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Short communication

Antiviral activity of an extract derived from roots of *Eleutherococcus senticosus*

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Abstract

A liquid extract from *Eleutherococcus senticosus* roots inhibited the productive replication of human rhinovirus (HRV), respiratory syncytial virus (RSV) and influenza A virus in cell cultures infected with these viruses, all of which belong to the RNA type viruses. Analysis of virus production after treatment of the infected cells using plaque-reduction assays showed a strong antiviral activity of the *Eleutherococcus* extract. In contrast, no effect was detected using the same protocol for cells infected with the DNA viruses, adenovirus (Adeno 5) or herpes simplex type 1 virus (HSV 1). Pre-treatment of cells did not inhibit either virus adsorption or virus replication. The results of the study demonstrate that the *Eleutherococcus* extract inhibited the replication of all RNA viruses studied so far. This antiviral activity remained stable under the conditions used for drug preparation and storage. © 2001 Elsevier Science B.V. All rights reserved.

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During the last few years efforts were made to increase the number of substances with antiviral activity. Few substances are known which provide an effective treatment of viral infections in vivo (Balfour, 1999). Most of these substances with antiviral activity belong to the class of nucleoside analogues, e.g. aciclovir and ribavirin (Fyfe et al., 1978; Hruska et al., 1990). Amantadine, a cyclic amine, has been widely used in prophylaxis and therapy of influenza virus infections due to its unique antiviral activity (Hay et al., 1985). Recently, important progress in the therapy of influenza was made with Zanamivir and Oseltamivir, both inhibitors of the viral neuraminidase (Calfee and Hayden, 1998). However, the therapeutic po-

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tency of most of the antiviral agents encountered so far is counteracted by their severe side effects in humans (Hayden et al., 1983; Janai et al., 1990; Englund et al., 1990). Thus, the search for anti-viral substances with high efficacy, low toxicity and minor side effects must continue.

In addition to the development of synthetic drugs as mentioned above, another approach is based on the screening of plant-derived substances for antiviral activity. During the last 30 years extracts from more than 4000 different species were studied and about 10% of them showed a significant antiviral activity in vitro (Che, 1991). However, only a very small number were systematically investigated further for their reactivity.

Among the plants with known antiviral activity, *Eleutherococcus senticosus* is mentioned repeatedly. *E. senticosus*, growing in the south eastern part of Russia, mainly in Siberia, is used in traditional medicine in China and Russia to stimulate the immune system (Wacker, 1983; Aicher and Wozniewski, 1998) and for prophylaxis of infectious diseases (Wacker and Eilmes, 1978; Wacker, 1983; Protasova and Zykov, 1984).

The aim of the study presented here was to determine the antiviral activity of *E. senticosus* against a panel of human pathogenic viruses in more detail. This study included both DNA and RNA viruses: herpes simplex type 1 virus (HSV 1, strain Thea), which belongs to enveloped double-stranded DNA viruses, adenovirus (Adeno 5), a member of a double-stranded DNA virus lacking an outer membrane, human rhinovirus (HRV 14), a non-enveloped, single-stranded RNA virus, and two enveloped, single-stranded RNA viruses, influenza A virus (Chile 1/83, H1N1) and the respiratory syncytial virus (RSV, strain Long).

Before the antiviral activity of an *E. senticosus* extract (Boehringer Ingelheim Pharma KG, Germany, 33% (v/v) ethanol) was studied, its influence on cell cultures used for virus replication was analyzed. To assay for possible toxic effects of the different drug preparations, confluent monolayers of MDCK, HEp-2 and HeLa cells were incubated without (controls) or with increasing dilutions (1:20; 1:40 etc.) of the ethanolic extracts for five days. Analyses of the cytotoxicity of the substances were performed either with an enzymatic assay (MTT-assay, Mosmann, 1983) or fluorescein diacetate (FDA) exclusion and ethidium bromide (EtBr) incorporation (Jackson and Pappas, 1974). The results of the analyses shown in Table 1, measuring the mitochondrial activity and physiologically active cell membranes, demonstrated a low toxicity of the extract for the different cell lines. The 50% inhibitory dilution (ID_{50}) for *Eleutherococcus* extract calculated from several experiments was in a dilution of less than 1:32. To exclude the possibility of any concentration-dependent artifacts, dilutions of 1:80 in the highest concentration were used in assays for antiviral activity.

