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Protective efficacy of an aerosol preparation, obtained from *Geranium sanguineum* L., in experimental influenza infection

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A polyphenol-rich extract from the aerial roots of the medicinal plant *Geranium sanguineum* L. (PC) protected mice from mortality in the experimental influenza A/Aichi/2/68 (H3N2) virus infection. To provide evidence how a maximum therapeutic benefit can be derived of this preparation, it was inoculated by 6 different routes. It was found that the aerosol application of PC was highly effective. In the dose 5.4 mg/ml, applied according to a prophylactic-therapeutic schedule, the extract exhibited a marked protective effect. The protective index reached the value of 70.1% and the mean survival time was prolonged with 2.9–4.9 days. The lung infectious virus titres and the lung consolidation of virus-infected and PC-treated animals were all reduced in comparison with control. The application of PC according to schedules, excluding the pretreatment of mice, proved that this condition was essential for protection.

1. Introduction

Despite the considerable achievements of antiviral chemotherapy, the options for control and treatment of influenza are limited and an obvious need continues to exist for effective therapies. In the search of novel antiviral drugs for influenza virus infection the extracts and products of plant origin provide an alternative source for substances with influenza virus-inhibitory activities. Often the virus inhibitory effect has been attributed to the presence of polyphenol compounds, a large family of natural compounds widely distributed in plants and exhibiting a variety of biological activities (Che 1991).

Earlier research showed that a polyphenol-rich extract, defined as polyphenolic complex (PC), isolated from the medicinal plant *Geranium sanguineum* L. protected mice from mortality in the experimental influenza A/Aichi/2/68 (H3N2) virus infection (Serkedjieva and Manolova 1992). To provide evidence how a maximum therapeutic benefit can be derived of the extract, it has been inoculated by 6 different routes (orally, intranasally, by aerosol, subcutaneously, intraperitoneally or intravenously) (Serkedjieva et al. 2002). It was found that the aerosol application of PC conferred a marked protective effect. In addition it has been suggested that the aerosol treatment with polyphenols could be the therapy of choice for influenza virus infection because the bioavailability of high molecular weight polyphenols is poor and a local administration of an aerosol formulation would be an advantage (Droebner et al. 2007). It has been shown also that the aerosol route of inoculation might be useful in the treatment of viral respiratory infections due to an increased amount of the drug reaching the viral-targeted tissues (Sidwell et al. 1994).

Based on this rationale we undertook the current study to investigate on the protective effect of the plant preparation, applied by the aerosol route of administration.

2. Investigations, results and discussion

The present experiments provided information about the effect of the aerosol application of PC in the course of the murine experimental influenza infection. In preliminary experiments the acute and chronic toxicity of PC was evaluated. LD_{50} for the intranasal route of inoculation was >80 mg/kg (>32 mg/ml).

In the current study we used 3 different schedules of treatment with the aerosolized preparation. The schedules of treatment were chosen on the base of previous experiments where the preparation was inoculated by 6 different routes according to 29 schedules of treatment (Serkedjieva et al. 2002). The prophylactic-therapeutic schedule of treatment proved to be most suitable. Thus applied by aerosol according to this schedule, in the dose 5.4 mg/ml, the extract exhibited a marked protective effect. The results are presented in Table 1 and the Fig. From the survival curve (Fig.) it becomes evident that the animals from the group, treated according to the third experimental schedule (-24 h treatment) survived best, the mortality rate was much slower. The protective index reached the value of 69.9% and the mean day to death was extended with 5.2 days, the reduction of lung infectious titre was 3.7 $\log_{10}$ $TCID_{50}$ (Table 1). The inoculation of the preparation at the time of viral challenge also reduced the lung infectious virus (δ $\log_{10}TCID_{50} = 2.4$) and increased defense (PI = 34.6; MST = +3.6); the application of PC after viral

Table 1: Protective effect of aerosolized PC in the murine influenza virus A/Aichi infection[a]

