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INHIBITION OF HSV-1 MULTIPLICATION BY FIVE SPECIES OF MEDICINAL PLANTS

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ABSTRACT

Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. As viral resistance to available chemical drugs causes problems in the treatment of herpes simplex virus type 1 infection, there is an evolving need for new antiherpes drugs. Therefore in the present study 5 species of medicinal plants with ethno-medical background were screened for antiherpes effect against HSV-1in Hep-2(Human epithelial type 2) cells. Different parts of the plants were collected and aqueous extract of them were prepared. These extracts were screened for their cytotoxicity against Hep-2 cell line by cytopathic effect (CPE) assay at concentrations 50-1000 µg/ml. Antiherpes properties of the extracts were determined by cytopathic effect inhibition assay. Four plants extract; *Thymus kotschyanus*, *Echinacea purpurea*, *Camellia sinensis* and *Echium amoenum L* exhibited significant antiherpes effect against HSV-1 at nontoxic concentrations to the cell lines used. The extracts of *Thymus kotschyanus* and *Camellia sinensis* showed highest antiherpes activity against HSV-1 at most concentrations. Our findings indicated that *Camellia sinensis* extract has inhibit HSV-1 multiplication at concentrations 50-1000 µg/ml while this figure for *Thymus kotschyanus* is 100-800 µg/ml and for *Echinacea purpurea* and *Echium amoenum L* are >400 µg/ml. Four plants extract of assay exhibited significant antiherpes activity at a concentration nontoxic to the cell line used. EC50 of *Camellia sinensis* extract was best sample and findings showed *Camellia sinensis* has most selectivity indices. Further research is needed to elucidate the active constituents of these plants which may be useful in the development of new antiviral drugs.

Keywords: Medicinal plants, antiherpes effect, HSV-1

INTRODUCTION

Medicinal plants have been used for different diseases in past. Found fossils showed application Thyme and Cumin for treatment diseases in Sumerians at 5000 years ago **(Mehrabani *et al.*, 2005)**. Iran ancient civilization has inveterate history in cognition and treatment by medicinal plants and Iranian scientists such as Avicenna have tried in improvement and dehisced this science.
According to estimation of WHO, 80% of world people use herbal drugs for treatment diseases, because most of Chemical drugs are expensive **(Borris, 1996)**. So 30% of modern drugs come from plants **(Robert *et al.*, 2000)** and more of 66% plant species have medical value **(Abolhassani, 2004; Torres *et al.*, 2006).**
Modern studies showed some of the medicinal plants with therapeutic application in traditional medicine have antiviral effects **(Hayashi *et al.*, 1997); Isaacs *et al.* (2008, 2011)** and in many studies have been seen plants flushed of flavones, tannin **(Tsuchiya *et al.*, 1994; Kaul *et al.*, 1985)** and alkaloid have antiviral, antibacterial, antifungal and antiparasite effects **(Sindambiwe *et al.*, 1999; Dorman *et al.*, 2000; Sydiskis *et al.*, 1991).**
There is an requirement for new antiviral drugs since the treatment of viral infections with the available chemical drugs often leads to the problems to viral resistance **(Field *et al.*, 1994; Vlietinck *et al.*, 1991)** and virus latency duration **(Wozniak *et al.*, 2009; Fatahzadeh *et al.*,2007; Smith *et al.*,2002).** Studying antimicrobial components of medicinal plants is useful way **(Cseke *et al.*, 2006; Zargari, 1997)**. Therefore in the present study 5 species of medicinal plants were screened for antiherpes effect against HSV-1.

MATERIAL AND METHODS

Extraction

In this study, *Thymus kotschyanus*, *Echinacea purpurea*, *Camellia sinensis*, *Echium amoenumL* and *Nerium oleander* plants were provided from bazaar of medicinal plants of Tehran city. Different parts of the plants were collected and dried at environment temperature 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. Filtered extracts were lyophilized. From herbal extracts were provided working solutions with concentration 1000 µg /ml and were stored in a cool place **(Jewell, 2009)**.

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 microtiterplate 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 TCID50 **(Cragg *et al.*, 1997)**.

Cytotoxicity assay

In a 96-well microtiterplate, Hep-2 cells propagated and incubated at 37ºC in a humidified incubator with 5 per cent CO2 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 37ºC for a week. The morphology of 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.

Antiviral assay

Nontoxic concentrations of plant extracts, *i.e.*, lower than CC_{50} were checked for antiviral activity by CPE inhibition assay **(Hu *et al.*, 1989)**. In this assay, cells were seeded in a 96-well microtiterplate and incubated at 37ºC in a humidified incubator with 5 per cent CO2 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 was treated with concentrations 50-1000 µg /ml of plant extracts. Microtiterplate incubated at 37ºC for seven days. Antiviral activity was determined by the inhibition of CPE compared with cell and virus control wells. The antiviral effective concentration was expressed as the 50% effective concentration (EC50)

which is the concentration of the sample required to inhibit virus-induced CPE by 50% and Selectivity indices for herbal extracts were calculated as CC50/EC50 ratios and were given in Table 3.

RESULTS AND DISCUSSION

Cytotoxicity assay

In cytotoxicity assay of herbal extracts *Nerium oleander* extract was shown more toxicity and all concentration of plant were toxic to Hep-2 cell line (more than 50 µg/ml), while four other studied extracts were good tolerated by cells (Table1). Especially two plants; *Camellia sinensis* and *Echium amoenum L.* were not toxic for cell lines at highest concentration (CC_{50}=1000 µg/ml).

