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Anti-Herpes Simplex Virus Effect of *Camellia sinesis*, *Echium*amoenum and *Nerium oleander*

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Abstract As viral resistance to available chemical drugs and virus latency duration causes problems in the treatment of herpes simplex virus type 1 infections, there is an evolving need for new anti-Herpes drugs. The present study evaluated the antiviral effect of *Camellia sinesis*, *Echiumamoenum*L and *Nerium oleander* with ethno medical background on HSV-1 multiplication. Plants were extracted with decoction method to obtain aqueous extracts. These extracts were screened for their cytotoxicity against Hep-2 (Human epithelial type 2) cell line by cytopathic effect (CPE) assay. Antiviral effects of the plant extracts were determined by Neutralization Test (NT) at times one, two and three hour. *Nerium oleander* extract had most toxicity (> 5 µg/ml) on cell line, and *Camelliasinesis* showed the most anti-Herpes property at inhibition of HSV-1 multiplication at one and two hour that decreased at three hour. *Echiumamoenum*L had lowest anti-Herpes effect that at two hour was similar antiviral property of *Camelliasinesi*s at three hour. *Camellia sinesi*s and *Echiumamoenum*L showed the most anti-Herpes effect when they used an hour after virus inoculation. Further research is needed to elucidate the active constituents of these plants which may be useful in the development of new anti-Herpes drugs.

Keywords: *Anti-Herpes effect, cell line, Camellia sinesis, EchiumamoenumL, Nerium oleander, HSV-1*

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1. Introduction

Herpes simplex virus (HSV) infections are very common worldwide. HSV-1 is the main cause of herpes infections on the mouth and lips, including cold sores and fever blisters. It is transmitted through kissing or sharing drinking utensils. HSV-1 can also cause genital herpes, although HSV-2 is the main cause of genital herpes. Today, the treatment of HSV-1 diseases with chemical drugs have faced with challenges because of the emergence of drug resistant viruses due to mutations in viral TK gene or viral DNA polymerase gene [1,2], its latency and recurrence [3,4,5].So we must seek new anti-Herpes drugs. Inmany researches have shown that plants contain tannins, flavonoids [6,7,8]and alkaloid have antiviral properties [9,10,11]. Therefore, medicinal plants can be used to treat viral infections [12,13].

In this study, the inhibitory effect of green tea (*Camellia sinesis*), Iranian borage (*Echiumamoenum*L) and oleander (*Nerium oleander*) on HSV-1 virus replication was investigated. Greentea leaves in traditional medicine for heart diseases, jaundice, and stoppedthe urine [14] and in modern medicine for nervous headaches, treatment of amoebic and bacterial dysentery and hepatitis, reduced fat and sugar is used due to its antimicrobial properties, antioxidant, anticancer and antiviral [15]. In the past the borage to have smear-binding properties and softening used in the cold [16], increased urinary, strengthens the heart and nervous system. Iranian traditional medicine used oleander plant for tinea, itching and scaling skin [17]. In modern medicine, oleander plant glycosides Instead of the digitalis glycosides for the treatment of heart disease. Oleander can cure cancers of the bladder, brain, neck, kidney, ovary, pancreas and uterus. Also, this plant use for viral diseases such as hepatitis B and C, influenza and AIDS [18].

2. Material and Methods

2.1. Extraction

Camellia sinensis, *Echiumamoenum*L and *Nerium oleander* plants were provided from School of Pharmacy, Medical Sciences of shahid Beheshti University, Tehran. Different parts of the plants were collected and dried in room temperatureat 25°C± 5 and then were ground. Briefly, 100 g of dried plants were boiled in 100 ml of distilled water for 10 min. The aqueous extracts were filtered with 0.22 µm pore size. Filtered extracts were lyophilized by EyelaF ryzdrayr machine (model 81, Japan) for 24 h. The dry powder of the plants was dissolved in DMEM (Dulbeccos Modified Eagles Medium)with the ratio 1:20. From herbal extracts were provided working solutions with concentration 1000 µg /ml and were stored in a refrigerator at -4°C until experiment [19,20].

2.2. Virus and Cell lines

The virus used in this study was herpes simplex virus type I (HSV-1, KOS strain)and Hep-2 cell that obtained from virology lab, School of Public Health, Medical Sciences of Tehran University.

