts for 10 to 15% of all genital herpetic infections. This virus can produce latent infection in the host for life and is reactivated by stimulus to cause recurrent infections and lesions (Cunningham et al., 2006). Considering the complications of this virus, some synthetic antiviral compounds 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 development of some resistant mutations of
Figure 3. A: Monolayer of uninfected Vero cells; B: HSV-1 infected monolayer of Vero cell; C: Uninfected monolayer processed for Immunofluorescence [negative control]; D: HSV-1 infected monolayer processed for Immunofluorescence [positive control]; E: Infected Vero cells incubated for 16h in the presence of 1000 μg/ml of the extract.

this virus, especially during long term medication with antiviral drugs, were reported (Pottage and Kessler, 1995; Malvy et al., 2005).

New antiviral agents from the plant origin can have easy acceptability being non-toxic and inexpensive. Meanwhile, the extracts of some medicinal herbs were investigated in term of searching hopeful candidates for treating HSV infection (Yoosook et al., 2000; Guarino and Sciarrillo, 2003). *C. majus* L. contains various isoquinoline alkaloids, flavonoids and phenolic acids that exhibit multiple biological activities, such as antiviral, antitumor, antibacterial/antifungal or anti-inflammatory effects (Colombo and Bosisio, 1996; Jang et al., 2004; Pieroni et al., 2005).

The present study was initiated to evaluate inhibitory effect of the extract of *C. majus* L against herpes simplex virus type-1 (HSV-1) using cytoxicity assay, virus yield reduction assay, virus infectivity and antigen expression assay. In this study, the effect of different concentrations of the extract on Vero cells was determined after 72 h incubation by neutral red staining method. *C. majus* L seed extract had no cytotoxicity on Vero cells up to concentration of 1000 μg/ml. The inhibition of virus yield showed that treating the cells with the extract 1 h after infection can significantly reduce virus titer in the first passage. Regarding the HSV replication cycle, soon after infection, approximately 2 to 4 h post infection (hpi), α or immediate early (IE) genes are expressed. Although transcription of α genes require no prior viral protein synthesis, an HSV protein brought in with the virion tegument, VP16, stimulates transcription of α genes (Cunningham et al., 2006). Therefore, inhibitory effect of the extract 1 h after infection can be caused by preventing α genes protein synthesis or possibly repressing function of VP16 tegument protein. Evaluation of HSV-1 protein expression in treated infected cells by
immunofluorescence assay showed significant reduction in protein synthesis at first passage which means that the extract can prevent viral gene expression at transcription or translation level. In this preliminary study, the extract of *C. majus* L. showed antiviral properties against Herpes simplex virus type-1. Further studies are required to know the mechanism of action using suitable animal models.

ACKNOWLEDGEMENTS

Authors thank Tehran University of Medical Sciences for financial support (Project code: 963).

REFERENCES