Experimental group (PC 5.4 mg/ml)	Lung parameters[b] Schedule of treatment	Score[c]	Virus titre[d] (EID_{50}/ml)	Mortality[e] (%)	Protective index[f] (%)	MST[g] (days)
Virus control		3.7 ± 0.4	5.5 ± 0.5	77.7 ± 2.9		8.2 ± 1.2
+ PC1	+24, +48, +72 h	3.0 ± 0.0	5.1 ± 0.3	59.6 ± 2.9	23.4	11.1 ± 2.1[i]
+ PC2	0, +24, +48, +72 h	2.7 ± 0.3[h]	3.1 ± 0.3[i]	50.0 ± 0.0[i]	34.6	11.8 ± 1.2[i]
+ PC3	−24, 0, +24, +48 h	1.3 ± 0.5[h]	1.8 ± 0.3[i]	23.3 ± 3.5[i]	70.1	13.4 ± 2.8[i]

[a] the results are the mean from three separate experiments
[b] determined on day 6 p.i.
[c, d, e, f] determined as described in Materials and Methods section
[g] mean survival time
[h] the difference between control and treated group is significant ($p < 0.05$, Wilcoxon ranked sum analysis test)
[i] the difference between control and treated group is significant ($p < 0.05$, Student's *t* test)

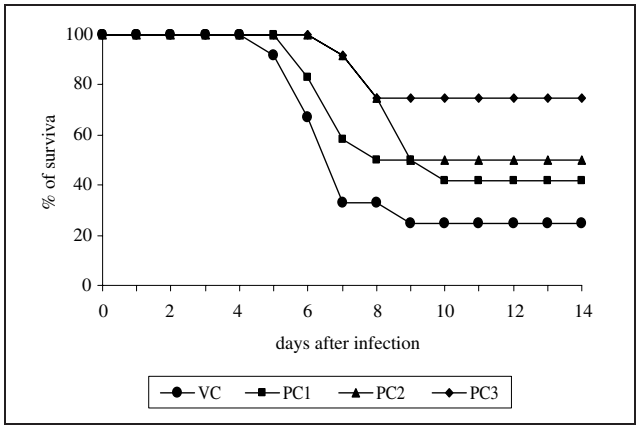

Fig.: Survival rates of influenza virus A/Aichi infected mice, treated with aerosolized PC*.
* the results are from one representative experiment, PC1, 2, 3 – as in Table 1

load did not achieve significant protection. As this group was treated three times in contrast with the other two groups, treated four times, one might speculate that a longer treatment regimen might be of some additional benefit. Obviously most successful was the −24 h treatment or the prophylactic-therapeutic regimen of treatment; the pretreatment of mice with PC proved to be essential for the protection from mortality in the experimental influenza infection. We have shown previously that this is also a requirement for the intranasal route of application (Serkedjieva et al. 2002). Sidwell et al. (1994), treating mice with an aerosolized plant flavonoid in influenza A/WSN/33 (H1N1) virus infection, also observed that the greatest protective effect was achieved using a treatment regimen beginning prior to virus exposure.

In an additional experiment lung parameters were determined (Table 1). The superior efficacy of the prophylactic-therapeutic schedule was confirmed. The lesions of the lung due to infection as well as lung infectious virus titres were significantly reduced in the virus-infected and PC-treated animals in comparison to control (virus-infected and PC not-treated) mice.

We have previously investigated the mode of the anti-influenza virus activity of the partially standardized plant preparation, isolated from *Geranium sanguineum* L (PC). Its *in vitro* virus-inhibitory effect was studied in respect to its specificity and selectivity (Serkedjieva and Hay 1998). It was shown that the inhibitory effect was strain-dependent, consistent with a selective antiviral action. In one-cycle experiments of viral growth with A/chicken/Germany/34, strain Rostock (A/Rostock) in CEF cells, PC was most effective applied 1 to 3 h after virus infection; presumably it affected the early synthetic stages of viral replication. Virus-specific protein synthesis (Serkedjieva 1995) as well as virus-specific RNA synthesis in RK cells, infected with A/WSN (Serkedjieva and Tonew 1996) was selectively inhibited. In addition the selectivity of inhibition was confirmed by the generation of PC-resistant variants of A/Rostock (Serkedjieva 2003).