2.3. Virus Culture

In a 96-well microplate Hep-2 cells propagated and virus was inoculated to culture. While virus permeated 80% of monolayer cells, viruses were harvested. Then virus titer was compared with the 100 TCID 50 [21].

2.4. Cytotoxicity Assay

In a 96-well microplate, Hep-2 cells propagated and incubated at 37°C in ahumidified incubator with 5 per cent CO_2 for a period of 48 hour. Then different concentrations of herbal extracts (50-1000 µg /ml) were added to cells to DMEM culture. The microtiterplate was incubated at 37°C for a week. The morphologyof the cells were checked daily for cytopathic effect (CPE). The 50% cytotoxic concentration (CC_{50}) was determined by evaluation of CPE. The CPE of all wells were evaluated compared with cell control well.

2.5. Antiviral Assay

Nontoxic concentrations of plant extracts, *i.e*., lower than CC_{50} were checked for antiviral activity by Neutralization Test (NT) at times one, two and three hour [22]. In this assay, cellswere seeded in a 96-well microplate and incubated at 37°C in a humidified incubator with 5 per cent CO_2 for a period of 48 hour. Then the 100 TCID 50 of virus was rushed on cell culture after to appear monolayer cells. The culture wastreated with concentrations 50-1000 µg /ml of plant extracts. Micro plate incubated at 37°C for seven days. Antiviral activity was determined by theinhibition of CPE compared with cell and virus control wells by using SPSS analysis, and IC_{50} values were determined. The antiviral inhibition concentration was expressed as the 50% inhibitory concentration (IC_{50})which is the concentration of the sample required to inhibit virus-induced CPE by 50% were brought in Table 1.

3. Results

3.1. Cytotoxicity Assay

In cytotoxicity assay of plant extracts *Nerium oleander* extract was shown moretoxicity and all concentration of plant were toxic to Hep-2 cell line (more than 50 µg/ml), while two other extracts were good tolerated by cells (Table 1).*Camellia sinensis* and *Echiumamoenum L.*were not toxic for cell lines at highest concentration (CC_{50}=1000 µg/ml).

3.2. Antiviral Assay

In antiviral assay, two plants extract; *Camellia sinensis* and *Echiumamoenum*L. exhibited significant anti-Herpes effect against HSV-1 at nontoxic concentrations to the cell lines used (Table 1). The extract of *Camellia sinensis* showed highest anti-Herpes effect against HSV-1 at one and two hour that decreased at three hour (Figure 1). *Echiumamoenum*L had lowest anti-Herpes effect that at two hour was similar antiviral property of Camellia sinesis at three hour (Figure 2).

Table 1. CC_{50}, IC_{50} of plants on Hep-2 cells against HSV-1 atvarioustimes determined by cytopathic effect (CPE) inhibition assay

Plant extracts	CC_{50}*	IC_{50}* (1 hour)	IC_{50}* (2 hour)	IC_{50}* (3 hour)
Camellia sinensis	1000	20	60	480
***Echiumamoenum*L.**	1000	350	500	750
Nerium oleander	<50	Toxic	Toxic	Toxic

*: CC50 – 50% cytotoxic effect concentration, IC50 – 50% effective concentration, HSV-1 – herpes simplex virus type 1, KOS strain.

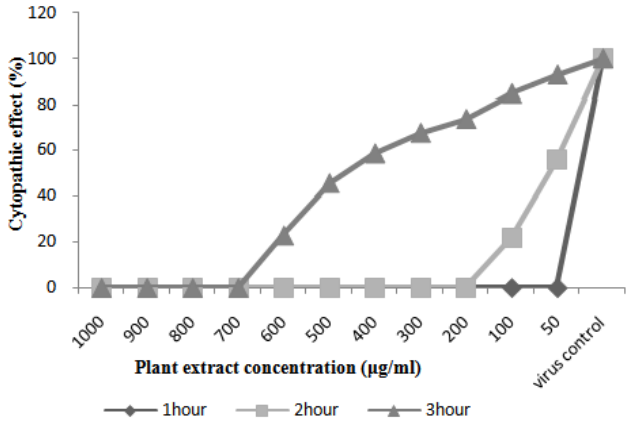

Figure 1. Antiviral effects of*Camellia sinensis* extract against HSV-1at various times

