

The influence of extracts from *Peucedanum salinum* on the replication of adenovirus type 5

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Submitted: 16 August 2010

Accepted: 26 October 2010

Arch Med Sci 2012; 8, 1: 43-46

DOI: 10.5114/aoms.2012.27279

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Abstract

Introduction: The aim of the study was to examine the cytotoxicity and evaluate the antiviral and virucidal activity of methanol and methanol/H₂O extracts from the herb of *Peucedanum salinum*. Plants belonging to the genus *Peucedanum* (*Apiaceae* family) have been used in traditional medicine for a long time.

Material and methods: The examination of the cytotoxicity of the extracts in the concentrations of 0.1, 0.2, 0.5, 1, 2, 4 mg/ml was carried out on the cell culture line HEK-293. Cytotoxicity of the examined extracts was measured by the colorimetric MTT (tetrazolium) method. After determination of the highest non-toxic concentration of examined extracts, antiviral and virucidal activity against adenovirus type 5 (*Adenoviridae*) was established.

Results: The non-toxic doses were as follows: 1 mg/ml of methanol extract and 2 mg/ml of methanol/H₂O extract (1 : 1 v/v). Antiviral activity was observed for the methanol extract of *Peucedanum salinum* in concentrations of 0.5 and 1 mg/ml. The extract caused the decrease of titre of the virus by 2 log and 1.33 log, respectively. The methanol/H₂O extract (1 : 1 v/v) decreased the titre of the virus by 1.33 log and 1.5 log in concentrations of 1 and 2 mg/ml, respectively. The examined extracts had no virucidal activity against adenovirus type 5.

Conclusions: The examined extract is a new, potentially active source of active substances possessing antiviral activity and further studies are needed.

Key words: cytotoxicity, antiviral activity, virucidal activity.

Introduction

The genus *Peucedanum*, belonging to the *Apiaceae* family, comprises numerous species widely distributed in Europe. Some of these plants have been used in traditional medicine for a long time. The leaves and roots of *Peucedanum japonicum* were used in ancient times as a medicine for the treatment of patients with sore throat in the Ryukyu Islands and in Taiwan. Further experiments show that the extract, by reducing the cell proliferation activity, has the chemopreventive potential in rat colon carcinogenesis [1]. The leaves are also traditionally used for food preservation, probably due to antioxidant activity [2]. Roots of *Peucedanum palustre* are known in folk medicine as being effective in the treatment of epilepsy and gastrointestinal disorders. Moreover, a strong inhibitory effect of the prolactin release from rat pituitary tumour cells was demonstrated [3]. The aerial parts and the roots of *Peucedanum ostruthium*, boiled in wine and drunk, help in joint pain. Furthermore, the roots, seeds, leaves and juice expel thick slime responsible for such pain [4].

It is well known that plants from the *Apiaceae* family exhibit antiviral and virucidal activity [5, 6]. Methanol and methanol/H₂O extracts contain mostly coumarins and phenolic acids which are known as strong antiviral agents; e.g. chlorogenic and caffeic acids, which the *Peucedanum* genus is abundant with [7], possess a strong antiviral effect either against DNA (*Herpes simplex*) or RNA virus (*HIV*, *Influenza*) [8, 9]. Strong anti-HIV activity is also characteristic of a series of coumarins [10]. Because adenoviruses are responsible for about 10% of acute respiratory infections, especially in children, the first step of our experiments was to examine the influence of extracts from *Peucedanum salinum* on the replication of adenovirus type 5. Human adenoviruses are a frequent cause of potentially fatal infections in patients after allogeneic stem cell transplantation [11]. Human adenoviruses, including adenovirus type 5, have often been used as model viruses for testing virucidal efficacy of disinfectants against all human pathogenic viruses [12]. As far as we know, these kinds of experiments were performed for the first time and no information is available on biological activity and chemical content of the examined plant.

Material and methods

Plant material

The herb of *Peucedanum salinum* was collected in 2006, in Mongolia. It was identified by a specialist from the Herbarium of the Botanical Institute of the Mongolian Academy of Science, Ulaanbaatar, Mongolia, where voucher specimens of the plant have been deposited. The plant material was dried and immediately milled according to the accepted standards.

Extraction

Ten grams of milled herb of *Peucedanum salinum* were refluxed for 30 min with 100 ml of the following solvents: methanol and methanol/H₂O (1 : 1 v/v). Obtained extracts were filtered and further extraction was repeated twice. Obtained extracts were combined and concentrated under the vacuum to dry residues. Next, the initial examined solutions from each extract were prepared in concentrations of 19 mg/ml and 17 mg/ml (dissolved in methanol and methanol/H₂O (1 : 1 v/v), respectively).

