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 antiviral drugs. Therefore in the present study 5 species of medicinal plants with ethno-medical background were screened for antiviral effect against HSV-1 and 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 cytopathic effects against Hep-2 cell line by cytopathic effect (CPE) assay at concentrations 50-1000 μg/ml. Antiviral properties of the extracts were determined by cytopathic effect inhibition assay. Four plants extract (Thymus kotschyanus, Echinacea purpurea, Camellia sinensis and Echium amoenum) Exhibited significant antiviral effects against HSV-1 at nontoxic concentrations to the cell lines used. The extracts of Thymus kotschyanus and Camellia sinensis showed highest antiviral activity against HSV-1 at most concentrations. Our findings indicated that Camellia sinensis extract has inhibits 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 plant species exhibited significant antiviral 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, antiviral 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 invertebrate history in cognition and treatment by medicinal plants and Iranian scientists such as Avicenna have tried in improvement and devised 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 (Tschiehy et al., 1994; Kaul et al., 1985) and alkaloid have antiviral, antibacterial, antifungal and antiparasite effects (Sindambiwe et al., 1999; Dorman et al., 2000; Sydikis et al., 1991). 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 (Field et al., 1994; Vlietinck et al., 1991) and virus latency duration (Woźniak et al., 2009; Fatahazadeh et al.,2007; Smith et al.2002). Studying antimicrobial components of medicinal plants is useful way (Cséke et al., 2006; Zargari, 1997). Therefore in the present study 5 species of medicinal plants were screened for antiviral effects against HSV-1.

MATERIAL AND METHODS

Extraction

In this study, Thymus kotschyanus, Echinacea purpurea, Camellia sinensis, Echium amoenum L. 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 microtitrate plate 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 microtitrate plate, Hep-2 cells propagated and incubated at 37°C in a humidified incubator with 5 per cent CO2 for a period of 48 hours. Then different concentrations of herbal extracts (50-1000 μg /ml) were added to cells to DMEM culture. The microtitrate plate was incubated at 37°C for a week. The morphology of the cells were checked daily for cytopathic effect (CPE). The 50% cytotical concentration (CC50) 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 CC50 were checked for antiviral activity by CPE inhibition assay (Hu et al., 1989). In this assay, cells were seeded in a 96-well microtitrate plate and incubated at 37°C in a humidified incubator with 5 per cent CO2 for a period of 48 hours. Then the 100 TCID50 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. Microtitrate incubated at 37°C 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 (CC50=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 amoenum are 500 μg/ml. So Camellia sinensis exhibited most selectivity indices in this study and for Thyme kotschyanus was 8, Echinacea purpurea and Echium amoenum had minimum SI (Table3).

Table 1: Extract preparation manner and cytotoxicity concentration, C50 of selected medicinal plants

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Part used</th>
<th>Local uses</th>
<th>Extract prepared</th>
<th>CC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus kotschyanus</td>
<td>Flower leaf</td>
<td>P/ UR/ T/ C/ / CS/ AC/ EI</td>
<td>decoction</td>
<td>800</td>
</tr>
<tr>
<td>Echinacea purpurea</td>
<td>Root</td>
<td>T/ C/ RI/ CS/ EI</td>
<td></td>
<td>900</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Leaf</td>
<td>P/ UR/ T/ C/ RI/ CS/ AC/ EI</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Nerium oleander L.</td>
<td>Flower leaf</td>
<td>CS/ AC</td>
<td></td>
<td>&lt;50</td>
</tr>
<tr>
<td>Echium amoenumL.</td>
<td>Flower</td>
<td>T/ C/ RI/ CS</td>
<td></td>
<td>1000</td>
</tr>
</tbody>
</table>


Table 2: assay of antiviral effect of selected medicinal plants against HSV-1

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Part used</th>
<th>Local uses</th>
<th>Extract prepared</th>
<th>CC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus kotschyanus</td>
<td>Flower leaf</td>
<td>P/ UR/ T/ C/ / CS/ AC/ EI</td>
<td>decoction</td>
<td>800</td>
</tr>
<tr>
<td>Echinacea purpurea</td>
<td>Root</td>
<td>T/ C/ RI/ CS/ EI</td>
<td></td>
<td>900</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Leaf</td>
<td>P/ UR/ T/ C/ RI/ CS/ AC/ EI</td>
<td></td>
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<td></td>
<td>&lt;50</td>
</tr>
<tr>
<td>Echium amoenumL.</td>
<td>Flower</td>
<td>T/ C/ RI/ CS</td>
<td></td>
<td>1000</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>CC50</th>
<th>EC50</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus kotschyanus</td>
<td>800</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>Echinacea purpurea</td>
<td>900</td>
<td>500</td>
<td>1.8</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>1000</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Echium amoenumL.</td>
<td>1000</td>
<td>500</td>
<td>2</td>
</tr>
</tbody>
</table>

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 methods 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 amoenum has not toxic effect at highest concentrations (CC50=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 amoenum 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 antiherpetic 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; Schnitzer 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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REFERENCES