Antiviral assay

In antiviral assay, four plants extract; *Thymus kotschyanus*, *Echinacea purpurea*, *Camellia sinensis* and *Echium amoenumL.* exhibited significant antiherpes effect against HSV-1 at nontoxic concentrations to the cell lines used (Table2). The extracts of *Thymus kotschyanus* and *Camellia sinensis* showed highest antiherpes effect against HSV-1 at most concentrations. Our findings indicated that *Camellia sinensis* extract has inhibit HSV-1 multiplication at concentrations 50-1000 µg/ml while this figure for *Thymus kotschyanus* is 100-800 µg/ml and for *Echinacea purpurea* and *Echium amoenum L* are >400 µg/ml when are used immediately after virus adsorption(Table2). EC50 of *Camellia sinensis* extract was 50 µg/ml and for *Thymus kotschyanus* is 100 µg/ml and for *Echinacea purpurea* and *Echium amoenumL* are 500 µg/ml. So *Camellia sinensis* exhibited most selectivity indices in this study and for *Thymus kotschyanus* was 8, *Echinacea purpurea* and *Echium amoenumL* had minimum SI (Table3).

Table1 Extract preparation manner and cytotoxicity concentration$_{50}$ of selected medicinal plants

Medicinal plants	Part used	Local uses	Extract prepared	CC_{50}
Thymus kotschyanus	Flower+ leaf	P/ UR/ T/ C/ / CS/ AC/ EI	decoction	800
Echinacea purpurea	Root	T/ C/ RI/ CS/ EI	″	900
Camellia sinensis	Leaf	P/ UR/ T/ C/ RI/ CS/ AC/ EI	″	1000
Nerium oleander L.	Flower+ leaf	CS/ AC	″	<50
Echium amoenumL.	Flower	UR/ T/ C/ RI/ CS	″	1000

Legend: CC50 – cytotoxicity concentration(µg/ml), P – pneumonia, UR – Upper respiratory infections, T – Tonsillitis, C – croup, RI – respiratory infections, CS – canker sores, AC – anti cancer, EI – enteric infections

Table2 assay of antiviral effect of selected medicinal plants against HSV-1

Medicinal plants	1000	900	800	700	600	500	400	300	200	100	50
Thymus kotschyanus			n	n	n	n	n	n	n	n	CPE
Echinacea purpurea		n	n	n	n	n	CPE	CPE	CPE	CPE	CPE
Echium amoenumL.	n	n	n	n	n	n	CPE	CPE	CPE	CPE	CPE
Camellia sinensis	n	n	n	n	n	n	n	n	n	n	n

Legend: HSV-1 – herpes simplex virus type 1, KOS strain, (50-1000) – concentrations used (µg /ml), CPE – cytopathic effect of virus, n – not cytopathic effect of virus

Table 3 CC50, EC50, and SI of plants on Hep-2 cells against HSV-1 determined by cytopathic effect (CPE) inhibition assay.

Medicinal plants	CC_{50}	EC50	SI
Thymus kotschyanus	800	100	8
Echinacea purpurea	900	500	1.8
Camellia sinensis	1000	50	20
Echium amoenum L.	1000	500	2

Legend: CC50 – 50% cytotoxic effect concentration, EC50 – 50% effective concentration, SI – Selectivity index (CC50/EC50), HSV-1 – herpes simplex virus type 1, KOS strain.

CONCLUSION

There is an 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 **(Field *et al*.,1994;Vlietinck *et al*.,1991;Frobert *et al*.,2005)** and virus latency duration**(Wozniak *et al*.,2009;Fatahzadeh *et al*.,2007; Smith *et al*.,2002)**. Modern studies showed some of the medicinal plants with therapeutic application in traditional medicine have antiviral effects **(Hayashi *et al*., 1997; Isaacs *et al*. (2008, 2011); Hu *et al*., 1989)**. So studying medicinal plants may be modern way for treatment of HSV-1 illness **(Cseke *et al*., 2006)**.

In this study, Of the 5 plant extracts tested in vitro, herbal extracts of *Camellia sinensis* and *Echium amoenumL* has not toxic effect at highest concentrations (CC_{50}=1000 µg/ml) to the cell lines used and all concentration of *Nerium oleander* extract was toxic on Hep-2 cell line. Findings indicated that *Camellia sinensis* was HSV-1 multiplication inhibitor at concentrations 50-1000 µg/ml. but antiherpes effect of *Thymus kotschyanus* was seen at concentrations 100-800 µg/ml. while the extracts of *Echinacea purpurea* and *Echium amoenumL* have inhibit HSV-1 multiplication at concentrations >400 µg/ml. So EC50 of *Camellia sinensis* extract was best of sample and findings showed *Camellia sinensis* has most selectivity indices.

Because antiherpes effect of 5 plant extracts has been studied on Hep-2 cells (has been derived from epithelial of human pharynx) and so well antiviral effect of four plants extract; *Thymus kotschyanus*, *Camellia sinensis* **(Isaacs *et al*.(2008,2011); Koch *et al*.,2008; Schnitzler *et al*.,2007)**, *Echinacea purpurea* **(Ghaemi *et al*.,2007; Hudson *et al*.,2005)** and *Echium amoenum L.* have been seen on HSV-1, can be useful way for treatment of HSV-1 infections. Further research is needed to elucidate the active constituents of these plants which may be useful in the development of new and effective antiviral agents.

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