To test the effect of the *Eleutherococcus* extract on virus replication, the cells (HeLa, HEp-2,

Table 1
Determination of the cytotoxicity of the *Eleutherococcus* extract [a]

Inhibitory dilution (ID)	Eleutherococcus extract dilution ($1/x$)					
	MTT			FDA/EtBr		
	HeLa	HEp-2	MDCK	HeLa	HEp-2	MDCK
ID_{90}	<20	<20	<20	<20	<20	<20
ID_{50}	27	29	30	30	32	26
Lowest dilution used in the antiviral tests	1:80	1:80	1:80	1:80	1:80	1:80

[a] The cytotoxicity of *Eleutherococcus* extract on confluent cell cultures was measured five days after the addition of the substance. The viability of the cells (HeLa, HEp-2, MDCK) cultivated with different dilutions of *Eleutherococcus* was quantified either using an MTT-test (left column) or by determination of the ratio FDA versus EtBr positive cells (right column). The relative cytotoxicity was standardized by the medium control representing 100% viability. All data represent six replicates derived from two experiments. Standard deviations were less than 14%.

Table 2
Activity of *Eleutherococcus* extract against DNA and RNA viruses [a]

Virus	Time of treatment [b]	Effective dilution (ED)	
		ED_{50}	ED_{90}
DNA-viruses			
Adenovirus 5	+1	<80 [c]	<80 [c]
HSV 1 (strain Thea)	+1	<80 [c]	<80 [c]
RNA-viruses			
Rhinovirus (HRV 14)	+1	120	<80
Rhinovirus (HRV 14)	−1	<80 [c]	<80 [c]
RSV (strain Long)	+1	2240	<640
RSV (strain Long)	−1	<80 [c]	<80 [c]
Influenza A (Chile 1/83, H1N1)	+1	120	<80
Influenza A (Chile 1/83, H1N1)	−1	<80 [c]	<80 [c]

[a] HEp-2 cells were infected with HSV-1, Adeno 5 (MOI of 0.0002) or RSV (MOI of 0.0004); HeLa cells with HRV-14 (MOI of 0.0002) and MDCK cells with influenza A virus (MOI of 0.0002). After infection, cell monolayers were incubated without or in the presence of different dilutions of *Eleutherococcus* extract (+1). In the case of HSV 1, HRV, RSV the antiviral activity was determined in plaque-reduction assays. In the case of Adeno 5, the virus synthesis was quantified in the supernatants by an enzyme immunoassay (ELISA). By using a prophylactic protocol, cells were incubated with different dilutions of *Eleutherococcus* extract for 1 hour before infection (−1). Thereafter cells were washed intensively and infected as described above. All data represent six replicates derived from two experiments. Standard deviations were less than 12%.

[b] Relative to virus addition to cells. (−) Different dilutions of the *Eleutherococcus* extract were added 1 h before infection; (+) different dilutions of the *Eleutherococcus* extract were added 1 h after infection and left on the cells throughout the incubation period.

[c] No inhibition at 1:80 dilution of extract.

MDCK) were infected with a multiplicity of infection (MOI) of 0.0002 (HSV 1; Adeno; HRV; influenza A) or 0.0004 (RSV), without or in the presence of different dilutions of the *Eleutherococcus* extract. The antiviral activity of the extract against HSV-1, HRV, RSV and influenza A was determined in plaque-reduction assays (Cooper, 1955). Therefore infected cell cultures were cultivated for three days (HeLa; HRV), five days (MDCK; influenza A), seven days (HEp-2; HSV-1), or eight days (HEp-2; RSV) until virus plaques were visible. In case of the adenovirus, the amount of newly synthesized virus was determined by titrations and enzyme immunoassays (ELISA) after six days of cultivation. The data are summarized in Table 2.

A therapeutic treatment of cell cultures infected with two different DNA viruses — the enveloped HSV-1 and the non-enveloped adenovirus 5 — with the *Eleutherococcus* extract had no effect on the virus replication in vitro (Table 2). In contrast, a clear antiviral effect of the *Eleutherococcus* extract against pathogenic RNA viruses like HRV, RSV and influenza A virus could be detected.

The *Eleutherococcus* extract showed a significant antiviral activity against HRV-infected cell cultures (Table 2; Fig. 1). At a dilution of 1:80, a 62% reduction of the HRV plaques was detectable. The dose-dependent anti-viral effect of the *Eleutherococcus* extract with a 50% effective dilution (ED_{50}) was reached using a dilution of 1:120.