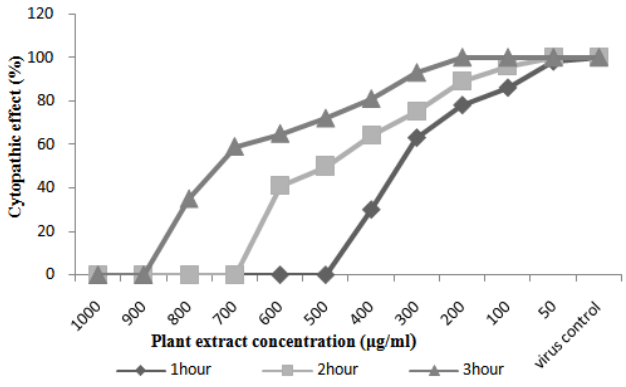

Figure 2. Antiviral effects of EchiumamoenumL. extract against HSV-1 at various times

Our findings indicated that *Camellia sinensis* extract has inhibit HSV-1 multiplication completelyat

concentrations 50-1000 µg/ml at one hour while this figure for *Echiumamoenum L* is>400 µg/ml.

IC_{50} of *Camellia sinensis* extract was 20 µg/ml and for *Echiumamoenum*L was 350 µg/ml at one hour which at two hour IC50 of *Camellia sinensis* was 60 µg/ml and for *Echiumamoenum*L was 500 and at three hour for *Camellia sinensis* extract was 480 µg/ml and for *Echiumamoenum*L was 750 (Table 1).

4. Discussion

The treatment of HSV-1 infections with the available chemical drugs often leads to the problems to viral resistance [1,2] and virus latency duration [3,4,5]. Modern studies showed some of the medicinal plants with therapeutic application in traditional medicine have antiviral effects [23,24,25]. So studying medicinal plants may be modern way for treatment of HSV-1 illness [26,27].

In this study, Of the 3 plant extracts tested in vitro, herbal extracts of *Camelliasinensis* and *EchiumamoenumL* has not toxic effect at highest concentrations(CC_{50}=1000 µg/ml) to the cell lines used and all concentration of *Neriumoleander* extract was toxic on Hep-2 cell line. Findings indicated that *Camelliasinensis* was HSV-1 multiplication full inhibitor at concentrations 50-1000 µg/ml at one hour (Figure 1). While the extracts of *EchiumamoenumL* was inhibit HSV-1 multiplication completely at concentrations >400 µg/ml (Figure 2). So IC_{50} of *Camellia sinensis* extract at one hour was best of sample (Table 1). Thus, Camellia sinesis and EchiumamoenumL showed the most anti-Herpes effect when they used an hour after virus inoculation.

In another study evaluating catechins of green tea on HSV-1 and HSV-2 in genital showed that herpes simplex virus type II in 10 to 20 minutes, and herpes simplex virus type I in 30 to 40 minutes multiplication were inhibited [23]. Our findings confirm their findings about inhibitory effect of green tea extract on HSV-1 multiplication. In another experiment, antiviral property of green tea on HSV-2 virus in the late (latency) virus infection was demonstrated [28]. So far antiviral effect of *EchiumamoenumL*, particularly on the virus HSV-1 has not been investigated. However, many researchers have been done about the other characteristics of plant. In a study on the aqueous extract of *EchiumamoenumL*, antibacterial effect on Staphylococcus aureus strain 8327 was observed [29].

The decoction of the plant has shown antioxidant effects on human [30]. In another study, the methanol extract of borage flowers on white male rats were investigated and the findings of its Indicated analgesic properties [16].

5. Conclusion

Inhibitory effect of the aqueous extracts of *Camelliasinensis* and *Echiumamoenum*L. against HSV-1 infection on Hep-2 cells was demonstrated. Plant extracts showed the most anti-Herpes effect when they used an hour after virus inoculation. In summary, because anti-Herpes effect of 3 plant extracts has been studied on Hep-2 cells (has been derived from epithelial of human pharynx) and so well antiviral effect of two plants extract; *Camellia sinensis* and *Echiumamoenum*L. have been seen on HSV-1 multiplication, can be useful way for treatment of HSV-1 infections. Further research is needed to elucidate the mechanismof these plants which may be useful in the development of new and effective antiviral agents.

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Conflict of Interest

The authors declare no conflict of interest

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