The significant protective effect of preparation in the murine experimental influenza virus infection has not been investigated thoroughly. We presupposed that the protection might be attributed to the combination of more than one biological activities of the extract – selective antiviral effect, non-selective immunomodulatory activity and some non-specific biological and pharmacological interactions, known for polyphenols, such as protein binding, radical scavenging and antioxidant activities. To provide evidence how a maximum therapeutic advantage can be derived of this preparation, it was inoculated by 6 different routes according to 29 schedules of treatment (Serkedjieva et al. 2002). It was found that the nasal application of PC was highly effective. The aerosol treatment, applied in the present experiments, proved to be effective as well. This might be due to an increased amount of the extract reaching the viral-targeted tissues; similar enhancement of the inhibitory effect of ribavirin against viral respiratory infections has been achieved using the aerosol route of inoculation (Smee et al. 2000).

In model systems it has been found that the plant preparation possessed some other biological activities: a stimulating effect on cell type immune response, induction of serum interferon after intraperitoneal application (Toshkova et al. 2004), O_2^- radical scavenging activity (Sokmen et al. 2005) and enzyme-inhibitory effect (Antonova-Nikolova et al. 2002). The immunomodulatory effect has been confirmed *in vivo* (Ivanova et al. 2005). The variety of biological activities of the plant extract is related to the presence of large quantities of polyphenolic compounds.

Phytochemical analysis of PC showed that the total polyphenol content of the extract was 167.8 μg/ml; it contained tannins (34%), flavonoids (0.17%), catechins and proanthocyanidines (2 mg/kg). The identification of individual compounds showed that flavonoids – aglycones and glycosides (quercetin, quercetin 3-O-galactoside, morin, myricetin, kaempferol, rhamnasin, retusin, apigenin), phenolic acids (caffeic, ellagic, quinic, chlorogenic), gallotannins and catechins were present (Table 2). The chemical composition was confirmed by HPLC analysis (Pantev et al. 2006). Most of the biologically active compounds with established antiinfluenza virus activity, identified in the preparation, belong to chemical groups, known as in-

Table 2: Antiviral effect of polyphenol compounds, identified in PC on the replication of influenza virus A/Aichi in MDCK

Substance	TC_{50}[a] (μg/ml)	EC_{50}[b] (μg/ml)	SI[c]
Total EtOH extract	100.0	6.2	16.13
Quercetin	60.0	8.4	7.14
Kaempferol	60.0	12.1	4.96
Myricetin	100.0	20.0	5.0
Morin	100.0	$>TC_{50}$	
Apigenin	60.0	10.0	6.0
Quercetin-3-*O*-galactoside	100.0	8.7	11.5
(+)Catechin	100.0	10.3	9.71
(−)Catechin	120.0	8.7	13.8
(−)Epicatechin	120.0	10.0	12.0
Caffeic acid	150.0	15.0	10.0
Rimantadine	>32	0.2	>160

[a] TC_{50}, 50% toxic concentration to MDCK cells
[b] EC_{50}, 50% effective virus-inhibitory concentration
[c] selectivity index = TC_{50}/EC_{50}

hibitors of viral growth. Apigenin, quercetin and their glycosides, found in *V. thapsiphorme* (Skwarek 1979), quercetin and catechins, from *H. perforatum* (Derebenzeva et al. 1972), kaempferol, quercetin and myricetin, discovered in *E. hirsutum* (Ivancheva et al. 1992), tea catechins (Nakayama et al. 1993) and catechins from *E. nebrodensis* (Cottiglia et al. 2005), epicatechins from Chinese quince (Hamauzu et al. 2005) were shown to inhibit influenza virus replication *in vitro*. Data on the protective effect of polyphenols in experimental influenza infection were reported by Polikoff et al. (1966, caffeic acid), Vickanova et al. (1970, flavonoid gossipol from *G. hirsutum*), Nagai et al. (1992, flavonoid F36 from *S. baicaliensis*), Sidwell et al. (1994, flavonoid from Euphorbiaceae).