Cell cultures and viruses

The HEK-293 cell culture (human embryonic kidney) from the American Type Culture Collection (ATCC CRL – 1573) was used in the experiment. The media in the culture (Minimum Essential Medium Eagle, Sigma) were supplemented with 10% fetal

bovine serum (FBS, Sigma), 100 U/ml of penicillin and 0.1 mg/ml of streptomycin (Polfa-Tarchomin, Poland). The cell culture was incubated at 37°C in a 5% CO₂ atmosphere.

For antiviral and virucidal activity of examined extracts the human adenovirus type 5 (ATCC VR – 1516) from the American Type Culture Collection was used. The virus was propagated in the HEK-293 cell culture. Virus stock was stored at –70°C until used. The titre of the virus was 2×10^4 TCID₅₀/ml.

Cytotoxicity assay

The concentration of the examined extract was 19 mg/ml for methanol and 17 mg/ml of methanol/H₂O, respectively. Extracts were sterilised by the use of 0.2 µm filters (SARSTEDT) and then stored at 4°C. One hundred microlitres of the prepared HEK-293 cell culture were plated into 96-well plastic plates (NUNC) at a cell density of 2×10^4 cells per well. After 24 h incubation at 37°C the media were removed and the cells were treated with a solution of the examined substance diluted in the media with 2% serum. The following final concentrations were applied: 0.1, 0.2, 0.5, 1, 2, 4 mg/ml. The culture cells were incubated for 72 h at 37°C in a 5% CO₂ atmosphere.

Cytotoxicity of tested extracts was estimated by the MTT method, described by Takenouchi and Munekata [13]. The MTT method is a quantitative colorimetric toxicity test, based on the transformation of yellow, soluble tetrazolium salts (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to purple-blue insoluble formazan. This process occurs naturally in mitochondria of living cells. After dissolution in an organic solvent, formazan is quantified spectrophotometrically at two wavelengths, 540 and 620 nm, in a multi-well scanning spectrophotometer (96-well plastic plates, Organon Teknika). On the basis of the test results the cytotoxic concentration (CC₅₀), which is the amount of tested substance that caused 50% decrease of cell activity compared to the control culture, was determined.

Antiviral activity assay

After 24 h incubation, the cell culture was infected with a virus in the dose of 100 TCID₅₀/ml. After 1 h incubation at 37°C the suspension of the virus was removed and the media with 2% serum together with the tested extracts were added to the cell cultures in the following concentrations: methanol extract 0.2, 0.5, 1 mg/ml; methanol/H₂O extract 0.5, 1, 2 mg/ml. The virus diluted in the culture media without tested extracts was used as a control. After 48 h incubation at 37°C the cells were frozen and after thawing the virus was titrated in the HEK-293

cell culture. The cytopathic effect (CPE) of the virus was examined by a light microscope and the titre of the virus was estimated according to the Reed-Muench method [14]. The investigation was carried out in triplicate.

Virucidal activity

For the evaluation of the virucidal activity of *Peucedanum salinum* extracts, non-toxic for cell culture (HEK-293) concentrations of these extracts were applied. Viral suspensions in the dose of 100 TCID₅₀/ml were mixed (1 : 1, v/v) with extracts which were diluted in media without fetal bovine serum to appropriate final concentrations. Mixtures were incubated at 37°C for 1 h and then the virus was titrated in cell culture. The viruses' suspension of adenovirus type 5 with media but without tested extracts was a control. The cytopathic effect (CPE) of each virus occurring after 24 h of incubation was measured using the Reed-Muench method [14]. Methanol and methanol/H₂O (1 : 1 v/v), used as a solvent, at the applied concentration was toxic neither to the cell culture nor the virus.

The investigation was carried out in triplicate.

Results

The influence of methanol and methanol/H₂O extracts from the herb of *Peucedanum salinum* on the HEK-293 cell culture after incubation for 72 h is presented in Table I.

Methanol used as an eluent for examined extracts in the tested concentration had no toxic effect on cell cultures. The investigated extracts proved to be non-toxic for cells, and did not cause any visible changes in the morphology of the cells, at the concentration range of 0.1-1 mg/ml for methanol extract and 0.1-2 mg/ml for 50% methanol extract.

For the determination of antiviral and virucidal activity, three different, non-toxic concentrations of examined extracts were chosen: 0.2, 0.5, 1 mg/ml of methanol extract, and 0.5, 1, 2 mg/ml of 50% methanol extract. Methanol used as an eluent for examined extracts in tested concentrations had no antiviral or virucidal activity against adenovirus type 5.