To extend the studies to other RNA-viruses, the antiviral activity of the *Eleutherococcus* liquid extract was studied in RSV-infected cell cultures (Table 2, Fig. 1). The extract showed a marked antiviral activity against RSV with nearly 100% (ED_{90}) reduction of the RSV-infectivity at a dilution of < 1:640 (Table 2; Fig. 1). The 50% effective dilution (ED_{50}) was reached at a dilution of 1:2240. The antiviral activity of ribavirin (Virazole®, ICN Pharmaceuticals, Frankfurt, Germany), a synthetic molecule with known activity

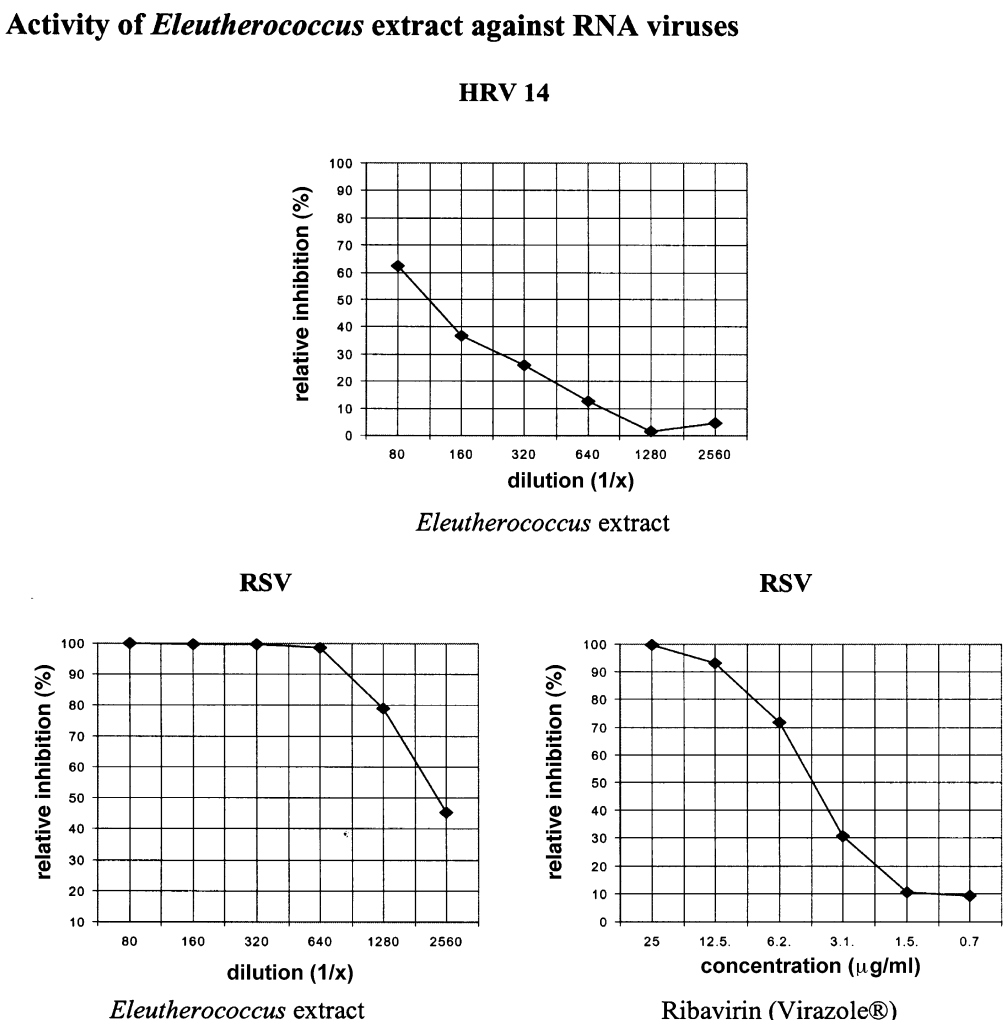

Fig. 1. Activity of *Eleutherococcus* extract against RNA viruses. HeLa cells were infected with HRV-14 (MOI of 0.0002), HEp-2-cells with RSV (MOI. of 0.0004) and MDCK cells with influenza A virus (MOI of 0.0002). After infection, cell monolayers were incubated without or in the presence of different dilutions of *Eleutherococcus* extract (dilution $(1/x)$ (abscissa). The antiviral activity of *Eleutherococcus* extract against the viruses (HRV, three days p.i.; RSV, seven days p.i.; influenza A, five days p.i.) was determined in plaque reduction assays. The relative inhibition (%) by *Eleutherococcus* extract (ordinate) was calculated by counting the number of plaques of the respective groups. Also shown is the activity of ribavirin (Virazole®) against RSV and of amantadine hydrochloride against influenza A. All data represent six replicates derived from two experiments. Standard deviations were less than 12%.