It has been demonstrated that the virus-inhibitory effect of the extract could not be attributed to one or few separate ingredients (Table 2). The presence of a diversity of biologically active compounds, as well as the possible synergistic action between them seemed to be more significant for the total protective effect.

With regard to the need for new anti-influenza therapeutics, an inhalable formulation of the polyphenol-rich extract from *Geranium sanguineum* L. appears to have a potential as an option as a supplementary remedy to currently available anti-influenza drugs.

3. Experimental

3.1. Compounds

Geranium sanguineum L. (Geraniaceae) has been introduced into the experimental field of the Institute of Botany, Bulg. Acad. Sci., Sofia and a voucher specimen was deposited in the Herbarium of the same institute (N SOM 5/96). The preparation of the extract has been described in detail previously (Serkedjieva and Manolova 1992). In short – ground air-dried aerial roots, collected during the flowering period, were defatted with petroleum ether and treated with ethanol to fully extract the polyphenolic components. The extract was lyophilized (yield 16%); the obtained preparation (PC) was a dark red powder, odorless, soluble in water. The polyphenol content of PC was controlled by thin-layer chromatography and by quantitative determination of tannins, flavonoids and catechins (Ivancheva et al. 1996).

Quercetin, kaempferol, myricetin, morin, apigenin, retusin, quercetin-3-O-galactoside (hyperoside), (−)catechin, (+)catechin, (−)epicatechin, chlorogenic, ellagic, quinic and caffeic acids were from Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany. Rimantadine hydrochloride was from Hoffman-La Roche, Nutley, NJ, USA. Dr. N. Mahmood and Dr. A.J. Hay (NIMR, Mill Hill, London, UK) provided some of the polyphenolic compounds.

3.2. Cells, media and viruses

Cell cultures from chicken embryo fibroblasts (CEF) were obtained from 11 days old fertile hens' eggs by a standard procedure and maintained as in Serkedjieva and Hay (1998). Madin-Darby canine kidney (MDCK) were passaged in Dulbecco's Eagle medium (GibcoBRL, Scotland, UK), supplemented with 5% fetal calf serum (FCS) (BioWhittaker Europe, Germany and antibiotics; cell cultures were cultivated at 37 °C in the presence of 5% CO_2 until confluent monolayers were formed. In the antiviral experiments 0.5% FCS was added. MDCK were provided by Mrs. I. Roeva, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia. The human influenza virus A/Aichi/2/68 (H3N2) (A/Aichi), adapted to MDCK and cultivated in the presence of 2 μg/ml trypsin (Sigma) was used as a test virus. A/Aichi, adapted to mice lungs (A/Aichi-ad) was used for the animal experiments in the dose 5 LD_{50}. The virus stocks were stored at −80 ^{0}C. The virus infectious titres were in the range $10^{6.3}$–$10^{7.5}$ $TCID_{50}$ (50% tissue culture infectious doses)/0.2 ml and infection was induced with 100 $TCID_{50}$/0.2 ml. The viruses were from the collection of the Institute of Microbiology, Bulgarian Academy of Sciences, Sofia.

3.3. Mice

Male and female inbred ICR mice, body weight 16–18 g, were obtained from the Experimental Animal Station, Bulgarian Academy of Sciences, Slivnitsa. They were quarantined 24 h prior to use and maintained on standard laboratory chow and tap water *ad libitum* for the duration of the studies. Specialized personnel took care of the welfare of the animals. The animals were bred under standard conditions, accepted by the Bulgarian Veterinary Health Service.