Antiviral activity against adenovirus type 5 (DNA virus, *Adenoviridae*) for both extracts obtained from the herb of *Peucedanum salinum* was observed. The methanol extract in the concentrations of 0.5 and 1 mg/ml decreased the titre of the virus by 2 log and 1.33 log (66.6% and 44.3%), respectively. The methanol/H₂O extract in the concentrations of 1 and 2 mg/ml decreased the titre of the virus by 1.33 log and 1.5 log (44.3% and 50%), respectively (Table II).

Virucidal activity of examined extracts is presented in Table III. Methanol and methanol/H₂O

Table I. The influence of *Peucedanum salinum* on the viability of HEK-293 cells. The results are given as the percentage of viability of HEK-293 cells to the control cell culture

Extracts [mg/ml]	Exposure time (72 h)	
	Methanol [%]	Methanol/H ₂ O (1 : 1 v/v) [%]
0 (control)	100.0	100.0
0.1	100.0	100.0
0.2	100.0	99.7
0.5	100.0	100.0
1	71.9	88.8
2	43.3	60.6
4	0.0	35.3

Table II. Antiviral activity of the extracts from *Peucedanum salinum* against adenovirus type 5

Extracts [mg/ml]	Adenovirus type 5 [TCID ₅₀ /ml]*	
	Methanol	Methanol/H ₂ O (1 : 1 v/v)
0 (control)	3.0 ± 0.53	3.0 ± 0.53
0.2	2.0 ± 0.45	–
0.5	1.0 ± 0.45	2.5 ± 0.41
1	1.67 ± 0.37	1.67 ± 0.37
2	–	1.5 ± 0.32

*The virus titres are shown in log: mean ± SD

extracts in the concentrations of 0.2, 0.5, 1 mg/ml were incubated with adenovirus type 5 suspension. After 1 h incubation at 37°C, decrease of the virus titration was not observed compared to the control.

The methanol extract at the concentrations of 0.5 and 1 mg/ml decreased the virus titre by 0.27 log while the methanol/H₂O extract in all tested concentrations decreased the virus titre by 1.17 log.

Discussion

The increasing danger of viral infections requires searching for new non-toxic antiviral drugs. The pre-

Table III. Virucidal activity of the extracts from *Peucedanum salinum* against adenovirus type 5

Extracts [mg/ml]	Adenovirus type 5 [TCID ₅₀ /ml]*	
	Methanol	Methanol/H ₂ O (1 : 1 v/v)
0 (control)	1.5 ± 0.41	1.5 ± 0.41
0.2	1.5 ± 0.41	–
0.5	1.23 ± 0.49	1.33 ± 0.36
1	1.23 ± 0.49	1.33 ± 0.36
2	–	1.33 ± 0.36

*The virus titres are shown in log: mean ± SD

sented results are a preliminary stage of further studies on the biological activity of compounds from the herb of *Peucedanum salinum*. Plants represent a large potential source of antiviral agents. About 30% of tropical plants have been observed to possess such activity. A large number of compounds responsible for this activity have been isolated, such as coumarins, phenolic acids and essential oils [15-17]. At the same time, these compounds are dominant in the genus *Peucedanum* and probably can share responsibility for the antiviral activity [7, 18, 19].

Our investigations proved the slight virucidal activity of the *Peucedanum salinum* extracts. Adenoviruses belong to a group of non-enveloped viruses. The research of Sauerbrei *et al.* proved that hexon, the capsid protein of adenoviruses, is strongly resistant to chemical biocides [12]. This can explain our research results, whereas the observed activity of extracts can be explained by the fact that extracts were added to cell cultures with already infected cells. The virus affected by extracts then had already been devoid of the protein envelope. In order to explain the mechanism of the extract activity against adenovirus type 5, further studies are advised to determine which viral protein synthesis is inhibited by agents present in tested extracts. So far, the examination of antiviral activity of extracts from the herb of *Peucedanum salinum* has been focused on the influence on virus replication in infected cells. The next step is the examination of its influence on other parts of virus replication, such as adsorption or penetration.

An antiviral compound could protect cells against virus infection in several ways, by directly inactivating the virus or by interfering with the replication cycle [20-22]. Plant extracts reveal the virucidal and antiviral activity definitely more often against DNA viruses than RNA ones [23-25].

Because the examined extract is a new, potentially active source of the active substances, further studies should be focused on the isolation of compounds and examination of their antiviral activity against other viruses.

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