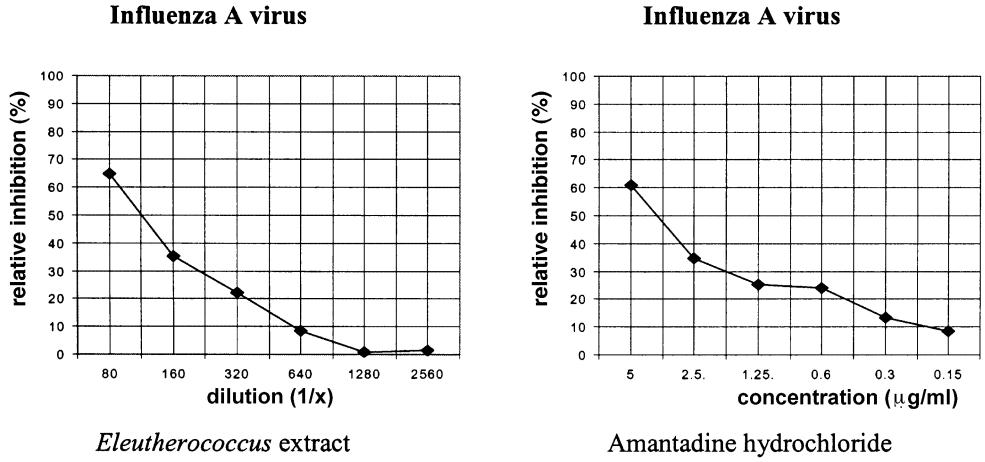

Fig. 1. *Continued*

against RSV (Hruska et al., 1990) (also shown in Fig. 1) enabled a partial quantification of the antiviral effect of *Eleutherococcus* extract. The *Eleutherococcus* extract at a dilution of 1:2240 (Table 2; Fig. 1) showed the same effect as about 5 µg/ml ribavirin, resulting in a 50% reduction of the antiviral activity. These data, derived from plaque-reduction assays, could be confirmed by microscopic examinations for the generation of RSV-induced syncytia in cell cultures and by quantification of the newly synthesized viral proteins in RSV-specific ELISA (data not shown).

On the basis of these results the antiviral activity of *Eleutherococcus* extract was further studied against influenza A infection. The *Eleutherococcus* extract showed significant activity against influenza A virus comparable to its activity against other RNA viruses (i.e. HRV and RSV). At a dilution of 1:80 a reduction of 64% of the virus plaques was visible (Table 2; Fig. 1). Interestingly, amantadine hydrochloride (Ratiopharm, Ulm, Germany; Fig. 1) which served as positive control against the influenza A virus infection (Hay et al., 1985), showed nearly the same antiviral effect as the *Eleutherococcus* extract. This means that the activity of *Eleutherococcus* extract against influenza A in a dilution of 1:80 was comparable to the activity of 5 µg/ml amantadine.

It was then examined whether the *Eleutherococcus* extract might also have a prophylactic effect on HRV, RSV and influenza virus infections and whether a dose-dependent treatment of cell cultures before infection would inhibit the individual virus infections. In none of these experiments any prophylactic effect against HRV, RSV or the influenza A virus could be detected (Table 2).

This antiviral activity could not be affected by routine production steps of herbal drug preparation. Neither storage of the liquid extract prepared from *E. senticosus* roots at 50°C for one month nor the conversion of the liquid extract to a dry extract preparation used in Eleu-Kokk® sugar-coated tablets (Pharmaton, Biberach, Germany) had any influence on the efficacy of the extract. This is an important finding with regard to the stability of the active substance(s) in the liquid extract during production, storage and usage.

Taken together, the results of this study demonstrated a significant in vitro antiviral activity of an extract derived from roots of *E. senticosus* against the RNA viruses, HRV, RSV and influenza A virus. In contrast to the reactivity against RNA viruses, no activity against DNA viruses HSV-1 and Adeno 5 was detected.

The nature of the component(s) of *E. senticosus* responsible for its antiviral activity remains to be determined. This is currently under investigation together with the characterization of the target molecules and the molecular basis of the antiviral efficacy of *E. senticosus*.

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