3.4. Cellular toxicity

The cell-toxic effect was examined following the cytopathic effect of the preparations (CPE) as described before (Serkedjieva and Hay 1998). The dose, causing visible changes in cell morphology or sheet density in 50% of intact cells was evaluated from graphic plots (50% toxic concentration, TC_{50}).

3.5. Cytopathogenic effect (CPE) reduction assay

The assay was as described by Serkedjieva and Hay 1998. The antiviral effect was studied in multicycle experiments of viral growth. The virus-induced CPE was used as a measure of viral replication. The substances were inoculated simultaneously with viral infection. The dose, reducing CPE by 50% with respect to virus control was estimated (50% effective concentration, EC_{50}). The selectivity index (SI) was found from the equation TC_{50}/EC_{50}. Selectivity index >4 was considered to indicate a significant selective antiviral effect. For comparative reasons the effects of caffeic acid and respective solvents were tested. All antiviral experiments were carried out in parallel with the selective antiinfluenza drug rimantadine hydrochloride.

3.6. Viral infection

The infection was induced under light ether anesthesia by intranasal inoculation of influenza virus A/Aichi/2/68 (H3N2), adapted to mouse lung. This virus causes hemorrhagic pneumonia in mice. The virus is from the collection of the Institute of Microbiology, Bulgarian Academy of Sciences. It is maintained by passages in mice lungs and in fertile hen's eggs. The viral stock is kept at −80 °C. To cause lethal infection, mice were infected with 5–10 LD_{50} (5.10^2–10^3 EID_{50}) of the virus in the volume of 0.05 ml phosphate buffered physiological saline (PBS)/mouse.

3.7. Aerosol camera

The experiments were performed in an original aerosol generator system, based on the flow-dynamic principle, the ratio V/Q (chamber volume/amount of air influx = 520 l/400 l/min) being 1.3 min. This ensures equal quantities of continuously influxed and released conditioned air and aerosol. Thus the conditions of aerosol influx with respect to time, temperature, humidity, air pressure and concentration are standardized. The aerosol system consists of an exposure chamber with a connecting cylinder to a satellite chamber, a desk for distance command and a block with control measuring appliances. The nebulizer, situated in the exposure chamber, controls the aerosol quantity/min, the concentration and the size of aerosol particles. The nebulizing of the suspension is accomplished with airflow from an electric generator under 0.3–0.4 atmospheres and a rate of nebulizing of 1.0 ml/min. The size as well as the distribution of the aerosol particles is measured automatically with a specific device to "Opton" microscope. Impingers, containing liquid collecting medium, are used to perform quantitative and qualitative assessment of aerosol samples from the chamber. The inhaled dose (D_{inh}) is $D_{imp} \times R$, where D_{imp} is the dose in the impingers after exposure of 10 min, measured spectrophotometricaly and plotted against a calibration curve and R is the coefficient of respiration, evaluated from the animals' respiratory volume. R for a mouse of 20 g for 10 min is 0.3 (Libich 1962; Rosebury 1947). As the aerosol particles, retained in the lungs, represent 27% of all particles, the effective dose of aerosol treatment (D_{eff}) is evaluated from the equation $D_{eff} = D_{inh}/100 \times 27$ (Libich 1962; Rosebury 1947). The determinations are done according a mathematical model, developed by Libich (1962).

