

Antiviral Effect Assay of Aqueous Extract of *Echium amoenum*-L against HSV-1

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Article information	Abstract
<p>Article history: Received: 5 Apr 2012 Accepted: 5 Aug 2012 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 46-48</p> <p>Keywords: Antiviral effect <i>Echium amoenum</i> L HSV-1</p> <p>*Corresponding author at: Department of Microbiology of Qom Branch, Islamic Azad University, Qom, Iran. E-mail: ami.airia@gmail.com</p>	<p>Background: Medicinal plants have been used for different diseases in past. 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. Therefore in the present study <i>Echium amoenum</i> L plant with ethnomedical background was screened for antiviral activity against HSV-1 in different times.</p> <p>Materials and Methods: Flower part of <i>Echium amoenum</i> L plant collected from Iran was extracted with different methods to obtain crude aqueous extract. This extract was screened for its cytotoxicity against Hep II cell line by CPE assay. Antiviral properties of the plant extract were determined by cytopathic effect inhibition assay.</p> <p>Results: <i>Echium amoenum</i> L extract exhibited significant antiviral activity at non toxic concentrations to the cell line used. Findings indicated that plant extract has the most antiviral activity when it used an hour after virus inoculation.</p> <p>Conclusion: <i>Echium amoenum</i> L plant had not toxic effect at highest concentrations to the cell lines used and showed the most antiviral activity when it used an hour after virus inoculation. Further research is needed to elucidate the active constituents of this plant which may be useful in the development of new and effective antiviral agents.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Medicinal plants have been used for different diseases in past. Found fossils showed application thymus and cuminum for treatment diseases in 5000 years ago on Sumerians [1]. According to estimation of WHO, 80% of world people use herbal drugs for treatment diseases, because most of chemical drugs are expensive [2]. Therefore 30% of modern drugs come from plants [3] and more than 66% plant species have medical value [4, 5]. Iran ancient civilization has chronic history in cognition and treatment by medicinal plants and Iranian scientists such as Avicenna have tried in improvement and dehisced this science.

Modern studies showed some of the medicinal plants with therapeutic application in traditional medicine [6-8] have antiviral effects and in many studies been seen plants flushed of flavones, tannin [9, 10] and alkaloid have antiviral, antibacterial, antifungal and antiparasite effects [11-13].

There is a requirement for new antiviral drugs since the treatment of viral infections with the available chemical drugs often leads to the problems to viral resistance [14, 15] and virus latency duration [16-18]. Studing antimicrobial components of medicinal plants is useful way [19]. Therefore in the present study *Echium amoenum* L plant with ethnomedical background was screened for antiviral activity against HSV-1 in different times. This plant is used for influ, urine addition, and stomach and nerve system amplification in Iran [20]. So

Avicenna used it for mouth ulcers, heart failure and cough [21].

Materials and Methods

Extraction: In this study, *Echium amoenum* L plant was provided from market of medicinal plants of Tehran city. The flower part of plant was dried at environment temperature and then was ground. Briefly, 100 g of dried plant was boiled in 100 ml of distilled water for 10 min. The aqueous extract was filtered [22, 23]. Filtered extract was lyophilized. From herbal extract was provided a working solution with concentration 1000 µg/ml and was stored in a cool place [24].

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.

Virus culture: In a 96-well micro titer plate with Hep-2 cells propagated and virus was inoculated to culture. While virus permeated 80% of monolayer cells, viruses was harvested. Then virus titre was compared with the 100 TCID₅₀ [25]. **Cytotoxicity assay:** In a 96-well micro titer plate with Hep-2 cells propagated and different concentrations of herbal extract was added to cells in DMEM culture. The micro titer plate was incubated at 37°C in incubator for a week. The morphology of the cells was checked daily and observed for cytopathic effect (CPE).

Table 1. Assay of antiviral effect of *Echium amoenum* L against HSV-1 at different times

Time of extraction effect	1000	900	800	700	600	500*	400	300	200	100	50
1 hour	-	-	-	-	-	**	+	+	+	+	+
2 hour	-	-	-	-	+	+	+	+	+	+	+
3 hour	-	-	+	+	+	+	***+	+	+	+	+

*. Concentration of extract ($\mu\text{g/ml}$) ** not to see CPE *** to see CPE

The CPE of all wells were evaluated and scored in compare to cell control well. Antiviral assay: In this assay, cells were seeded in a 96-well micro titer plate and then virus was rushed on cell culture after to appear monolayer cells. The culture were treated with concentrations 50-1000 $\mu\text{g/ml}$ of plant extract in 1, 2 and 3 h. micro titer plate incubated at 37°C for seven days. Antiviral activity was determined by the inhibition of CPE compared with cell and virus control wells.

Results

In cytotoxicity assay of *Echium amoenum* L extract was seen highest concentration was not toxic for cell lines (1000 $\mu\text{g/ml}$). In antiviral assay, *Echium amoenum* L extract exhibited significant antiviral activity at non toxic concentrations to the cell line used and herbal extract was inhibitor of virus replication at concentrations higher than 400 $\mu\text{g/ml}$. but inhibition of virus replication was reduced at concentrations lower than 400 $\mu\text{g/ml}$ in 2h. Findings indicated that plant extract has the most antiviral activity when it used an hour after virus inoculation (Table1). So in this study, preservation role of herbal extract was seen in terminal day of assay at 1 and 2 h as if this role was reduced at 3 h.

Discussion

Echium amoenum L plant had not has toxic effect at highest concentrations (1000 $\mu\text{g/ml}$) to the cell lines used. Findings indicated that plant extract was inhibitor of virus replication at 1, 2 and 3 h. So showed the most of antiviral activity when it used an hour after virus inoculation that this property was reduced at 2 and 3 h (Table 1).

Medicinal plants have been used for different diseases from past to now. Modern studies showed some of the medicinal plants with therapeutic application in traditional medicine [6-8] have antiviral effects [6-8, 26].

There is a requirement for new antiviral drugs since the treatment of HSV-1 infections with the available chemical drugs often leads to the problems to viral resistance [14, 15, 27] and virus latency duration [16, 17, 18]. Studying

medicinal plants may be modern way for treatment of HSV-1 illness [19].

Antiviral effect of *Echium amoenum* L has been not studied in Iran so far. But another property of plant has been studied that in a study was seen antibacterial effect of aqueous extract of *Echium amoenum* L on *Staphylococcus aureus* [4]. So antioxidant effect of plant was seen in human that this property been for rosmarinic acid and flavones [28]. In another study, methanol extract of *Echium amoenum* L had preservation property on liver cells of rat [29]. In our study, preservation property of aqueous extract of *Echium amoenum* L was seen for Hep-2 cells at 1 and 2 hour as if this effect was reduced at 3h. So relaxaion effect of herbal extract at treatment of chronic illness was studied in rat [30, 31]. In another study, extract of *Echium amoenum* L had immunomodulatory properties in Balb/c mice [32].

Because antiherpes effect of *Echium amoenum* L has been studied on Hep-2 cells (has been derived from epithelial of human pharynx) and so antiviral effect of *Echium amoenum* L was seen on HSV-1, can be useful way for treatment of HSV-1 infections. Further research is needed to elucidate the active constituents of this plant which may be useful in the development of new and effective antiviral agents.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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