3.8. Experimental design

The presented experiments were done with aerosolized liquid suspension, containing aerosol particles with controlled size (2–5 μm). The input dose was 18% solution of the substance. The dose in the impingers was 69 mg/ml, D_{inh} = 20 mg/ml, D_{eff} = 5.4 mg/ml. PC was applied according to different treatment schedules. The experimental groups were of 20 animals each. The animals were kept in plastic cages and were exposed to the aerosolized extract for 10 min at every time point (Table 1). Mice were observed for death daily for 14 days after viral challenge. After the end of the experiments surviving mice were sacrificed by cervical dislocation. To determine lung parameters on days 6 after infection groups of animals from each experimental group were sacrificed, lungs were removed aseptically and *lung consolidation* (score) was scored: 0 – normal, 1–25% consolidation, 2–50% consolidation, 3–75% consolidation, 4–100% consolidation. *Infectious virus titres* were determined in embryonated hen's eggs. Mice lungs (3 for each determination) were homogenized to a 10% suspension in phosphate buffered saline (PBS) and ten-fold dilutions (0.2 ml) were assayed for infectivity in quadruplicate; hemagglutination (HA) was used as an end point. The infectious titre was estimated as the reciprocal value of the last viral dilution in which hemagglutination was observed in 50% of the samples and the titres were expressed as $\log_{10}$ 50% egg infectious doses (EID_{50})/ml. The *HA-assay* was done *per se*: 0.5 ml of virus suspensions were incubated with an equal volume of 1% hen erythrocyte suspension for 30 min at the room temperature. Virus-infected, not-treated with PC animals were used as virus control.

The *protective effect* was estimated by the reduction of the lung virus infectious titre, lung consolidation, rate of mortality; the increase of indices of protection and prolongation of mean survival time (MST) as described in Serkedjieva and Ivanova (1997). The index of protection (PI) was determined from the equation (PR-1)/PRx100, where PR (ratio of protection) is $M_{control}/M_{experiment}$ and M is mortality.

3.9. Statistical evaluations

Differences in numbers of survivors, mean survival times and mean lung virus titres between control and treated experimental groups were analyzed by Student's t-test. The Wilcoxon ranked sum analysis test was used to compare mean lung scores. $P < 0.05$ was accepted for statistical significance.

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References

Antonova-Nikolova S, Ivanova I, Ivancheva S, Tsvetkova R, Serkedjieva J (2002) Protease-inhibitory activity of a plant preparation with anti-influenza virus effect. In: Proceedings of Xth Congress of Bulgarian Microbiologists, Plovdiv, 9–12 October 2002, v. 1, pp. 358–362.

Che CT (1991) Plants as a source of potential antiviral agents. Econ Med Plant Res 5: 167–251.

Cottiglia F, Bonsignore L, Casu L, Deidda D, Pompei R, Casu M, Floris C (2005) Phenolic constituents from *Ephedra nebrodensis*. Nat Prod Res 19: 117–123.

Derebenzeva NA, Mishenkova EL, Garagulja OD (1972) Microbiologicheski J 34: 768–772 (in Ukrainian).

Droebner K, Ehrhardt C, Poetter A, Ludwig S, Planz O (2007) CYSTUS052, A polyphenol-rich plant extract, exerts anti-influenza virus activity in mice. Antiviral Res doi:10.1016/j.antiviral.2007.05.002.

Hamauzu Y, Yasui H, Inno T, Kume C, Omanyuda M (2005) Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. J Agric Food Chem 53: 928–934.

Ivancheva S, Manolova N, Serkedjieva J, Dimov V, Ivanovska N (1992) Basic Life Sci 59: 717–729.

Ivancheva S, Stancheva B, Serkedjieva J (1996) Polyphenol compounds in *Geranium sanguineum* L. Comptes Rendus Bulgarian Acad Sci 49: 41–43.

Ivanova E, Toshkova R, Serkedjieva J (2005) A plant polyphenol-rich extract restores the suppressed functions of phagocytes in influenza virus-infected mice. Microbes and Infection 7: 391–398.

Libich J (1962) Effect of the administration of streptomycin in the incubation and manifest phase on the course of inhalation tularaemia in guinea pigs. Folia Microbiologica (Praha) 7: 320–325.

Naga, T, Miyaichi Y, Tomomori T, Suzuki Y, Yamada H (1992) In vivo antiinfluenza virus activity of plant flavonoids possessing inhibitory activity for influenza virus sialidase. Antiviral Res 19: 207–219.

Nakayama M, Suzuki K, Toda M, Okubo S, Hara Y, Shimamura T (1993) Antiviral Res 21: 289–299.

Pantev A, Ivancheva S, Staneva L, Serkedjieva J (2006) Biologically active constituents of a polyphenol extract from *Geranium sanguineum* L. with antiviral activity. Z Naturforsch C 61c: 508–516.

Pollikoff R, Liberman M, Cochran KW, Pascale AM (1966) Effect of caffeic acid on mouse and ferret lung infected with influenza A virus. Antimicrob Agents Chemother 5: 561–566.

Rosebury T (1947) Experimental air-born infection. William and Wilkins Co., Baltimore.

Serkedjieva J, Manolova N (1992) A plant polyphenolic complex inhibits the reproduction of influenza and herpes simplex viruses. Basic Life Sci 59: 705–715.

Serkedjieva J (1995) Inhibition of influenza virus protein synthesis by a plant preparation from *Geranium sanguineum* L. Acta Virol 39: 5–10.

Serkedjieva J, Tonew EC (1996) Inhibitory effect of a plant preparation from *Geranium sanguineum* L. on virus specific RNA synthesis. Comptes Rendus Bulgarian Acad Sci 49: 73–76.

Serkedjieva J, Ivanova E (1997) Combined protective effect of an immunostimulatory bacterial preparation and rimantadine in experimental influenza A virus infection. Acta Virol 41: 65–70.

Serkedjieva J, Hay AJ (1998) *In vitro* anti-influenza virus activity of a plant preparation from *Geranium sanguineum* L. Antiviral Res 37: 221–230.

Serkedjieva J, Roeva I, Ivancheva S, Tzvetkova R, Mladenov K, Balabanov T, Mechkueva L. (2002) Influence of treatment schedules and route of inoculation on the protective effect of a polyphenol extract, isolated from *Geranium sanguineum* L. in the murine influenza A virus infection. In: Proceedings Xth Congress of Bulgarian Microbiologists, Plovdiv, 9–12 October 2002, v. 2, pp. 310–315.

Serkedjieva J (2003) Influenza virus variants with reduced susceptibility to inhibition with a polyphenol extract from *Geranium sanguineum* L. Pharmazie 58: 53–57.

Sidwell RW, Huffman JH, Moscon BJ, Warren RP (1994) Influenza virus-inhibitory effects of intraperitoneally and aerosol-administered SP-303, a plant flavonoid. Chemotherapy 40: 42–50.

Skwarek T (1979) Effect of plant extracts on the growth of influenza viruses. I. Effect of plant extracts on the growth of influenza viruses in cultures of chick embryo fibroblasts and in chick embryos. Acta Polon Pharmacol 36: 605–612 (in Polish).

Smee DF, Bailey KW, Sidwell RW (2000) Treatment of cowpox virus respiratory infections in mice with ribavirin as a single agent or followed sequentially by cidofovir. Antiviral Chem Chemother 11: 303–309.

Sokmen M, Angelova M, Krumova E, Pashova S, Ivancheva S, Sokmen A, Serkedjieva J (2005) *In vitro* antioxidant activity of a plant polyphenol extract with antiviral properties. Life Sci 76: 2981–2993.

Toshkova R Nikolova N, Ivanova E, Ivancheva S, Serkedjieva J (2004) *In vitro* investigation on the effect of a plant polyphenol extract with antiviral activity on the functions of mice phagocyte cells. Pharmazie 59: 150–154.

Vickanova, SA, Oifa AI, Gorjunova LV (1970) On the antiviral properties of gossipol in experimental influenza infection. Antibiotiki 12, 1071–1076 (in Russian).